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Preparation of Porcine Allogenic Acellular Diaphragm Matrix and its Application for Umbilical Hernioplasty in Pig

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ABSTRACT

Background: The surgical reconstruction of large abdominal defects with application of surgical mesh of synthetic origin resulted a number of potential clinical complications; although it is having good tensile strength. Acellular *bio-mesh* derived from slaughtered animals could be better alternative for surgical management abdominal wall defects. The present study aimed to evaluate bio-detergent based decellularized allogenic porcine acellular diaphragm matrix in umbilical hernioplasty in pig.

Methods: For the present study sodium deoxycholate was used as bio-detergent for preparation of porcine allogenic acellular diaphragm matrix (ADM). All total eighteen (18) numbers of piglets aged 2-3 months, body weight 8-14 kg and either sex with umbilical hernia equally divided in to three groups. Group A was subjected to application of ADM; while in Group B injection of methylprednisolone along with ADM application and Autologous Tunica Vaginalis was used for hernioplasty in Group C. Various parameters *viz* clinical, gross evaluation of surgical wound, haemato-biochemical and indirect ELISA of blood and serum was carried out as per standard methods.

Result: Decellularization protocol carried out with sodium deoxycholate as bio-detergent@ 2% concentration resulted complete decellularization of porcine diaphragm and preserved the distinctive, natural, three-dimensional collagen structure within the prepared matrix. Clinical and haemato-biochemical observations following umbilical hernioplasty in pig with ADM promises its clinical utility. The immune response of host tissue to *bio-mesh* as revealed by Indirect ELISA was negligible.

Key words: Bio-mesh, Decellularization, Hernioplasty, Pig, Umbilical hernia.

INTRODUCTION

The herniation of abdominal organ *viz*. rumen, intestine, omentum etc may result due to defects in abdominal wall as a result of trauma or diseases requires immediate corrections (Sankar *et al.,* 2010). The common complications of surgical reconstruction of abdominal wall defects include incisional hernias with additional complications of adhesions, suture sinus, fistula formation, gangrene *etc* (Penttinen and Gronroos, 2008). Despite the development of improved surgical techniques, the repair of the abdominal wall defects remains a clinical challenge for the veterinary surgeon and the use of prosthetic materials for surgical management of abdominal wall defect is required when the size of the defect or hernial ring size exceeds 3 cm in diameter (Vilar *et al.,* 2011). More than 80 different types of synthetic mesh have been used till date in the repair of abdominal wall reconstruction; however, although these synthetic meshes present excellent tensile strength and easy to handle, but with potential clinical complications could result following abdominal wall repair includes wound infections, bowel fistula, adhesion, erosion into the abdominal viscera, increased recurrence rate, persistent abdominal pain, mesh extrusion, seroma formation *etc* (Karrouf *et al*., 2016). The added advantages of biological mesh over synthetic prosthetic mesh such as minimal adhesion formation, providing better framework for fibroblast proliferation, release of antimicrobial peptides and non-complement fixing antibodies, induction of mild inflammatory response, host cell migration and angiogenesis neovascularization and building of multidirectional fibrous tissue proliferation (Pillay *et al.,* 2002 and Bellows *et al.,* 2007). The preparation of biological mesh involves the removal of cells (Decellularization) from a tissue or an organ leaves the complex mixture of structural and functional proteins that constitute the extracellular matrix (ECM). The goal of decellularization of biological materials is to ameliorate the antigenicity of biological graft by efficiently removing all its cellular and nuclear material while minimizing any adverse effects on the composition, biological activity, and mechanical integrity of the remaining ECM (Gilbert *et al.,* 2006). Use of chemical detergents like sodium deoxycholate solution under physical agitation can be used for complete decellularization of biological tissues like diaphragm, urinary

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bladder, pericardium *etc*. In the present study, sodium deoxycholate based decellularized allogenic acellular porcine diaphragm matrix (ADM) was evaluated following application in umbilical hernioplasty in piglets.

MATERIALS AND METHODS

The experiment was carried out in the Department of Surgery and Radiology, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam during January 2018 to December 2019. All total eighteen (18) numbers of young piglets of age group 2-3 months of either sex, of weighing 8-14 kg clinically affected with umbilical hernia requiring hernioplasty was selected (Fig 1). The animals were divided randomly into three (3) groups consisting of six animals in each, *viz* Group A, Group B and Group C. Surgical hernioplasty was carried out with application of ADM in Group A; while hernioplasty was done in Group B animals with application of ADM along with Methylprednisolone @ 1 mg kg^{-1} IM at weekly interval for three occasions. Hernioplasty in Group C animals were carried out with application of Autologous Tunica Vaginalis harvested from the same animal after open covered method of castration and was treated as control.

