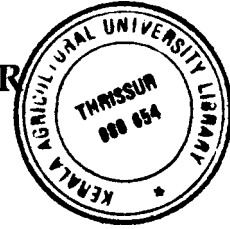# PROCESSED OESOPHAGEAL ALLOGRAFTS FOR HERNIOPLASTY IN PIGS

By
S. SENTHIL KUMAR

THESIS

Submitted in partial fulfillment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

DEPARTMENT OF SURGERY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR
KERALA, INDIA
2000

DECLARATION

I hereby declare that the thesis entitled **"PROCESSED OESOPHAGEAL ALLOGRAFTS FOR HERNIOPLASTY IN PIGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.
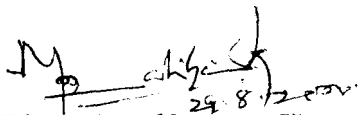
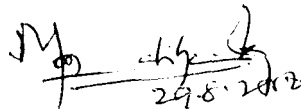Mannuthy,
29 Aug' 2000.

S. SENTHIL KUMAR

CERTIFICATE

Certified that the thesis entitled "**PROCESSED OESOPHAGEAL ALLOGRAFTS FOR HERNIOPLASTY IN PIGS**" is a record of research work done independently by **Shri. S. Senthil Kumar**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Mannuthy,
29ᵗʰ Aug' 2000.

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CERTIFICATE

We, the undersigned members of the Advisory Committee of **Shri. S. Senthil Kumar**, a candidate for the degree of Master of Veterinary Science in Veterinary Surgery, agree that the thesis entitled **"PROCESSED OESOPHAGEAL ALLOGRAFTS FOR HERNIOPLASTY IN PIGS"** may be submitted by Shri. S. Senthil Kumar, in partial fulfillment of the requirement for the degree.
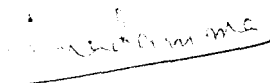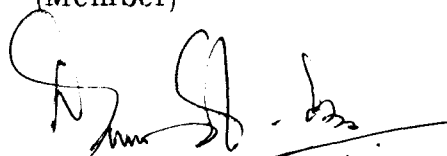
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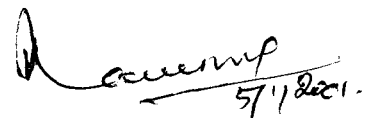Department of Surgery
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ACKNOWLEDGEMENT

S. SENTHIL KUMAR

Dedicated to my
beloved parents

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Introduction

1. INTRODUCTION

Umbilical hernia is one of the most frequent developmental defects in pigs. In swine herds, the reported frequency of umbilical hernia ranges from 0.4 to 1.2 per cent. Pigs with umbilical hernia often grow more slowly than non-affected littermates and sometimes die from strangulation of intestine in the umbilical ring (Searcy- Bernal *et al.*, 1994).

Counter irritants applied topically or injected subcutaneously was an early method of hernia correction (O' Connor, 1960). Infection and loss of clamps were common when metal or wooden clamps were applied to the hernial sac (Frank, 1964). Radical surgery of hernia is the most effective method of correcting it and minimising the aftercare required (Johnson, 1970).

Hernioplasty is a technique in which grafting of materials is used when the hernial ring is too large to close by simple suturing or when the suture line require additional support. Prosthetic materials such as ceramics, synthetic polymers, natural macromolecules and metals have been used as implants. Many synthetic materials like nylon, orlon and Dacron have been implanted in the abdominal wall to repair tissue defects. Tantalum mesh has been used more

commonly but reported to fragment after two to twelve months of implantation (Johnson, 1969). Wear particles released from metal prosthesis and degraded material from polymers cause sensitisation when they are used as *in vivo* implants (Mohanty, 1995). Besides, metallic implants are also painful to patients (Hurov, 1961). Synthetic fibres of nylon, orlon and Dacron are prone to infection resulting in sloughing of the graft.

Biological materials like split thickness skin graft, fascia lata, pericardium, peritoneum and bladder were used in hernioplasty either fresh or after processing and preservation. Split thickness skin grafts and homologous grafts of fascia lata undergo early dissolution in the host tissue resulting in the breakdown of repair (Mayou *et al.*, 1988).

Natural materials are better for *in vivo* applications and collagen seems to offer many of the desirable characteristics that are important for a biomaterial (Sastry, 1989). Collagen is being used in increasing amounts as an important prosthetic material in clinical surgery. Minimal tissue reaction after implantation is a significant factor favouring the use of collagen as a biomaterial (Simpson, 1983). Oesophageal collagen has higher tensile strength (Sastry *et al.*, 1998) and can be used as graft for closure of any body wall defect.

The safety evaluation of prosthetic materials is of paramount importance in the development of medical devices. Materials on contact with tissues evoke a variety of responses, which determine the ultimate fate of an implant or the compatibility of a device. Biological evaluation in addition to functional acceptance of the implant is important for selection of a material (Mohanty, 1995).

Glutaraldehyde is being used extensively as a cross-linking agent, which reduces biodegradation of collagen graft making them biocompatible and non-thrombogenic. Glutaraldehyde cross-linking has been found to be the most effective method of preparation of biological grafts (Jayakrishnan and Jameela, 1996).

The use of prosthetic materials in hernioplasty has been reported extensively, but literature on the use of collagen in pigs has been scanty. Hence the present study was conducted with the following objectives:

i)   To evaluate the suitability of processed collagen based oesophageal allograft as a biological tissue substitute for hernioplasty in pigs and

ii)   To compare the healing in herniorrhaphy and in hernioplasty where collagen based oesophageal graft was used.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Umbilical hernia in pigs

2.1.1 Incidence

Warwick (1926) reported that the incidence of umbilical hernia in pigs was about one per cent in females and 0.6 per cent in males.

Priester *et al.* (1970) studied the occurrence of congenital defects in domestic animals. They observed that swines had the highest proportion of congenital defects of all domesticated species, mainly in the form of umbilical hernia, inguinal hernia, cryptorchidism and anal atresia.

Hayes (1974) reported that the incidence of umbilical hernia and inguinal hernia was very high in pigs compared to other animals.

Bille and Nielson (1977) made an extensive post-mortem survey of piglets and observed that inguinal hernia was twice as common as umbilical hernia in them.

Huston *et al.* (1978) studied the incidence of various congenital defects in pigs. It was reported that

0.5 per cent of males and one per cent of females were affected with umbilical hernia.

