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Xenogenic Acellular Pericardium Matrix of Caprine and Porcine Origin for Abdominal Wall Reconstruction in Rabbits

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ABSTRACT

Background: The abdominal wall defects require urgent medical attention owing to the voluminous visceral content and uneven wound edges, which remains a clinical challenge for the surgeon. In the present study, xenogenic porcine and caprine acellular pericardium matrix was evaluated for surgical reconstruction of the induced abdominal wall in rabbits.

Methods: All total eighteen adult rabbits were divided into three equal groups. The defects were corrected with acellular caprine and porcine pericardium matrix in Group A and B, respectively and Group C was kept as control. Various parameters *viz* clinical, gross evaluation of surgical wound, degree of adhesion and histopathological changes were evaluated as per standard methods.

Result: Clinical parameters and gross changes of the surgical wound showed significant changes on the 3rd and 5th day in all the groups. TLC, Serum Alkaline Phosphatase and Total Protein showed significant variation in Group A and B on the 10th day. Macroscopic and histopathological evaluation of implanted mesh has shows better host tissue proliferation in both the groups. Acellular caprine and porcine pericardium can be good options as *bio-mesh* for the repair of the abdominal wall and the subject may be considered for in-depth studies and further research programs.

Key words: Abdominal defects, Acellular pericardium, Caprine, Porcine, Rabbits.

INTRODUCTION

Due to voluminous visceral content, large abdominal wall defects require immediate medical attention, a delayed approach that might prove malicious or life-threatening (Slatter, 2003). The abdominal wall defects with uneven edges and contamination account majority of presented cases in veterinary practiceand makes reconstruction challenging to surgeons. Reconstruction of such defects with synthetic mesh may be a quick solution but may lead to strong inflammatory reactions, adhesion, chronic pain, adhesion with abdominal viscera with seroma and suture sinus formation (Baylon *et al.,* 2017). The application of biological mesh as an alternate tool, in the recent past, became popular as these materials are cheaper, less prone to adhesion formation and provides better fibroblast proliferation with neovascularization to release peptides and antibodies (Nath *et al*., 2022). These meshes are used to be absorbed completely in the body following host tissue proliferation (Singh *et al.,* 2008). Though autologous graft is best for tissue reconstruction, with potential/ donor site morbidity; however, the allogenic and xenogenic needs decellularization to minimize the immune rejection (Broyles *et al.,* 2013). Therefore, a pre-prepared acellular allogenic or xenogenic tissue matrix will help save time in an emergency situation as well as render less trauma in patients. The cellular and nuclear component of the tissue matrix is the potential antigen for the host. Hence the process of decellularization reduces the antigenicity to a great extent and immune rejection can be prevented (Crapro *et al*., 2011). In the present study, an acellular pericardium matrix of caprine and porcine origin was evaluated as a biological alternative to synthetic mesh in the surgical reconstruction of abdominal defects. Therefore, this study aims to explore the future prospect of acellular biological mesh for surgical management of clinical cases of abdominal wall defects.

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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, 2020 to December, 2020. The present study was in full compliance with the Institutional Animal Ethics Committee,

College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam.

All total eighteen (18) numbers of adult healthy New Zealand White rabbits of either sex was used for the study. The animals were maintained under similar managerial and environmental conditions throughout the experiment period. The animals were randomly divided into three groups consisting of six animals in each. Following creation of lateral abdominal wall defects in all the animals of Group A and B were corrected with acellular caprine pericardium matrix (ACP) and acellular porcine pericardium matrix (APP) respectively; while Group C animals were corrected with autologous tissue harvested from the same site and was treated as control. The preparation of ACP and APP was done as per the method described by Kumar *et al*., (2015).