Fresh diaphragm of porcine origin was collected from a local abattoir and immediately preserved in chilled (4°C) sterile 1× phosphate buffer saline (PBS, pH 7.4) containing 0.1% Amikacin (Mikacin, Aristo Pharmaceuticals Private Limited, Mumbai, India) and 0.2025% Ethylene Diamine Tetra Acetic Acid (EDTA). The tendinous portion of each diaphragm was excised and washed thoroughly with sterile 1X PBS to remove all the adherent blood and dirt and subjected to different concentrations of sodium deoxycholate solution (0.5%, 1%, 2% and 4%) at a constant temperature (37°C) on an orbital shaker (Orbitek, Scigenics Biotech Pvt. Ltd.) under physical agitation at rate of 180 rotations per minute. The sodium deoxycholate solution was changed in every 6 hours and representative samples were collected at interval of 12 hours till 72 hours of detergent treatment and fixed in 10% neutral buffered formalin for histomorphological analysis to confirm decellularization. Following decellularization the resulting porcine ADM was ringed extensively (6 times of 2 h each) with 1× PBS under constant agitation (180 rotations per minute) and temperature (37°C) on an orbital shaker to remove the residual chemicals and cellular debris. Further the prepared matrices were stored in a sterile 1× PBS solution containing 0.1% Amikacin and 0.1% Sodium Azide at -20°C until use. The present study was in full compliance with the Institutional Animal Ethics Committee, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. The decellularization protocol carried out in 2% sodium deoxycholate solution, incubated for duration of 24 h at 37°C under physical agitation in orbital shaker was found to best in preserving the distinctive, natural, three-dimensional collagen structure within the prepared matrix (Fig 2). Before hernioplasty each piglet was kept off-fed for 12 hours and deprived of water for 4 hours and surgical site was prepared as per standard protocol. The general anaesthesia was produced with atropine sulphate @ 20 µg/kg IM followed by diazepam @ 2 mg/kg IV and kg ketamine hydrochloride@ 10 mg/kg IV. The animal was then positioned in dorsal recumbency and externally the hernial ring was assessed by pressing with thumb (Fig 3). Following application of an elliptical skin incision over the swelling the hernial ring was exposed (Fig 4) and any adhesion remained was removed. In Group A and B hernioplasty was carried out with ADM (Fig 5). The bio-mesh was cut into appropriate size and sutured with abdominal wall in “inlay” fashion using polypropylene suture and skin incision was closed with horizontal mattress suture. In Groups C animals, the male piglets with clinical umbilical hernia were selected; where before surgical reconstruction of hernia autologous tunica vaginalis (Fig 6) was harvested flowing open covered methods of castration and was kept in chilled PBS before implanted in the hernial correction (Fig 7). In all the animals a course of antibiotic, regular dressing the skin wound with application of antiseptic ointment was followed till 5th day of operation and the external sutures were removed after 7th post-operative day.

The clinical parameters *viz*. rectal temperature, heart beat and respiration rate and gross evaluation of the surgical wound *viz*. degree of swelling, degree of exudation, degree of warmth and degree of pain on palpation were recorded as per the methods described by Kumar *et.al* (2002), on 0th, 3rd, 5th, 7th and 10th day of post-hernioplasty. The haemato-biochemical examination of whole blood and blood serum *viz*. Haemoglobin (%), Packed Cell Volume (%), Total Leukocyte Count ($10^3/mm^3$), Differential Leucocytic Count, Alanine Aminotransferase (ALT- U/L), Creatine Kinase (CK-U/L), Gama-glutamyl transferase (GGT-U/L), Serum Cortisol (ng/ml), Serum Total Protein (g/dl) and Serum Albumin (g/dl)was carried out on 0th, 3rd, 5th, 7th and 10th day following surgical hernioplasty. The blood serum was subjected to indirect ELISA as per method described by Mohsina *et al.* (2014) on 0th, 5th, 10th, 15th, 20th and 30th days of surgical reconstruction.