Falkenberg *et al.* (1991) studied the incidence of various congenital defects in pigs and reported that hernia had higher frequency of occurrence compared to other congenital defects.

2.1.2 Etiology

Warwick (1926) suggested that umbilical hernia in pigs could be a heritable character and stated that hernia in boars resulted from double recessive genotype.

Wrathall (1975) reported that umbilical hernia in pigs was a polygenically inherited condition.

Freeman and Spencer (1991) studied the risk factors for umbilical hernia in horses. It was suggested that sex linkage could be a predisposing factor for umbilical hernia.

Edwards (1992) reported the possible hereditary basis of umbilical hernia in calves. It was suggested that male calves with hernia should be castrated at the same time as repairing the hernia if no other predisposing factors such as cord infection could be identified.

Stigler *et al.* (1992) studied the heritability of congenital defects in pigs and suggested two female carrier traits as the probable cause for umbilical hernia.

Bampton (1994) made an extensive study on the incidence of congenital defects in pigs. It was suggested that umbilical hernia had a genetic component, but was probably environmentally determined.

Searcy-Bernal *et al.* (1994) studied the factors associated with umbilical hernias in a swine herd. The results of the study supported the existing genetic and infectious hypotheses about causes of umbilical hernia.

Satio *et al.* (1995) reported that umbilical hernia in Japanese black breed calves were associated with autosomal trisomy. It was suggested that high maternal age could be a contributing factor for the chromosomal anomaly.

2.2 Surgical correction of hernia
2.2.1 Herniorrhaphy

Fretz *et al.* (1983) reported that the imbricating technique of hernia correction did not have any added advantage over simple appositional closure. Bursting strength of wound sutured by imbricating technique decreased to a degree proportional to the amount of

overlapping. Simple interrupted closure was preferred as it required less time and dissection, and was easy to perform.

Kanade *et al.* (1984) compared four surgical techniques of repair of umbilical hernia in calves. The techniques were simple interrupted sutures, overlapping sutures, figure of eight sutures and closure using stainless steel mesh. Simple interrupted sutures were found suitable for closing small sized hernial ring. Overlapping sutures provided additional support and was found suitable where the ring was large and the edges were thin and pliable. Tissue reaction was minimal following stainless steel implantation. Histopathological examination revealed thick, fibrous gelatinous layer completely enclosing the mesh by 14th postoperative day.

Nguhiu–Mwangi *et al.* (1991) developed a modified overlapping technique for correction of ventral abdominal hernia in large animals. The modification consisted of separating the tissues at the hernial ring into two or three layers. Each layer was apposed by overlapping sutures separately. The outermost layer was over sewn with Cushing's suture pattern followed by horizontal mattress suture for skin closure. Recurrent hernia following conventional overlapping mattress suture was also repaired by this modified technique and healing was satisfactory.

Gahlot *et al.* (1994) reported a case of umbilical hernia with rumen fistulation in a camel. The hernial ring being small was closed by conventional overlapping suture pattern and the animal had an uneventful recovery.

Riley *et al.* (1996) made a retrospective study and compared herniorrhaphy and clamping of umbilical hernia in horses. Majority of the surgically treated cases were in males, while majority of the cases in which clamps were used were in females. It was concluded that hernia with large hernial ring of size more than eight cm could be treated surgically rather than by use of clamps.

2.2.2 Hernioplasty
2.2.2.1 Synthetic materials

Paatsama (1954) described a method for the repair of umbilical, ventral and traumatic hernias in cattle by implanting a plastic mesh between the peritoneum and the abdominal muscles. A second fold of skin was sutured over the skin suture to protect it and to prevent exudation. It was concluded that this technique could be employed effectively in the surgical correction of hernia in large animals.

Johnson (1969) reported that marlex mesh could be used for the repair of experimentally created abdominal wall defect in ponies. It was observed that the mesh was

suitable for surgical manipulation and was incorporated in the tissues with very little foreign body reaction.

Philip (1973) reported successful treatment of three clinical cases of hernia with marlex mesh. In two cases the mesh was implanted as inlay graft and in the third case, as an onlay graft. It was concluded that suturing the mesh as an onlay graft was simpler, easier and time saving than the inlay graft technique. However, inlay grafting provided the firmest and strongest hold of the graft to the body wall.

Rajendran *et al.* (1974) reported the use of Polyethylene mesh in the surgical correction of porcine umbilical hernia where total apposition of the edges was not possible due to large hernial ring. Hence, herniorrhaphy with reinforcement using Polyethylene mesh was performed. Postoperative observation of animal for seven months did not reveal any recurrence of hernia. The implanted mesh was indistinguishable from the surrounding tissues and was interwoven with fibrous tissue.

Matera *et al.* (1976) described a technique of lateral overlapping with propylene mesh reinforcement for surgical correction of umbilical hernia in cattle. Histopathological examination after 110 days of mesh implantation revealed a mild chronic inflammatory process in

the vicinity of mesh with formation of hyalinized connective tissue.

Kanade *et al.* (1988) reported that ordinary cotton mesh could be used for the repair of defects of ventral body wall in buffalo calves. Mild to moderate degree of inflammation was observed up to 15th postoperative day. Moderate degree of collagen deposition was observed at the site of implantation in specimen collected after 90th postoperative day.

Sen and Paul (1989) suggested that nylon mesh could be used to repair defects of ventral abdominal wall. The commonly available nylon mosquito net was used as a mesh to correct two clinical cases of ventral hernia in buffalo calves. The material was well tolerated and no evidence of infection was observed.

Varshney and Singh (1991) reported that nylon mesh could be employed by the inlay graft method for correction of abdominal wall defect in clinical cases of ventral hernia in buffaloes.

Velden and Klein (1994) reported that implantation of polypropylene mesh on the outer side of the hernial defect was simple and effective. It was suggested that recurrence of hernia could be effectively treated by

implantation of a second and larger mesh across the previously implanted mesh.

Shoukry *et al.* (1997) reported that commercially available polyester fabric could be used in the repair of abdominal wall defects. Intraperitoneal implantation without omentalization was often associated with peritoneal adhesions. Histopathology at the sixth postoperative month revealed uniform infiltration of fibrous tissue and firm incorporation of mesh into the abdominal wall.

2.2.2.2 Biomaterials

Helphrey (1982) reported a surgical technique in which abdominal muscle graft was used for repair of chronic diaphragmatic hernia in two dogs. Transverse abdominal muscle flap of a size larger than the defect was employed to close the defect in the diaphragm. The method was successful in one dog. But the second dog died of torsion of liver.