Surgical reconstruction of abdominal defects was carried out under general anaesthesia with Xylazine Hydrochloride[1] and Ketamine Hydrochloride[2] @ 5 mg/kg and 35 mg/kg IM respectively in combination. Full thickness left abdominal was defect of size 2 × 2 cm^2 was created following standard aseptic procedure in each animal (Fig 1). In Group C the defect was immediately covered with the same autograft using synthetic absorbable suture (Fig 2). Pre-prepared ACP and APP were cut into appropriate sizes and was implanted as *bio-mesh* in Group A and B respectively with synthetic absorbable suture (Fig 3 and 4). In all the animals skin wound was closed using monofilament polyamide. Postoperatively, enrofloxacin (Flobac-SA, Intas Pharmaceuticals Ltd.) at the dose rate of 5 mg/kg body weight and meloxicam (Melonex, Intas Pharmaceuticals Ltd.) at the dose rate of 0.2 mg/kg body weight was injected intramuscularly and subcutaneously respectively. The skin sutures were removed on10thpostoperative day.

All the rabbits were observed for their general health, behaviour and alertness throughout the period of study. Gross evaluation of surgical wound *viz.* degree of swelling, exudation, warmth and pain was done as per method described by Singh *et al*., (2008) and clinical parameters were checked on 0th, 3rd,5th, 7th and10th day of operation. The haemato-biochemical evaluation was carried out on 0th, 10th, 20th and 30th day of operation as per the standard method using commercial kits. The surgical wounds were re-opened on 10th, 20th and 30thday to record the gross pathological changes such as adhesion score (if any) and representative tissue sample from each animal was collected for histopathological studies.

The statistical analysis was done as per the method described by Snedecor and Cochran (1989) with the help of statistical analysis system 9.3 (SAS9.3). The values were expressed non-significant difference ($p>0.05$) and highly significant changes ($p<0.01$) with similar and different superscripts in the tables.

RESULTS AND DISCUSSION

Mild anorexia, general inactivity was seen in all the animals after 2-3 days of surgical reconstruction; however, they recovered and seemed active and alert by 10th post-surgical day. None of the animal developed any sign of persistent

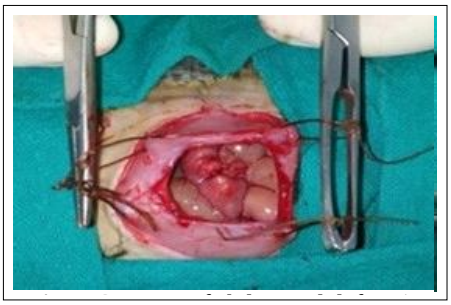

Fig 1: Creation of abdominal defect (2 × 2 cm^2).

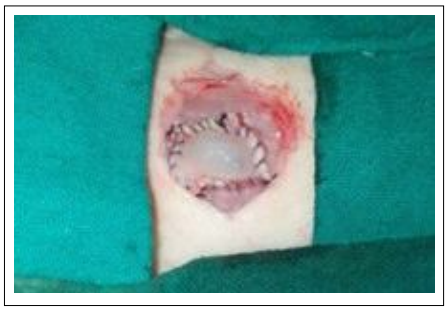

Fig 3: Repair of abdominal defect with ACP matrix (Group A).

Fig 2: Repair of abdominal defect with autologous graft (Group C).

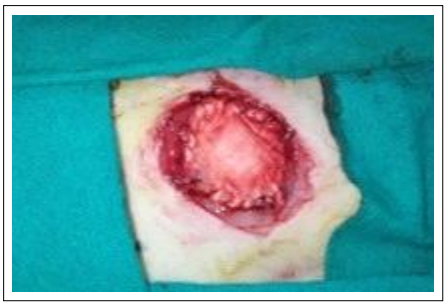

Fig 4: Repair of abdominal defect with APP matrix (Group B).