RESULTS AND DISCUSSION

The histomorphological examination of representative tissue sample collected after shorter duration (12 hours) under physical agitation in sodium deoxycholate of different concentration revealed presence of whole cells with no detectable changes in the architectural fibre pattern; however, the same treated with higher concentration (2.5%) for longer duration (24 to 48 hours) resulted complete decellularization with disintegration of tissue three-dimensional structure. Decellularization protocol run for 24 hours at 37°C in 2% sodium deoxycholate resulted complete removal of cells and preserving the distinctive, natural, three-dimensional collagen structure within the prepared matrix. Gilbert *et al.* (2006) reported that ionic biological detergent like sodium deoxycholate initiate decellularization with

disintegration of cell membrane and productively eliminate cellular residues; which accelerate by mechanical stirring. In contrary to author's findings Kumar *et al.* (2013 and 2015) standardized decellularization protocol for small intestine and diaphragm of bubaline origin with application of 2% sodium deoxycholate for a period of 36 and 48 hours at 37°C under physical agitation respectively. The changes of clinical parameters and gross changes of the surgical



Fig 1: Clinical case of umbilical hernia.



Fig 5: Hernioplasty with Bio-mesh.



Fig 2: Acellular bio-mesh.



Fig 6: Harvested autologous tunica vaginalis.



Fig 3: Assessing hernial ring.



Fig 7: Hernioplasty with tunica vaginalis



Fig 4: Hernial ring.



Fig 8: Animal after operation in group A on 7th day.

wound (Table 1) revealed that the rectal temperature (°C), heart rate (beat/minute) and respiration rate (rate/minute) were increased significantly on 3rd day in all the groups, thereafter the values were declining trend towards normalcy at the end of study. The elevated values immediately after surgical reconstruction might be due to surgical trauma. The author's findings were in accordance with the observations of Singh *et al.* (2008) in rabbits and Kumar *et al.* (2015) in pig. There was significant elevation of degree of swelling, exudation, warmth and pain on palpation score of surgical wounds in all the groups on 3rd post-surgical days followed by reduction towards end; however, there was significant difference of the values between Group A (Fig 8) and Group B on 5th and 7th post-surgical days (Fig 9).



Fig 9: Animal after operation in group B on 7th day.

This might be attributed to the effect of methylprednisolone administration in Group B. Kumar *et al.* (2013) reported mild to moderate inflammation, oedema and elevation of body physiological parameters following ventral hernioplasty in six (6) goats with application of acellular dermal matrix.

The changes of haematological parameters (Table 2) showed significant reduction of Hb%, PCV% and Lymphocyte %; while significant increase of TLC, Neutrophil % in all the groups on 3rd post-surgical day. The changes of Eosinophil %, Basophil % and Monocyte % remained non-significant variation in entire experiment periods. A significant variation of TLC, Neutrophil % and Lymphocyte % between Group A and Group B on 5th and 7th day, which might be due attributed to surgical trauma induced local inflammatory response of the host tissue and the result of neutrophilic leucocytosis and use of corticosteroids in Group B post-operatively resulting lyses of lymphoid tissue as well as lyses of circulating lymphocyte. The author's findings were in accordance with the findings of Tembhurne *et al.* (2010); Kumar *et al.* (2016) in goat; Toth *et al*. (2014) and Singh (2015) in canine.

The changes of biochemical parameters (Table 3) revealed significant elevation of Creatine Kinase (ul^{-1}), Serum Cortisol (ng ml^{-1}), Total Serum Protein (gdl^{-1}), Serum Globulin (gdl^{-1}) level of in all the groups on 3rd day following surgical reconstruction; however there was a significant variation of total serum protein level between Group A and

Table 1: Mean±SE of clinical parameters changes and gross evaluation of the surgical wound in different groups at different day of observation.