Sharmah and Holl-Allen (1984) used glutaraldehyde treated porcine dermal collagen sterilized by gamma radiation for the repair of incisional hernia in human patients. The technique was found successful and it was concluded that porcine dermal collagen implantation could be

used as a good alternative to other methods of incisional hernia repair.

Becker *et al.* (1985) studied the use of bovine pericardial tissue for closing abdominal wall defects in calves. Epigastric defect created in the umbilical region was closed using processed pericardial tissue and the healing was found to be satisfactory.

Frankland (1986) employed glutaraldehyde treated porcine dermal collagen for the repair of perineal hernia in 21 dogs. In one case, the wound broke down completely, porcine collagen and suture were extruded and had to be removed. Junctional histopathology 28 weeks later revealed slow absorption of the implant and ingrowth of host collagen into the implanted site.

Kanade *et al.* (1986) used frozen diaphragm as an implant for the correction of experimentally created defect in the ventral abdomen in buffalo calves. The breaking and tensile strength of implanted tissue gradually increased up to 90th postoperative day. Histopathological examination of the tissue collected on the 60th and 90th postoperative day revealed complete healing of the defect and extensive development of fibroblasts around the junction.

Purdy (1987) reported that sheet of fascia lata harvested from the lateral hip surface could be used to reinforce sutured abdominal wall defect in ponies. The experimentally created ventral abdominal hernias were successfully treated with the technique.

Deokiouliyar *et al.* (1988) used buffalo pericardial tissue preserved in glycerol to repair experimentally induced hernia in buffalo calves. The pericardial implant became firmly incorporated in the host tissue. The graft was gradually invaded by connective tissue and was completely replaced in 16 weeks.

Varshney *et al.* (1990) used frozen homologous prosthetic materials like diaphragm, pericardium, tensor fascia lata and peritoneum for the repair of abdominal wall defect in buffaloes. The materials were implanted by inlay graft technique after defrosting. The breaking strength and tensile strength on the 90th postoperative day were maximum in the case of diaphragm graft followed by pericardium, tensor fascia lata and peritoneum grafts. Histopathological examination revealed a well organised fibroblastic proliferation and bundles of collagen and mature connective tissue at 60 to 90 days after implantation indicating the complete uptake of the grafts.

Okamoto *et al.* (1993) observed that polymeric N-acetyl D-glucosamine (chitin) could be used for various surgical affections including umbilical hernia repair. Chitin was used as prosthetic material at the suture site of the hernial ring. Good healing was observed and no side effect was encountered.

Iqbal *et al.* (1994) used an autogenous jejunal graft with its intact mesenteric blood supply to repair abdominal wall defect in dogs. Experimentally induced full thickness abdominal wall defects in the right flank were repaired using jejunal graft. Neither rejection nor adverse tissue reaction was observed in any of the animals. Junctional histology revealed complete fibrous union between abdominal wall and jejunal graft within three to four weeks.

Matsumoto *et al.* (1996) used canine pericardium treated with poly-epoxy compounds as a graft for the correction of experimentally induced defect on the diaphragm in dogs. The patch graft was well tolerated by all the animals.

Jayakrishnan and Jameela (1996) reported that glutaraldehyde cross-linking of collagenous tissues significantly reduced the biodegradation, making them biocompatible and non-thrombogenic while preserving anatomic integrity, leaflet strength and flexibility.

Goud and Raghavender (1997) described the technique of using external abdominal oblique myofascial flap in the reconstruction of the abdominal wall defect in dogs. Postoperative observation for six months indicated that there was no complication in the animals.

Bhattacharya and Bose (1998) reported that autologous full thickness skin and dermis could be used in the surgical repair of experimentally created ventral hernia in dogs. It was found that dermal graft was more suitable than the full thickness skin graft.

Yuvru *et al.* (1999) reported that the outer epidermal layer of the hernial sac could be used as a graft material in the correction of large hernial defects. It was concluded that skin autograft was superior to other hernioplasty materials due to its elasticity, availability from the animals, ease of application and low cost.

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted in twelve clinical cases of umbilical hernia in two to three-month-old pigs (Fig.1) presented to the Veterinary College Hospital, Mannuthy from the Centre for Pig Production and Research, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy. All the animals were maintained under identical conditions of feeding and management prior to and after surgery.

The animals with umbilical hernia were divided into two groups viz., Group I and Group II based on the size of hernial ring. Group I animals had smaller hernial ring of size 5.00 ± 0.56 cm x 3.50 ± 0.37 cm and Group II animals had larger hernial ring of size 8.25 ± 0.42 cm x 6.80 ± 0.47 cm.

Group I

This group comprised of six animals. All the animals of this group had hernial ring of size 5.00 ± 0.56 cm x 3.50 ± 0.37 cm. In five animals the hernial contents were reducible and herniorrhaphy was performed after reduction of hernial contents. In one animal hernia was irreducible. It

was corrected and the hernial contents were reduced and herniorrhaphy was performed.

Group II

This group comprised of six animals. The animals of this group had hernial ring of size 8.25 ± 0.42 cm x 6.80 ± 0.47 cm. The hernial contents were reducible in all the animals. After reducing the contents, the hernial ring was sutured using primary simple interrupted sutures and hernioplasty was performed using processed oesophageal allografts.

3.1   Preparation of graft

3.1.1 Collection

Fresh pieces of oesophagus about 30 to 35cm length were collected from pigs at the time of slaughter. The collected pieces of oesophagus were thoroughly cleaned in running water. They were then washed with sterile normal saline and placed in sterile isotonic saline to which ampicillin sodium[1] (500 mg/l) was added. The tissue samples were processed at Bio-products Laboratory, Central Leather Research Institute, Adayar, Chennai.

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1   ROSCILLIN – Ampicillin Sodium – 500mg/vial, Ranbaxy Laboratories Ltd., New Delhi, India.

3.1.2 Preparation

The tissues were made into sheet form by cutting it lengthwise and thoroughly washed in running water. The muscular layer and adhering fat tissues were completely removed from submucosal layer manually. It was then washed in distilled water.

3.1.3 Cross-linking with glutaraldehyde

The tissues were treated for three hours in 0.5 per cent (v/v) aqueous glutaraldehyde solution (25 per cent) containing 0.5 per cent (w/v) sodium chloride and 0.01 per cent (w/v) sodium acetate. The pH of the solution was adjusted to 8.5 using dilute sodium hydroxide solution. The materials were stirred occasionally and were removed after three hours. It was then washed thoroughly in distilled water.