Note: [1]Hylazin, Indian Immunological Ltd.
[2]Aneket, Neon Lab. Ltd.:

inflammatory reaction, chronic pain, scar formation, suture sinuses, wound infection, wound dehiscence or herniation. The skin wounds in all the animals were completely healed as recorded on 10th post-surgical day and external stitches were removed. The changes of clinical parameters and gross evaluation of the surgical 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; however it was non-significant between the groups which indicates that biomaterial implant *i.e.* ACP and APP imparts little or no immediate effects on body clinical parameters. The post-surgical elevation of clinical parameters might be attributed to surgical pain, post-surgical fever and swelling. In agreement with the author's findings Nath (2018) recorded similar changes in clinical parameters in pigs in application of biomaterial for surgical reconstruction of abdominal wall. The gross evaluation of surgical wound revealed highest scores *viz.* degree of swelling, exudation, warmth and pain on 3rd day following operation; however, the changes remained non-significant between the groups. The gross changes of the surgical wound might be due to host tissue reaction as well as surgical stress. Similar observation was also noticed by Kumar *et al*., (2013) in goats with acellular dermal matrix and Abass *et al.* (2008) in sheep after correction of umbilical hernia using bovine tunica vaginalis. The changes in the haemato-biochemical parameters have been depicted in Table 2. There was mild decrease in blood haemoglobin concentration in all the groups on 10th post operative day, which might be due to blood loss during surgery. Munoz *et al*., (2015) stated that decrease of blood haemoglobin is of multifactorial origin following surgery. Kumar *et al.,* (2016) observed significant decline of haemoglobin level on day7 following fracture corrections in goat. There was mild level fall of PCV in all the groups on 10th day which was insignificant between the groups. It indicates that use of different biomaterial had a trifling effect on packed cell volume. A contemporaneous fall in blood haemoglobin concentration and regarded as post operative anaemia as discussed by Babu *et al.* (2014); Munoz *et al*., (2015) and Shander (2016). The elevated PCV on 20th and 30th might be due to regenerative action of haemopoetic system. TLC increased significantly in Group A and B and non-significantly in Group C on10th postoperative day which returned to normalcy towards the end. This could be due to local inflammatory reaction following surgical trauma. Similar findings were observed by Fazio *et al*., (2015) in bitch and cat; Kumar *et al*., (2016) in goats. In all the Groups the serum alkaline phosphatase (ALP) showed significant elevation on 10th day which returned gradually in subsequent observations. This might be attributed to ubiquitous nature of the enzyme as well as acute wound healing involves type III collagen formation. In agreement with the author's findings Vimalraj (2020) also mentioned ALP expression during hard tissue healing and inflammation. The magnitude of increase in enzyme activity in Group A and B was more as compared to Group C which could be due to extra cellular matrix regulated ALP induction. Similar observation recorded by