Parameter	Groups	Time intervals in days				
		0th	3rd	5th	7th	10th
Rectal temperature	A	$38.92\pm0.17^{a}_{A}$	$42.45\pm0.14^{b}_{A}$	$42.28\pm0.16^{b}_{A}$	40.80 ± 0.28^{c}	$39.18\pm0.13^{a}_{A}$
(°C)	B	$39.07\pm0.10^{a}_{A}$	$42.37\pm0.11^{b}_{A}$	$41.60\pm0.08^{cd}_{A}$	39.20 ± 0.17^{a}	$38.93\pm0.15^{ad}_{B}$
	C	$39.03\pm0.10^{a}_{A}$	$40.86\pm0.39^{b}_{B}$	$39.12\pm0.13^{ad}_{B}$	38.83 ± 0.12^{ae}	$38.52\pm0.08^{cde}_{C}$
Heart rate	A	$84.17\pm2.51^{a}_{A}$	$111.50\pm1.28^{b}_{A}$	$108.83\pm1.17^{bd}_{A}$	$103.16\pm2.02^{cd}_{A}$	$94.50\pm2.70^{e}_{A}$
(Beat/Minute)	B	$87.33\pm2.14^{a}_{AC}$	$105.83\pm2.79^{b}_{AC}$	$103.33\pm1.89^{bd}_{AC}$	$97.66\pm2.66^{cde}_{A}$	$92.83\pm2.02^{ae}_{AC}$
	C	$90.67\pm1.05^{a}_{BC}$	$100.83\pm1.17^{b}_{BC}$	$99.33\pm0.88^{b}_{BC}$	$89.50\pm3.23^{a}_{B}$	$87.83\pm3.33^{a}_{BC}$
Respiration rate	A	$29.67\pm1.70^{a}_{A}$	$47.33\pm1.12^{b}_{A}$	$47.83\pm1.16^{b}_{A}$	$39.16\pm1.58^{c}_{A}$	$34.33\pm0.95^{d}_{A}$
(Rate/Minute)	B	$30.83\pm1.19^{a}_{A}$	$45.67\pm1.23^{b}_{A}$	$43.33\pm1.63^{b}_{B}$	$37.67\pm1.54^{c}_{A}$	$33.33\pm0.80^{a}_{A}$
	C	$30.50\pm1.67^{a}_{A}$	$40.67\pm0.95^{b}_{B}$	$38.33\pm2.09^{b}_{C}$	$33.33\pm0.71^{a}_{A}$	$31.66\pm1.26^{a}_{A}$
Degree of swelling	A	$1.00\pm0.0^{a}_{A}$	$2.50\pm0.22^{b}_{A}$	$2.67\pm0.21^{b}_{A}$	$1.83\pm0.17^{c}_{A}$	$1.50\pm0.22^{a}_{A}$
Score (Scale of 1-4)	B	$1.00\pm0.00^{a}_{A}$	$2.17\pm0.17^{b}_{A}$	$1.83\pm0.31^{bc}_{B}$	$1.50\pm0.22^{ac}_{B}$	$1.17\pm0.17^{ac}_{A}$
	C	$1.00\pm0.00^{a}_{A}$	$1.50\pm0.22^{a}_{A}$	$1.33\pm0.21^{a}_{C}$	$1.00\pm0.00^{a}_{C}$	$1.17\pm0.17^{a}_{A}$
Degree of exudation	A	$1.00\pm0.00\ ^{a}_{A}$	$3.17\pm0.17^{b}_{A}$	$2.50\pm0.22^{c}_{A}$	$1.83\pm0.31^{d}_{A}$	$1.17\pm0.17^{a}_{A}$
Score (Scale of 1-4)	B	$1.00\pm0.00^{a}_{A}$	$2.83\pm0.31^{b}_{AB}$	$2.00\pm0.37^{c}_{B}$	$1.16\pm0.17^{a}_{B}$	$1.00\pm0.00^{a}_{A}$
	C	$1.00\pm0.00^{a}_{A}$	$2.50\pm0.23^{b}_{BC}$	$1.17\pm0.17^{a}_{B}$	$1.17\pm0.17^{a}_{B}$	$1.00\pm0.00^{a}_{A}$
Degree of warmth	A	$1.00\pm0.00^{a}_{A}$	$2.50\pm0.22^{b}_{A}$	$1.83\pm0.31^{c}_{A}$	$1.33\pm0.21^{a}_{A}$	$1.00\pm0.00^{a}_{A}$
Score (Scale of 1-4)	B	$1.00\pm0.00^{a}_{A}$	$2.16\pm0.17^{b}_{A}$	$1.33\pm0.21^{a}_{B}$	$1.00\pm0.00^{a}_{A}$	$1.00\pm0.00^{a}_{A}$
	C	$1.00\pm0.00^{a}_{A}$	$1.50\pm0.22^{bc}_{B}$	$1.17\pm0.17^{ac}_{B}$	$1.00\pm0.00^{a}_{A}$	$1.00\pm0.00^{a}_{A}$
Degree of pain on palpation	A	$1.00\pm0.00\ ^{a}_{A}$	$3.50\pm0.22^{b}_{A}$	$3.17\pm0.17^{b}_{A}$	$2.00\pm0.00^{c}_{A}$	$1.50\pm0.22^{d}_{A}$
score (Scale of 1-4)	B	$1.00\pm0.00^{a}_{A}$	$3.00\pm0.26^{b}_{B}$	$2.34\pm0.21^{c}_{B}$	$1.67\pm0.21^{d}_{A}$	$1.17\pm0.17^{c}_{AB}$
	C	$1.00\pm0.00^{a}_{A}$	$2.17\pm0.17^{b}_{C}$	$1.83\pm0.17^{b}_{C}$	$1.00\pm0.00^{a}_{B}$	$1.00\pm0.00^{a}_{BC}$