3.1.4 Packing

The processed tissue were aseptically sealed in polythene covers in 98 per cent isopropanol as preservative (Fig.2) and sterilized in gamma irradiation chamber at two M rads dose (Sastry, 1989) at Central Leather Research Institute, Adayar, Chennai.

3.2   Preoperative preparations

All the animals were kept off feed for twelve hours prior to surgery. Skin of the ventral abdominal area and the hernial swelling was shaved, washed with soap and water, mopped dry, applied 70 per cent alcohol, dried and painted with Tr. Iodine.

3.3   Anaesthesia

Triflupromazine hydrochloride[2] at the rate of 1.5mg/kg body weight was administered intramuscular (I/M) to all the animals. Fifteen minutes later local anesthesia was effected using two per cent solution of lignocaine hydrochloride[3] by subcutaneous (S/C) infiltration around the hernial ring.

3.4   Surgical technique
3.4.1 Herniorrhaphy

In group I animals, herniorrhaphy was performed. The animals were secured on dorsal recumbency. An elliptical skin incision was made over the hernial swelling. Blunt dissection was done to separate and remove the skin

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2   SIQUIL INJECTION–Veterinary–Triflupromazine hydrochloride inject- ion U.S.P – 20mg/ml, Sarabhai Chemicals, Baroda, India.
3   XYLOCAINE 2 per cent-Lignocaine hydrochloride injection I.P.– 20mg/ml, Astra – IDL Limited, Bangalore, India.

from the lining peritoneum (Fig.3). The peritoneal sac was dissected free up to the hernial ring (Fig.4). After reduction, the edges of the ring were scarified and sutured with overlapping mattress sutures using size No.2 braided silk thread (Fig.5). The subcutaneous tissue was apposed using size No. 1-0 braided silk with simple continuous sutures. The excess skin was trimmed and apposed with interrupted vertical mattress sutures using monofilament nylon.

In one animal where the contents were irreducible, simple reduction of hernial contents could not be achieved (Fig.6). The herniated loop of intestine was distended and hard. Enterotomy was performed and the intestinal contents were removed. Enterotomy incision was closed using No.2-0 chromic catgut[4] by Cushing's suture followed by Lembert's suture. The intestinal loop was cleaned and reduced. Closure of hernial ring and skin incision was done as in the other five animals.

3.4.2 Hernioplasty

In animals of group II, hernioplasty using processed oesophageal graft material was performed. The animals were secured on dorsal recumbency. An elliptical

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4   ETHICON – Absorbable Surgical Suture U.S.P., Catgut–Johnson & Johnson Ltd., Aurungabad, India.

skin incision was made over the hernial swelling. Blunt dissection was done to separate and remove the skin from the lining peritoneum. The hernial contents were reduced and the edges of the ring were scarified and primary sutures – simple interrupted sutures using size No.2 braided silk thread were applied (Fig.7). Total apposition of the hernial ring could not be achieved due to large size of the hernial ring. The processed oesophageal allograft was placed over the narrowed ring to close the ring and was fixed in position by suturing it to the muscle and fascia with size No.1 braided silk thread (Fig.8). The subcutaneous tissue was apposed using size No. 1-0 braided silk. The excess skin was trimmed and sutured with interrupted vertical mattress sutures using monofilament nylon.

3.5 *Postsurgical management*

Tr. Benzoin seal was applied over the suture line. The operated animals were housed individually and the amount of feed given was reduced to 75 per cent of normal ration till the seventh postoperative day.

Tetanus toxoid[5] 0.5ml was administered I/M after surgery and streptopenicillin[6] at the rate of 10000 IU/kg

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5 TETANUS TOXOID ADSORBED – 0.5 ml/amp, Serum Institute, Bombay, India.
6 DICRYSTICIN–S -Streptopenicillin – 2.5 G/vial, Sarabhai, Wadi Wadi, Baroda, India.

body weight was given I/M daily for seven days postoperatively.

The wound was cleaned daily with 70 per cent alcohol and zinc oxide ointment was applied. The skin sutures were removed on the seventh postoperative day.

The animals were kept under observation for 21 days and maintained up to a maximum period of six months.

Tissue specimens were collected from the site of surgery when the animals were slaughtered for meat six months after surgery.

3.6   Main items of observation

3.6.1 Physiological parameters

Rectal temperature (^{0}C), pulse rate (per min.) and respiration rate (per min.) were recorded just before surgery and for seven consecutive days postoperatively.

3.6.2 Clinical observations

a. *General condition:-* General condition of the animals were assessed and graded as active, dull, or depressed.

b. *Feed intake:-* Feed intake was graded as normal or reduced.

c. *Changes at the surgical site:-* Clinical evaluation of the surgical site was made on the basis of wound inflammation, infection, granulation and fibrosis of the sutured area.

d. Complications, if any

3.6.3 Haematological parameters

Blood smears were prepared and venous blood samples were collected from ear vein in EDTA[7] preoperatively and on the first, third, seventh, 14th, and 21st day postoperatively for estimation of the total leucocyte count (TLC) differential leucocyte count (DC), haemoglobin concentration (Hb) and packed cell volume (PCV).

a. Haemoglobin concentration

For estimation of haemoglobin acid haematin method as described by Benjamin (1985) was followed.

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7 EDTA – EDTA Disodium Salt-Nice Laboratory reagent, New India Chemical Enterprises, Kochi, India.

b.   Packed cell volume

The PCV was estimated by Wintrobe method as described by Schalm (1975).

c.   Total leucocyte count

The method described by Wintrobe (1981) was followed for total leucocyte count.

d.   Differential leucocyte count

The method described by Benjamin (1985) was followed for differential leucocyte count.

3.6.4 Biochemical parameters

Serum samples were obtained preoperatively and on the first, third, seventh, 14th and 21st day postoperatively for estimation of total serum protein, serum sodium and serum potassium.

a.   Total serum protein was estimated by total protein kit[8] (Biuret method – Inchiosa, 1964) using photometer 5010.

b.   Serum sodium and potassium concentration was estimated using flame photometer 128.

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8 TOTAL PROTEIN AND ALBUMIN KIT – Dr. Reddy's Laboratories, Hyderabad, India.