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

Parameter	Groups	Time intervals in days				
		0th	3rd	5th	7th	10th
Rectal temperature (°C)	A	39.14±0.27^{a}	40.50±0.22^{b}	40.27±0.36^{b}	39.77±0.32^{c}	39.22±0.24^{a}
	B	39.23±0.37^{a}	40.46±0.25^{b}	40.15±0.45^{bc}	39.79±0.46^{c}	39.19±0.25^{a}
	C	39.08±0.36^{a}	40.56±0.29^{b}	39.96±0.29^{c}	40.11±0.12^{bc}	39.17±0.32^{a}
Heart rate (Beat/Minute)	A	220.00±4.67^{Aa}	371.50±8.38^{Ab}	361.67±9.90^{Ab}	307.00±9.91^{Ac}	248.83±12.39^{Ad}
	B	222.67±5.91^{Aa}	368.83±9.59^{Ab}	364.67±14.05^{Ab}	292.33±10.60^{Ac}	203.33±8.42^{Bd}
	C	231.50±5.40^{Aa}	342.33±6.90^{Bb}	300.33±3.79^{Bc}	247.67±11.37^{Ba}	189.00±4.80^{Bd}
Respiration rate (Rate/Minute)	A	54.67±3.39^{a}	86.83±1.92^{b}	79.67±2.22^{b}	54.67±2.49^{a}	53.50±2.20^{a}
	B	54.83±3.75^{a}	84.00±2.54^{b}	83.00±0.97^{b}	59.83±3.37^{a}	56.33±2.01^{a}
	C	55.83±3.05^{a}	80.50±2.20^{b}	76.67±3.32^{b}	55.67±3.13^{a}	54.33±2.09^{a}
Degree of swelling Score (Scale of 1-4)	A	1.00±0.00^{Aa}	3.00±0.37^{ABb}	2.67±0.21^{Ab}	1.83±0.31^{Ac}	1.33±0.21^{Aac}
	B	1.00±0.0^{Aa}	3.17±0.40^{Ab}	2.67±0.33^{Ab}	1.83±0.31^{Ac}	1.50±0.22^{Aac}
	C	1.00±0.00^{Aa}	2.33±0.21^{Bb}	1.50±0.22^{Ba}	1.17±0.17^{Aa}	1.00±0.00^{Aa}
Degree of exudation score (Scale of 1-4)	A	1.00±0.00^{Aa}	3.17±0.31^{Ab}	2.83±0.31^{Ab}	1.67±0.21^{ABc}	1.33±0.21^{Aca}
	B	1.00±0.00^{Aa}	3.00±0.37^{Ab}	2.67±0.21^{Ab}	1.83±0.31^{Ac}	1.33±0.21^{Aca}
	C	1.00±0.00^{Aa}	2.33±0.21^{Bb}	1.67±0.21^{Bc}	1.17±0.17^{Bac}	1.00±0.00^{Aa}
Degree of warmth score (Scale of 1-4)	A	1.00±0.00^{Aa}	2.50±0.34^{ABb}	2.33±0.21^{Ab}	1.67±0.21^{ABc}	1.17±0.17^{Aac}
	B	1.00±0.00^{Aa}	2.83±0.31^{Ab}	2.50±0.22^{Abc}	2.00±0.26^{Ac}	1.33±0.21^{Aa}
	C	1.00±0.00^{Aa}	2.17±0.17^{Bb}	1.50±0.22^{Ba}	1.33±0.21^{Ba}	1.00±0.00^{Aa}
Degree of pain on palpation score (Scale of 1-4)	A	1.00±0.00^{a}	2.33±0.21^{b}	2.00±0.26^{b}	1.50±0.22^{c}	1.00±0.00^{a}
	B	1.00±0.00^{a}	2.50±0.22^{b}	2.17±0.17^{b}	1.50±0.22^{c}	1.00±0.00^{a}
	C	1.00±0.00^{a}	2.17±0.17^{b}	1.83±0.17^{b}	1.17±0.17^{a}	1.00±0.00^{a}

The means with common superscripts within a column (small letter) and within arrow (capital letter) does not differ significantly.

Vimalraj (2020) hard tissue healing. In contrary, Arun *et al.,* (2015) mentioned that there is an acute decrease in plasma ALP activity after brain injury. The total serum protein in all the animals exhibited significant elevation on 10th day following surgical reconstruction; however the values decreased on 20th and 30th day. There was non-significant difference of the values between control and treated groups might be attributed to comparative tissue reaction to xenograft. In accordance to the author's observation Munthe-Kaas *et al.,* (2018) recorded elevated serum total protein level in correction of inguinal hernia in pig. A state of hyperglycemia was developed in all the animals on 10th day after operation followed by declined significantly on 20th day and reached to base value on 30th day. This might be attributed to surgical trauma and metabolic stress response. The authors findings also supported by Duncan (2012).

The wounds were reopened on 10th, 20th and 30th post-surgical day in order to record the gross changes of the surgical site, implanted tissue, surrounding host tissue reaction, adhesions and the degree of incorporation of the engrafted biomaterial with the host tissue. In Group C the abdominal suture was slenderly palpable and moderately palpable in the other two groups on 10th post surgical day (Fig 5, 6 and 7). Mild adhesion of the grafted tissue viscera was seen in Group B which was easily separable (Fig 8). Moderate adhesion was recorded between biomaterial and

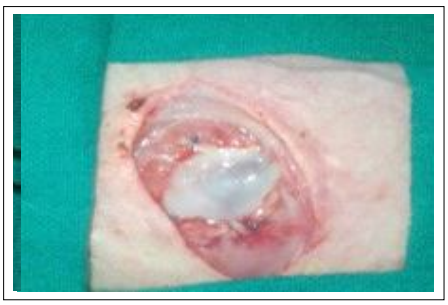

Fig 6: Abdominal wall defect on 10th day in Group A.