Levels bearing different superscript (between the intervals) and subscript (between the groups) letters vary significantly.

Table 2: Mean±SE of haematological changes in whole blood in different groups at different day of observations.

Parameter	Groups	Time intervals in days				
		0th	3rd	5th	7th	10th
Haemoglobin	A	11.83±0.37^{a}	9.69 ±0.51^{b}	10.23±0.68^{a}	10.25±0.50^{a}	11.88±0.66^{a}
(Hb= g/dl)	B	11.85±0.31^{a}	9.25±0.62^{bc}	10.43±0.69^{a}	10.77±0.57^{ac}	11.58±0.70^{a}
	C	11.00±0.72^{a}	9.22±0.76^{b}	10.83±0.70^{a}	10.28±0.78^{a}	10.95±0.66^{a}
Packed cell volume	A	32.90±0.70^{a}	28.03±1.32^{b}	28.52±1.53^{b}	30.95±0.94^{a}	32.25±0.80^{a}
(PCV) - (%)	B	31.33±0.75^{a}	28.63±1.69^{b}	27.25±1.83^{b}	30.95±0.94^{a}	32.75±0.94^{a}
	C	29.68±1.73^{a}	28.35±1.35^{a}	29.03±1.66^{a}	32.37±1.20^{a}	29.50±1.71^{a}
Total leukocyte count	A	24.38±1.15^{a}	34.43±1.00^{b}	32.51±0.94^{b}	28.52±0.33^{c}	24.42±1.22^{a}
(TLC)-(10^3/mm^3)	B	22.53±2.02^{a}	32.06±0.74^{b}	28.40±0.23^{c}	23.22±0.78^{a}	23.62±0.61^{a}
	C	23.02±1.18^{a}	31.04±0.95^{b}	24.19±0.75^{a}	23.53±1.65^{a}	21.22±1.06^{a}
Differential neutrophil	A	35.71±0.84$^{a}_{A}$	68.58±0.70$^{b}_{A}$	61.24±1.06$^{c}_{A}$	55.98±1.25$^{d}_{A}$	45.13±1.36$^{e}_{A}$
counts (%)	B	35.71±0.84$^{a}_{A}$	68.58±0.70$^{b}_{A}$	61.24±1.06$^{c}_{A}$	55.98±1.25$^{d}_{A}$	45.13±1.36$^{e}_{A}$
	C	33.57±1.01$^{a}_{A}$	53.88±0.72$^{b}_{B}$	42.30±1.58$^{c}_{C}$	34.37±1.32$^{a}_{C}$	34.26±1.05$^{a}_{C}$
Differential eosinophil	A	2.76±0.43	3.03±0.57	3.03±0.40	2.80±0.45	3.07±0.55
counts (%)	B	3.16±0.31	3.10±0.37	3.25±0.36	3.17±0.35	2.63±0.40
	C	3.48±0.28	2.84±0.35	3.37±0.61	3.11±0.37	2.73±0.46
Differential basophil	A	0.67±0.16	0.65±0.13	0.67±0.07	0.86±0.14	0.62±0.06
counts (%)	B	0.93±0.11	0.81±0.19	0.81±0.07	0.65±0.13	0.67±0.16
	C	0.52±0.10	0.81±0.07	0.65±0.07	0.70±0.17	0.86±0.14
Differential lymphocyte	A	54.22±0.70^{a}	22.52±1.79$^{b}_{A}$	31.20±0.86$^{c}_{A}$	34.60±0.29$^{c}_{A}$	45.55±1.51$^{d}_{A}$
cournt	B	53.03±0.96^{a}	25.68±1.34$^{b}_{AC}$	33.49±0.63$^{c}_{B}$	45.72±1.62$^{d}_{B}$	49.48±2.06$^{d}_{B}$
	C	55.90±0.97^{a}	29.36±1.43$^{b}_{BC}$	47.81±1.95$^{c}_{C}$	55.38±1.12$^{a}_{C}$	56.81±1.08$^{a}_{C}$
Differential monocyte	A	6.40±0.63$^{a}_{A}$	5.08±0.27$^{bd}_{A}$	3.81±0.12$^{c}_{A}$	5.66±0.13$^{ad}_{A}$	5.61±0.12$^{ad}_{A}$
counts (%)	B	8.29±0.22$^{a}_{B}$	8.29±0.22$^{a}_{B}$	6.07±0.12$^{b}_{B}$	6.21±0.27$^{b}_{A}$	5.20±0.48$^{c}_{A}$
	C	6.39±0.12$^{a}_{A}$	5.75±0.31$^{ac}_{A}$	5.68±0.17$^{a}_{B}$	6.33±0.27$^{a}_{A}$	5.20±0.35$^{bc}_{A}$