3.6.5 Tissue changes

a. Macroscopic/Gross changes

The gross changes, if any at the site of surgery were recorded.

b. Biomechanical studies

The tissue at the surgical site was collected from three animals of each group at slaughter and biomechanical factors were studied. Tissue samples from three normal animals were also collected from umbilical region at slaughter for comparison. The tensile strength and elongation at break were studied using "disruption from without method" which involves application of a force to a tissue edge while the opposite edge is fixed (Al-Sadi and Gourley, 1977).

i. Tensile strength (stress-kg/cm^2): The force required to disrupt the tissue per unit cross sectional area. Tensile strength was estimated using Instron 4501 tensile testing system (Sastry *et al.*, 1998).

ii. Elongation at break (Extensibility – per cent): The degree to which a tissue stretches before it disrupts. Elongation at break was estimated

using Instron 4501 tensile testing system (Sastry *et al.*, 1998).

c.   Histopathology

The tissue samples from the site of surgery were collected at the time of slaughter in 10 per cent neutral buffered formol saline and processed. The tissue samples were cut into 4μ thickness, stained with Haematoxylin and Eosin and examined for histomorphological changes at the site of surgery.

3.7 Statistical analysis

The data obtained in all the groups were analyzed using T-test and the means were compared with presurgical values (Snedecor and Cochran, 1967).

Fig.1 Piglet with umbilical hernia before surgery

Fig. 2 Polythene sachet containing processed oesophageal allograft in 98 per cent isopropanol

Results

4. RESULTS

Qualities of graft material

The oesophageal allografts, processed using glutaraldehyde and sterilized by gamma irradiation were used in the present study. The processed collagen sheets were creamy white in colour and measured 11.0 x 4.0 cm. The thickness of graft material was one mm and the flexibility of processed oesophageal collagen was satisfactory. The suture holding power of the graft material was good and sutures did not tear through the graft. The width of the collagen sheet was found to be sufficient to close the tissue defect after reducing the size of hernial ring by primary suture. The graft materials preserved for a period of 12 months were used for hernioplasty without any untoward effect. There was no deterioration in the quality of graft material or loss of functional strength within the period of storage of 12 months.

4.1   Herniorrhaphy (Group I)

This group had six animals of which four were female and the rest two were male. The observations are presented in tables 1 to 5.

4.1.1 Preoperative considerations

The average body weight (kilogram) of the animals was 12.16 ± 1.71. The size of hernial ring was measured after exposure (Table 1). The quantity of triflupromazine hydrochloride administered was 18.92 ± 2.58 mg. Local anaesthesia was effected using two per cent solution of lignocaine hydrochloride by S/C infiltration around the hernial ring. The anaesthesia was satisfactory for the duration of surgical procedure.

4.1.2 Physiological parameters

The physiological parameters like rectal temperature, pulse rate and respiration rate were recorded just before surgery and for seven consecutive days postoperatively (Table 2).

a. Rectal temperature

The rectal temperature (^{0}C) was 39.60 ± 0.27 before surgery. There was increase on first (40.17 ± 0.09) and second day (39.98 ± 0.20) followed by a decrease in temperature to reach near normal level (39.18 ± 0.21) on seventh postoperative day. The values on third, fourth, fifth and sixth postoperative day were 39.27 ± 0.23, 39.33 ± 0.07, 38.92 ± 0.13 and 38.93 ± 0.24 respectively.

Table 1. Body weight and size of hernial ring (Group I animals)

Animal No.	Body weight (kg)	Size of hernial ring (cm)
1	8	4.5 x 3.0
2	12	5.0 x 3.7
3	21	8.0 x4.5
4	10	4.5 x 3.0
5	12	4.0 x 3.0
6	10	4.0 x 3.0
Mean ± SE	12.16 ± 1.71	5.00 ± 0.56 x 3.50 ± 0.37

b. Pulse rate

The pulse rate (per min.) was 89.70 ± 5.39 before surgery. Increase in rate (100.70 ± 9.55) was noticed on the first day postoperatively which continued up to fourth postoperative day (101.00 ± 9.35) followed by a decrease to reach near normal value (86.30 ± 6.41) on the seventh postoperative day.

c. Respiration rate

The respiration rate (per min.) was 44.70 ± 6.04 before surgery. Increase in value (57.00 ± 2.90) was noticed on the first postoperative day. The respiration rate remained within the normal range thereafter. The values were 45.00 ± 3.71, 45.00 ± 6.73, 43.30 ± 5.31, 46.30 ± 4.82, 48.00 ± 5.35 and 47.00 ± 7.06 on the second, third, fourth, fifth, sixth and seventh postoperative day respectively.

4.1.3 Clinical observations

General condition, feed intake and changes at the surgical site were assessed and recorded daily up to seventh postoperative day.

Table 2. Rectal temperature, pulse rate and respiration rate before and after herniorrhaphy in Group I animals (Mean ± SE)

n=6

Parameters	Preop-erative	Postoperative (days)						
		1	2	3	4	5	6	7
Rectal temperature(°C)	39.60± 0.27	40.17± 0.09	39.98± 0.20	39.27± 0.23	39.33± 0.07	38.92*± 0.13	38.93± 0.24	39.18± 0.21
Pulse rate (per min.)	89.70± 5.39	100.70 ± 9.55	91.70± 4.73	94.30± 8.08	101.00 ± 9.35	85.30± 5.14	84.30± 6.33	86.30± 6.41
Respiration rate (per min.)	44.70± 6.04	57.00± 2.90	45.00± 3.71	45.00± 6.73	43.30± 5.31	46.30± 4.82	48.00± 5.35	47.00± 7.06

* Significant (P<0.05)

a) General condition

Five animals of this group were active and alert within 24 hours after herniorrhaphy. One animal, which underwent enterotomy and herniorrhaphy, was dull for the first four days postoperatively, but improved in condition afterwards.

b) Feed intake

The amount of feed given was reduced to 75 per cent of normal ration and it was given three times a day till seventh postoperative day. The feed given to the animals were fully consumed and there was no wastage. From the eighth day the feed ration was restored to the preoperative level in all the animals.