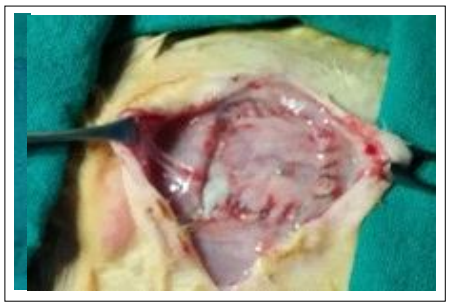

Fig 5: Abdominal wall defect on 10th day in Group C.

Fig 7: Abdominal wall defect on 10th day in Group B.

Table 2: Mean±SE of haemato-biochemical changesin whole blood in different groups at different day of observations.

Parameter	Groups	Time intervals in days			
		0th	10th	20th	30th
Haemoglobin (Hb= g/dl)	A	8.60±0.30^{ac}	8.08±0.31^{a}	10.38±0.37^{b}	9.37±0.41^{ab}
	B	9.26±0.32^{a}	8.12±0.32^{b}	9.34±0.54^{a}	8.91±0.23^{ab}
	C	8.83±0.36^{ab}	7.76±0.45^{a}	8.55±0.38^{ab}	9.03±0.47^{b}
Packed cell volume (PCV) - (%)	A	27.35±1.15^{ac}	24.60±1.00^{a}	31.48±1.29^{b}	28.90±1.20^{bc}
	B	28.33±0.84^{a}	25.70±0.83^{a}	28.63±1.43^{a}	27.82±0.63^{a}
	C	27.63±1.32^{a}	24.10±1.32^{b}	27.10±1.04^{a}	28.90±1.47^{a}
Total leukocyte count (TLC)-(10^3/mm^3)	A	6.30±0.32^{Aa}	9.32±0.27^{Ba}	6.94±0.32^{Aa}	6.46±0.31^{Aa}
	B	9.71±0.48^{Ab}	12.37±0.69^{Bb}	9.98±0.48^{Ab}	10.27±0.46^{Ab}
	C	8.25±0.39^{Ab}	9.19±1.66^{Aa}	8.46±0.23^{Aab}	8.81±0.34^{Ab}
Serum alkaline phosphatase (U/L)	A	59.15±2.72^{Aa}	104.28±2.45^{Ab}	81.90±2.00^{Ac}	76.47±1.72^{Ac}
	B	59.28±2.42^{Aa}	108.70±3.62^{Ab}	90.75±2.82^{Bc}	74.83±2.74^{ABd}
	C	59.32±3.62^{Aa}	78.68±4.19^{Bb}	72.23±3.65^{Cb}	66.15±3.99^{Bab}
Serum total protein (g/dl)	A	6.38±0.15^{a}	8.77±0.12^{b}	7.27±0.35^{c}	6.35±0.13^{a}
	B	6.28±0.15^{a}	8.80±0.12^{b}	7.25±0.35^{c}	6.48±0.22^{a}
	C	6.58±0.17^{a}	8.18±0.17^{b}	6.82±0.32^{a}	6.57±0.25^{a}
Random blood glucose (mg/dl)	A	94.38±0.15^{a}	122.45±0.12^{b}	97.88±0.35^{a}	92.35±0.13^{a}
	B	93.82±1.35^{a}	122.93±3.89^{b}	100.12±0.96^{c}	93.62±1.28^{a}
	C	94.38±1.79^{a}	111.12±0.97^{b}	104.23±1.72^{c}	90.12±1.39^{a}

The means with common superscripts within a column (small letter) and within arrow (capitalletter) does not differ significantly.