Levels bearing different superscript (between the intervals) and subscript (between the groups) letters vary significantly.

Table 3: Mean±se of biochemical changes in whole blood in different groups at different day of observations.

Parameter	Groups	Time intervals in days				
		0th	3rd	5th	7th	10th
Alanine amino transferase	A	48.51±3.33	53.05±4.41	52.73±3.01	49.67±4.49	49.38±2.01
(ALT)-(U/L)	B	48.66±4.05	55.50±3.57	51.72±2.40	50.73±3.90	50.30±5.11
	C	46.38±4.01	54.67±4.15	50.05±4.35	48.81±3.90	49.25±2.32
Creatine kinase	A	16.08±1.66^{a}	36.65±1.59^{b}	28.83±1.64^{c}	16.45±1.82^{a}	15.53±1.57^{a}
(CK)-(U/L)	B	15.55±1.41^{a}	35.42±1.37^{b}	24.80±1.63^{c}	16.63±1.37^{a}	17.61±1.72^{a}
	C	14.22±1.87^{a}	35.68±1.70^{b}	22.73±2.01^{c}	15.36±1.83^{a}	15.82±1.46^{a}
Gamma-glutamyl transferase	A	54.00±3.79	46.10±3.13	53.00±3.25	40.93±4.72	48.60±4.73
(GGT)-(U/L)	B	46.87±3.96	50.53±3.90	42.60±4.45	49.43±2.87	46.28±3.06
	C	46.87±3.96	50.53±3.90	42.60±4.45	49.43±2.87	46.28±3.06
Serum cortisol (ng/ml)	A	115.14±4.64$^{a}_{A}$	172.86±4.20$^{b}_{A}$	156.45±4.79$^{c}_{A}$	131.31±6.00$^{de}_{A}$	122.52±3.94$^{ae}_{A}$
	B	112.99±2.34$^{a}_{A}$	170.05±7.41$^{b}_{A}$	148.30±2.16$^{c}_{B}$	111.04±3.18$^{a}_{B}$	112.74±2.39$^{a}_{A}$
	C	116.49±3.96$^{a}_{A}$	174.65±7.31$^{b}_{A}$	134.20±7.25$^{c}_{B}$	112.50±4.71$^{a}_{B}$	106.24±2.13$^{a}_{A}$
Serum total protein (g/dl)	A	6.97±0.20$^{a}_{A}$	7.84±0.05$^{bc}_{A}$	8.20±0.18$^{b}_{A}$	7.69±0.26$^{bd}_{A}$	7.47±0.18$^{acd}_{A}$
	B	6.95±0.19$^{a}_{A}$	7.64±0.33$^{bc}_{A}$	7.62±0.18$^{ac}_{B}$	7.21±0.19$^{ac}_{A}$	7.18±0.19$^{ac}_{A}$
	C	6.96±0.17$^{a}_{A}$	7.14±0.22$^{a}_{A}$	7.32±0.27$^{a}_{A}$	7.24±0.21$^{a}_{A}$	7.01±0.18$^{a}_{A}$
Serum albumin (g/dl)	A	2.91±0.13	2.83±0.11	2.81±0.19	2.91±0.12	2.91±0.08
	B	2.79±0.15	2.73±0.16	2.78±0.21	2.70±0.12	2.87±0.13
	C	2.70±0.16	2.65±0.14	2.73±0.12	2.61±0.11	2.78±0.18
Serum globulin (g/dl)	A	4.09±0.06^{a}	4.95±0.16^{bc}	4.36±0.13^{b}	4.78±0.18^{bd}	4.56±0.23^{acd}
	B	4.18±0.27^{a}	4.84±0.39^{bc}	4.90±0.24^{bc}	4.55±0.28^{ac}	4.31±0.18^{ac}
	C	4.24±0.24^{a}	4.44±0.17^{a}	4.67±0.22^{a}	4.63±0.28^{a}	4.23±0.11^{a}

Levels bearing different superscript (between the intervals) and subscript (between the groups) letters vary significantly.