The animal, which underwent enterotomy and herniorrhaphy, was fed with rice gruel for the first four days postoperatively. Normal concentrate feed was given in reduced quantity (75 per cent) from fifth to seventh postoperative day. The quantity of feed was increased to normal level after seventh postoperative day.

c) Changes at the surgical site

Moderate swelling, signs of pain and inflammatory oedema were observed at the surgical site in the umbilical region postoperatively. The reaction gradually subsided and

disappeared completely by tenth postoperative day in five animals. The surgical site appeared normal after 30 days of surgery (Fig.10).

d) Complications

In one animal the inflammatory oedema was marked and purulent discharge was noticed after four days of surgery. The collected exudate was drained off and magnesium sulphate – glycerine paste was applied over the oedematous region. The skin wound got disrupted completely on seventh day and it was treated as open wound thereafter. Administration of streptopenicillin was continued up to tenth postoperative day and wound dressing till the healing was complete (12 days).

Recurrence of hernia was noticed in one animal 24 days after surgery and there was herniation of abdominal contents (Fig. 9). The animal was sent for slaughter on 30th day.

In the remaining four animals the healing was satisfactory and there was no complication for the period of observation.

4.1.4 Haematological parameters

Blood was collected preoperatively and on the first, third, seventh, 14th and 21st postoperative day to estimate the haemoglobin concentration, packed cell volume, total leucocyte count and differential leucocyte count (Table 3).

a) Haemoglobin concentration

The haemoglobin concentration (g/dl) was 11.42 ± 0.91 before surgery. Decrease in haemoglobin concentration was noticed throughout the period of observation up to 21st postoperative day. The values were 11.02 ± 0.38, 11.00 ± 0.45, 10.08 ± 0.27, 11.20 ± 0.31 and 11.00 ± 0.54 respectively on the first, third, seventh, 14th and 21st postoperative day.

b) Packed cell volume

The packed cell volume (per cent) was 30.80 ± 2.04 before surgery. Increase in value (33.80 ± 1.06) was noticed on the third postoperative day but it reached presurgical value on the seventh postoperative day (30.80 ± 2.00).

Table 3. Haemogram in before and after herniorrhaphy in Group I animals (Mean ± SE)

n=6

Parameters	Preoperative	Postoperative (days)				
		1	3	7	14	21
Haemoglobin (g/dl)	11.42± 0.91	11.02± 0.38	11.00± 0.45	10.08± 0.27	11.20± 0.31	11.00± 0.54
Packed cell volume (per cent)	30.80± 2.04	32.20± 0.73	33.80± 1.06	30.80± 2.00	31.80± 1.80	32.00± 1.14
Total leucocyte count (x 10^3/mm^3)	13.04± 1.71	15.93± 1.70	14.87± 2.69	12.12± 1.79	12.47± 1.07	13.23± 1.72
Differential count:						
Neutrophil count (per cent)	27.30± 1.59	48.50± 5.51	48.30± 4.61	36.00*± 2.94	29.20± 3.18	29.20± 2.94
Lymphocyte count (per cent)	69.67± 2.23	50.50± 6.22	51.33± 4.59	70.33**± 2.50	70.67± 3.17	70.67± 2.78
Monocyte count (per cent)	1.50± 0.66	0.34± 0.19	0.34± 0.19	0.34± 0.19	0.17± 0.15	0.17± 0.15
Eosinophil count (per cent)	0.50± 0.46	0.67± 0.61	0.00	0.00	0.67± 0.61	0.17± 0.15
Basophil count (per cent)	0.00	0.00	0.00	0.00	0.00	0.00

* Significant (P<0.05)
** Significant (P<0.01)

c) Total leucocyte count

The total leucocyte count (x $10^3/mm^3$) was 13.04 ± 1.71 before surgery. There was an increase on the first (15.93 ± 1.70) and third (14.87 ± 2.69) postoperative day followed by a decrease to reach near normal value (13.23 ± 1.72) on the 21st postoperative day. The counts observed on seventh and 14th day were 12.12 ± 1.79 and 12.47 ± 1.07 respectively.

d) Differential leucocyte count

The neutrophil count (per cent) was 27.30 ± 1.59 before surgery. Increase in neutrophil count was noticed on the first (48.50 ± 5.51) and third (48.30 ± 4.61) postoperative day. The neutrophil count reached the near normal value (29.20 ± 3.18) on the 14th postoperative day. The values observed on seventh and 21st day were 36.00 ± 2.94 and 29.20 ± 2.94 respectively.

The lymphocyte count (per cent) was 69.67 ± 2.23 before surgery. There was a decrease on the first (50.50 ± 6.22) and third (51.33 ± 4.59) day postoperatively followed by an increase to reach near normal value (70.33 ± 2.50) on the seventh postoperative day. The counts observed on 14th and 21st day respectively were 70.67 ± 3.17 and 70.67 ± 2.78.

The monocyte count (per cent) was 1.50 ± 0.66 before surgery. The count was 0.34 ± 0.19 on the first, third and seventh day postoperatively, and 0.17 ± 0.15 on the 14 and 21st postoperative day.

The eosinophil count (per cent) was 0.50 ± 0.46 before surgery and 0.67 ± 0.61 on the first and 14th postoperative day. The count was 0.17 ± 0.15 on the 21st postoperative day.

4.1.5 Biochemical parameters

The total serum protein, serum sodium and serum potassium were estimated preoperatively and on the first, third, seventh, 14th and 21st postoperative day (Table 4).

a) Total serum protein

The total serum protein (g/l) was 74.40 ± 7.22 before surgery. There was a decrease in total serum protein level on first (63.20 ± 5.92), third (63.80 ± 5.43) and seventh (63.90 ± 5.80) postoperative day. There was an increase in total protein on the 14th (71.50 ± 5.39) postoperative day and it reached near normal value (71.90 ± 4.16) on the 21st postoperative day.

Table 4.　Serum constituents before and after herniorrhaphy in Group I animals (Mean ± SE)

Parameters	Preoperative	Postoperative (days)				
		1	3	7	14	21
Total serum protein (g/l)	74.40± 7.22	63.20± 5.92	63.80± 5.43	63.90± 5.80	71.50± 5.39	71.90± 4.16
Serum sodium (mmol/l)	154.67± 3.79	155.50± 4.16	156.00± 4.71	157.00± 5.86	156.67± 6.92	160.00± 10.99
Serum potassium (mmol/l)	5.13± 0.37	5.61 ± 0.22	5.63 ± 0.39	5.64 ± 0.23	5.81 ± 0.28	5.56 ± 0.64

b) Serum sodium

The serum sodium level (mmol/l) was 154.67 ± 3.79 before surgery. There was no significant change in serum sodium up to 21st postoperative day. The values were 155.50 ± 4.16, 156.00 ± 4.71, 157.00 ± 5.86, 156.67 ± 6.92 and 160.00 ± 10.99 on the first, third, seventh, 14th and 21st postoperative day respectively.

c) Serum potassium

The serum potassium level (mmol/l) was 5.13 ± 0.37 before surgery. There was no significant change in the serum potassium level up to 21st postoperative day. The values were 5.61 ± 0.22, 5.63 ± 0.39, 5.64 ± 0.23, 5.81 ± 0.28 and 5.56 ± 0.64 respectively on the first, third, seventh, 14th and 21st days postoperatively.