omentum in Group A, which was comfortably separated by blunt dissection (Fig 9). On contrary, there was no adhesion noticed in Group C (Fig 10). On 20thpost-surgical day in Group A, the abdominal suture was inconspicuously detectable with the outline (Fig 11); there was also mild adhesion with visceral organs (Fig 12). In group B the sutures were no more visible although the outline of the pericardium matrix was clear; the host tissue proliferation towards the engrafted tissue was clearly visible without abdominal adhesion (Fig 13). On the other hand, in Group C there was complete integration between the graft and native tissue, abdominal suture was feebly visible with a mild elevation over the surgical knot without any adhesion (Fig 14). On 30th post-operative day significant host tissue proliferation over the biomaterial and fibroplasias with fuzzy line of demarcation without any adhesion was seen in Group A (Fig 15). In Group B the matrix was entirely concealed by host tissue proliferation without any visceral adhesion (Fig 16). Group C

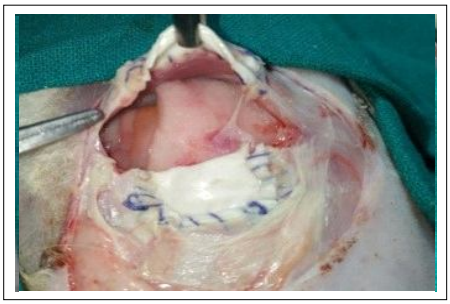

Fig 8: Mild visceral adhesion on 10th day in Group B.

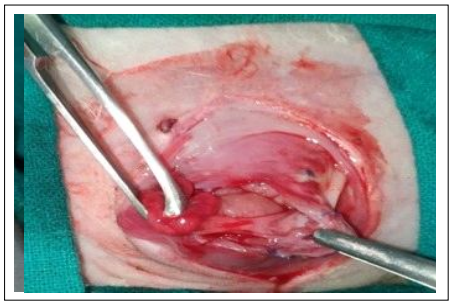

Fig 9: Moderate visceral adhesion on 10th day in Group A.

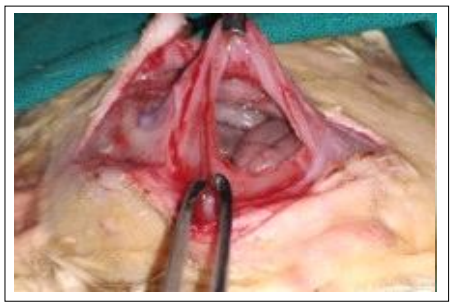

Fig 10: No visceral adhesion on 10th day in Group C.

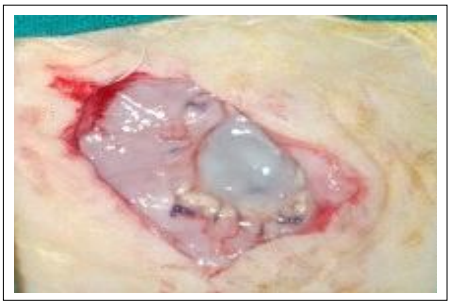

Fig 11: Abdominal defect on20th day in Group A.

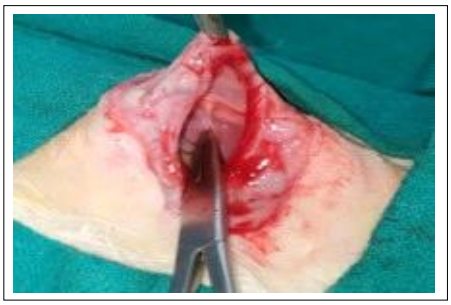

Fig 12: Abdominal defect on 20th with mild adhesion in Group A

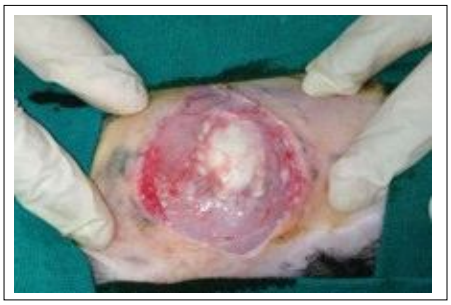

Fig 13: Abdominal defect on 20th day in Group B.