Table 4: Mean±SE absorbance at 492 nm waves length (od_{492}) of ELISA reaction in different groups at different days of observation.

Days	Indirect elisa (OD_{492})			
	Group A	Group B	Group C	Total
0th	$0.17\pm0.01^{a}_{A}$	$0.17\pm0.01^{a}_{A}$	$0.18\pm0.01^{ac}_{B}$	0.17 ± 0.01^{a}
5th	$0.20\pm0.01^{b}_{A}$	$0.19\pm0.01^{b}_{B}$	$0.17\pm0.01^{a}_{C}$	0.19 ± 0.01^{b}
10th	$0.30\pm0.01^{c}_{A}$	$0.29\pm0.01^{c}_{A}$	$0.19\pm0.01^{bc}_{B}$	0.26 ± 0.01^{c}
15th	$0.41\pm0.01^{d}_{A}$	$0.35\pm0.01^{d}_{B}$	$0.19\pm0.01^{bc}_{C}$	0.31 ± 0.02^{d}
20th	$0.41\pm0.01^{d}_{A}$	$0.33\pm0.01^{e}_{B}$	$0.19\pm0.01^{bc}_{C}$	0.31 ± 0.02^{d}
30th	$0.29\pm0.01^{e}_{A}$	$0.28\pm0.01^{c}_{A}$	$0.18\pm0.01^{a}_{B}$	0.25 ± 0.01^{e}
Total	$0.30\pm0.02_{A}$	$0.27\pm0.01_{B}$	$0.18\pm0.01_{C}$	

Levels bearing different superscript (between the intervals) and subscript (between the groups) letters vary significantly.

Group B was observed on 5th day. Tissue traumatisation during surgical intervention, stress, release of acute-phase protein and administration of methylprednisolone in Group B might be attributed to such changes. The values of Alanine Amino Transferase, Gamma-Glutamyle transferase and Serum Albumin were remained non-significant variation during entire experiment period. In agreement with the findings of the present experiment, Munthe-Kass *et al.,* (2018) and Nevill *et al.* (2010) in dog; Schreiber *et al.* (1982), Kumar *et al.* (2015) and Schulz *et al.* (2007) in piglet and Citil *et al.* (2004) and Jamma (2004) in cattle and buffaloes recorded similar results. Indirect ELISA (Table 4), absorbance at 492 nm (OD_{492}) revealed negligible antigenicity of autologous Tunica Vaginalis to host tissue (Group C); however minimal extracellular matrix elicits early immunogenic and inflammatory reaction in Group A and Group B, which decreased gradually. The difference of absorbance between Group A and Group B on 15th and 20th day of post implantation might be due to immunosuppressive action of methylprednisolone in Group B. Wu *et al.* (2002) observed that xenogenic acellular dermal matrix produced immunogenic and inflammatory reaction at early stage of implantation which decreased gradually. Coito and Kupiec-Weglinsky (1996) reported that even after remove the cellular components with nuclear debris from biomaterials the extracellular matrix of the acellular tissue itself may elicit some amount of immune response. In corroborated with the author's findings, Singh *et al.* (2008) recorded minimal ELISA reaction at 90th day of post implantation of acellular biomaterials of porcine origin in induced abdominal defect in rabbits following initial rise in antibody titre.

CONCLUSION

Chemical bio-detergent like sodium deoxycholate can effectively be used @ 2% concentration, for 24 hours treatment in orbital shaker for preparation of allogenic acellular diaphragm matrix (ADM) of porcine origin. Clinical, haemato-biochemical and immunological studies revealed that the umbilical hernioplasty with allogenic ADM promises clinical utility. The immune response of host tissue to ADM as revealed by Indirect ELISA was negligible.

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Conflict of interest

The authors declared that they have no any conflict of interest.

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