4.1.6 Tissue changes

Macroscopic examination of the surgical site and collection of tissue samples were done to evaluate the healing from three animals that were slaughtered for meat after 180 days of surgery. The collected tissue samples were subjected to biomechanical and histomorphological studies.

Table 5. Biomechanical characteristics of tissue at the site of herniorrhaphy and hernioplasty

Parameters with units	Normal animals	Group – I (Herniorrhaphy)	Group – II (Hernioplasty)
Tensile strength (kg/cm^2)	9.95 ± 0.33	12.04 ± 0.78	30.25 ± 0.83
Elongation at break (per cent)	63.38 ± 0.36	59.53 ± 0.24	75.78 ± 1.91

a) Macroscopic/Gross changes

The gross changes at the surgical site were observed at slaughter. There was no adhesion of viscera at the surgical site. Remnants of silk suture could be identified at the site. The suture material was completely encapsulated by fibrous tissue.

b) Biomechanical studies

The tensile strength and elongation at break were estimated using Instron 4501 tensile testing system (Table 5). The tensile strength (kg/cm^2) were 12.04 ± 0.78 and 9.95 ± 0.33 for Group I and normal animals respectively. The elongation at break (per cent) were 59.53 ± 0.24 and 63.38 ± 0.36 for Group I and normal animals, respectively.

c) Histopathology

The muscle bundles appeared intact and normal. Collagen fibrils predominated in the proliferating connective tissue (Fig 12). No inflammatory cells were seen at the site. The histopathological changes were suggestive of complete healing at the site.

4.2 Hernioplasty (Group II)

This group had six animals of which four were female and the rest two were male. The observations are presented in tables 5 to 9.

4.2.1 Preoperative considerations

The average body weight (kilogram) of the animals was 18.83 ± 3.27. The size of hernial ring was measured after exposure (Table 6). The quantity of triflupromazine hydrochloride administered was 28.83 ± 4.83 mg. Local anaesthesia was effected using two per cent solution of lignocaine hydrochloride by S/C infiltration around the hernial ring. The anaesthesia was satisfactory for the duration of surgical procedures.

4.2.2 Physiological parameters

The physiological parameters like rectal temperature, pulse rate and respiration rate were recorded just before surgery and for seven consecutive days postoperatively (Table 7).

Table 6.   Body weight and size of hernial ring (Group II animals)

Animal No.	Body weight (kg)	Size of hernial ring (cm)
1	32	9.0 x 6.0
2	13	6.5 x 5.5
3	12	7.5 x 8.0
4	10	9.5 x 5.5
5	20	8.0 x 8.0
6	26	9.0 x 8.0
Mean ± SE	18.83 ± 3.27	8.25 ± 0.42 x 6.80 ± 0.47

a) Rectal temperature

The rectal temperature (^{o}C) was 39.15 ± 0.24 before surgery. There was an increase (39.82 ± 0.18) on the first postoperative day followed by a decrease to reach near normal value (39.18 ± 0.21) on the seventh postoperative day. The values on second, third, fourth, fifth and sixth postoperative day respectively were 39.68 ± 0.36, 39.68 ± 0.32, 39.25 ± 0.23, 39.48 ± 0.15 and 39.23 ± 0.16.

b) Pulse rate

The pulse rate (per min.) was 84.70 ± 8.78 before surgery. Increase in pulse rate was noticed on the first (87.00 ± 5.76), second (90.00 ± 7.18), third (86.30 ± 7.67) and fifth (85.00 ± 3.59) postoperative day. The pulse rate reached the presurgical value on the sixth postoperative day (84.70 ± 3.14).

c) Respiration rate

The respiration rate (per min.) was 49.00 ± 5.55 before surgery. Increase in rate was noticed on the first (56.30 ± 3.35), second (53.00 ± 4.94), third (52.70 ± 2.65), fourth (51.30 ± 3.80), fifth (50.30 ± 3.59), and seventh (55.30 ± 3.39) postoperative day. The respiration rate was near normal 48.70 ± 2.82 on sixth postoperative day.

Table 7. Rectal temperature, pulse rate and respiration rate before and after hernioplasty in Group II animals (Mean ± SE)

n=6

Parameters with units	Preop-erative	Postoperative (days)						
		1	2	3	4	5	6	7
Rectal temperature(^{o}C)	39.15± 0.24	39.82± 0.18	39.68± 0.36	39.68± 0.32	39.25± 0.23	39.48* ±0.15	39.23± 0.16	39.18± 0.21
Pulser rate (per min.)	84.70± 8.78	87.00± 5.76	90.00± 7.18	86.30± 7.67	81.00± 4.65	85.00± 3.59	84.70± 3.14	85.30± 3.67
Respiration rate (per min.)	49.00± 5.55	56.30± 3.35	53.00± 4.94	52.70± 2.65	51.30± 3.80	50.30± 3.59	48.70± 2.82	55.30± 3.39

* Significant (P<0.05)

4.2.3 Clinical observations

General condition, feed intake and changes at the surgical site was assessed and recorded daily up to seventh postoperative day.

a) General condition

All the animals were active and alert within 24 hours postoperatively.

b) Feed intake

The amount of feed given was reduced to 75 per cent of normal ration and it was given three times a day till seventh postoperative day. The feed given to the animals were fully consumed and there was no wastage. The quantity of feed given was restored to normal by eighth postoperative day.

c) Changes at the surgical site

Moderate swelling and signs of pain were observed at the operated site in the umbilical region postoperatively. Inflammatory oedema was noticed in four animals in the umbilical region. In the other two animals the oedema was mild and disappeared by seventh postoperative day.

Magnesium sulphate – glycerine paste was applied over the oedematous region in four animals. The oedema/ swelling gradually subsided and completely disappeared by 14th postoperative day. Moderate thickening was noticed in the umbilical region in all the animals by 21st postoperative day. There was no sign of wound infection in any of the animals and the skin wound healing was satisfactory. The skin sutures were removed by seventh postoperative day.

d) Complications

The animals were observed for six months after surgery. Recurrence of hernia/ infection/ wound disruption was not observed in any of the animals.