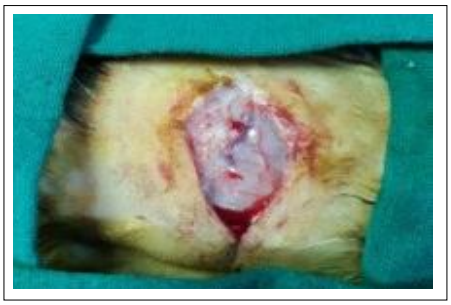

Fig 14: Abdominal defect on 20th day in Group C.

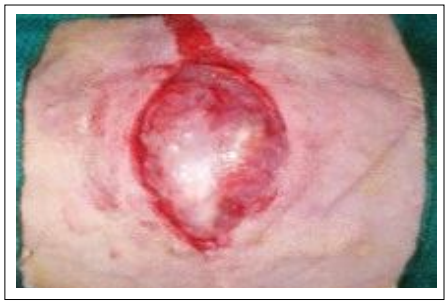

Fig 15: Abdominal defect on 30th day in Group A.

animals exhibited completely normalized abdominal wall with no traces of abdominal suture line and visceral adhesion (Fig 17). The initial mild to moderate visceral adhesion found resolved automatically in tested groups as observed on 30th day was also supported by findings of Singh *et al.,* (2008) in rabbits with biomaterial application and Nath *et al.* (2022) in rabbits with synthetic mesh application.

On 10th day, the histopathological examination of the implanted graft revealed early signs of neovascularization

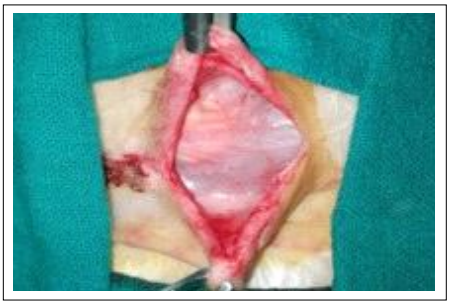

Fig 16: Abdominal defect on 30th day in Group B.

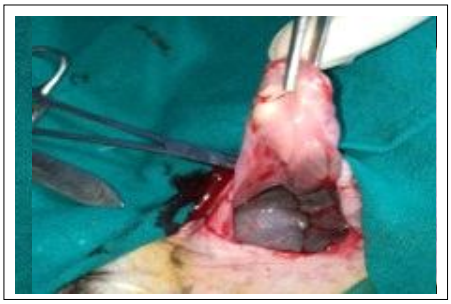

Fig 17: Abdominal defect on 30th day in Group C.

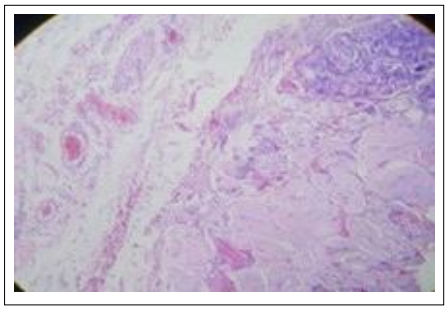

Fig 18: Photomicrograph showing neovascularisation in Group-C, day 10 (H and E, X10).

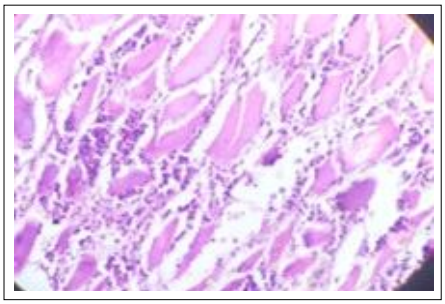

Fig 19: Photomicrograph showing angiogenes is in Group-A, day10 (H and E,X40).

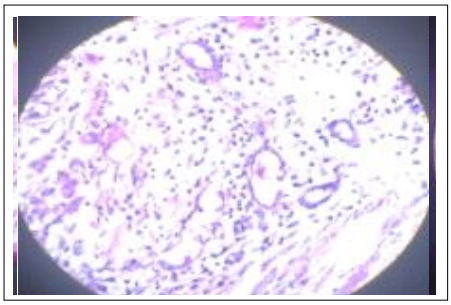

Fig 20: Photomicrograph showing cellular infiltration in Group-B, day 10 (H and E, X10)

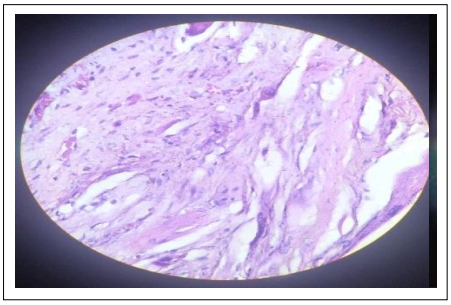

Fig 21: Photomicrograph showing angiogenes is in Group-C, day 20 (H and E, X40)

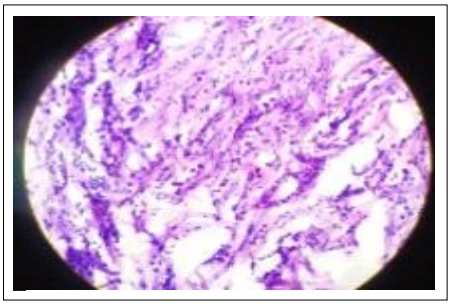

Fig 22: Photomicrograph showing well organised fibrous tissue in host tissue surrounding the implant in Group A day 20 (H and E, X 40).

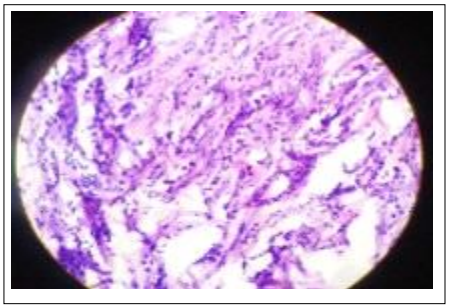

Fig 23: Photomicrograph showing cellular infiltration in Group B, day 20 (H and E, X 40).

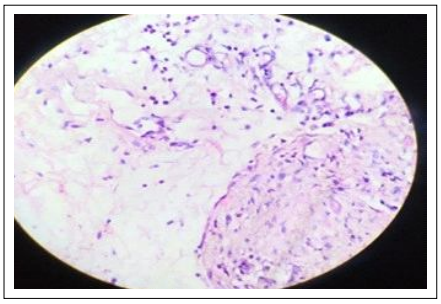

Fig 24: Photomicrograph showing angiogenesis and fibrosis with cellular infiltration on day 30 (H and E, X40).

with cellular infiltration (Fig 18) in Group C and B (Fig 19); while in Group A there were polymorphonuclear, mononuclear and fibroblast cellular infiltration (Fig 20). Fibrosis with marked angiogenesis was recorded on 20th day in Group C (Fig 21), which was well organized in Group A (Fig 22) and remained nearly unchanged in Group B (Fig 23). A mild degree of cellular infiltration along with neovascularization in all the groups was recorded till 30th day of examination (Fig 24). Cellular infiltration of polymorph nuclear cells, mononuclear cells and fibroblasts was observed in all the groups in entire experiment however highest was in Group A long with angiogenesis and fibrosis. This indicates that acellular caprine pericardium is well suited for integration with host tissue. The author's findings were in accordance with the observation of Singh *et al.,* (2008) after abdominal reconstruction with porcine bladder, diaphragm and dermal matrix in rabbits at day 7. Doria *et al*., (2018) found that inflammatory reaction had mostly subsided by 180 days after surgical repair of abdominal wall defects.

CONCLUSION

The application of ACP and APP matrix in induced abdominal wall reconstruction in rabbits showed encouraging results as observed in clinical, gross, haemoto-biochamical and histopathological studies. However, in-depth host tissue immune response has to be done in future to explore its clinical utility for surgical reconstruction of abdominal wall defects.

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Conflict of interest

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

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