4.2.4 Haemotological parameters

Blood was collected preoperatively and on the first, third, seventh, 14th and 21st postoperative day to estimate the haemoglobin concentration packed cell volume, total leucocyte count and differential leucocyte count (Table 8).

a) Haemoglobin concentration

The haemoglobin concentration (g/dl) was 10.60 ± 0.66 before surgery. Decreased haemoglobin value

was noticed up to 14th postoperative day. The values were 10.28 ± 0.69, 10.17 ± 0.56, 10.25 ± 0.40 and 10.47 ± 0.57 on the first, third, seventh and 14th postoperative day. The haemoglobin concentration reached a near normal value (10.58 ± 0.52) on the 21st postoperative day.

b) Packed cell volume

The packed cell volume (per cent) was 32.00 ± 0.90 before surgery. Increase in packed cell volume was noticed on the first (33.00 ± 0.98) and third (33.30 ± 0.65) postoperative day followed by a decrease to 31.80 ± 1.27 on the seventh postoperative day. The packed cell volume reached the presurgical value 32.00 ± 0.90 on the 14th postoperative day. The packed cell volume was 32.70 ± 0.86 on the 21st postoperative day.

c) Total leucocyte count

The total leucocyte count (x $10^3/mm^3$) was 12.02 ± 1.77 before surgery. The count increased to 14.35 ± 1.38, 14.59 ± 1.41 and 13.48 ± 1.18 on the first, third and seventh postoperative day. The count decreased to 11.35 ± 1.31 on the 14th postoperative day and reached near normal value 12.03 ± 2.48 on the 21st postoperative day.

Table 8. Haemogram before and after hernioplasty in Group II animals (Mean ± SE)

n=6

Parameters with units	Preoperative	Postoperative (days)				
		1	3	7	14	21
Haemoglobin (g/dl)	10.60± 0.66	10.28± 0.69	10.17± 0.56	10.25± 0.40	10.47± 0.57	10.58± 0.52
Packed cell volume (per cent)	32.00± 0.90	33.00± 0.98	33.30± 0.65	31.80± 1.27	32.00± 0.90	32.70± 0.86
Total leucocyte count (x 10^3/mm^3)	12.02± 1.77	14.35± 1.38	14.59± 1.41	13.48± 1.18	11.35± 1.31	12.03± 2.48
Differential count:						
Neutrophil count (per cent)	32.30± 1.31	48.00± 2.82	49.00± 2.82	45.70*± 1.76	34.30± 1.18	33.00± 1.22
Lymphocyte count (per cent)	66.00± 1.22	51.83± 2.76	50.83± 2.81	53.00**± 1.91	63.83± 1.94	66.83± 1.28
Monocyte count (per cent)	1.17± 0.68	0.17± 0.15	0.17± 0.15	1.17± 0.89	1.17± 1.06	0.00
Eosinophil count (per cent)	0.50± 0.46	0.00	0.00	1.17± 0.15	0.00	0.00
Basophil count (per cent)	0.00	0.00	0.00	0.00	0.00	0.00

* Significant (P<0.05)
** Significant (P<0.01)

d) Differential leucocyte count

The neutrophil count (per cent) was 32.30 ± 1.31 before surgery. Increase in values to 48.00 ± 2.82, 49.00 ± 2.82 and 45.70 ± 1.76 were observed on the first, third and seventh postoperative day. The count decreased to 34.30 ± 1.18 on the 14th postoperative day and reached near normal value 33.00 ± 1.22 on the 21st postoperative day.

The lymphocyte count (per cent) was 66.00 ± 1.22 before surgery. Decrease in values to 51.83 ± 2.76, 50.83 ± 2.81 and 53.00 ± 1.91 were noticed on the first, third and seventh postoperative day. The count increased to 63.83 ± 1.94 on the 14th postoperative day and reached near normal level 66.83 ± 1.28 on the 21st postoperative day.

The monocyte count (per cent) was 1.17 ± 0.68 before surgery. The count was 0.17 ± 0.15 on the first and third postoperative day. The count was 1.17 ± 0.89 and 1.17 ± 1.06 on the seventh and 14th postoperative day. No monocyte could be observed on the 21st postoperative day.

4.2.5 Biochemical parameters

The total serum protein, serum sodium and serum potassium were estimated preoperatively and on the first, third, seventh, 14th and 21st postoperative day (Table 9).

a) Total serum protein

The total serum protein (g/l) was 72.70 ± 6.61 before surgery. Decrease in value was noticed on the first (71.50 ± 6.57), third (72.20 ± 6.49) and seventh (71.70 ± 6.45) postoperative day. The total protein increased to 74.00 ± 5.59 on the 14th postoperative day. The value was 73.30 ± 6.24 on the 21st postoperative day.

b) Serum sodium

The serum sodium (mmol/l) was 164.67 ± 7.14 before surgery. There was no significant change in the serum sodium level up to 21st postoperative day. The values observed on first, third, seventh, 14th and 21st postoperative day respectively were 160.67 ± 7.10, 156.34 ± 8.06, 159.34 ± 10.59, 160.00 ± 10.41 and 159.67 ± 10.76.

c) Serum potassium

The serum potassium (mmol/l) was 5.25 ± 0.19 before surgery. There was no significant change in serum potassium level up to 21st postoperative day. The values observed on first, third, seventh, 14th and 21st postoperative day respectively were 5.12 ± 0.36, 5.41 ± 0.41, 5.52 ± 0.24, 5.82 ± 0.28 and 5.63 ± 0.54.

Table 9. Serum constituents before and after hernioplasty in Group II animals (Mean ± SE)

Parameters	Preoperative	Postoperative (days)				
		1	3	7	14	21
Total serum protein (g/l)	72.70± 6.61	71.50± 6.57	72.20± 6.49	71.70± 6.45	74.00± 5.59	73.30± 6.24
Serum sodium (mmol/l)	164.67± 7.14	160.67± 7.10	156.34± 8.06	159.34± 10.59	160.00± 10.41	159.67± 10.76
Serum potassium (mmol/l)	5.25± 0.19	5.12± 0.36	5.41± 0.41	5.52± 0.24	5.82± 0.28	5.63± 0.54

4.2.6 Tissue changes

Macroscopic examination of the surgical site and collection of tissue samples were done to evaluate the healing process from three animals that were slaughtered for meat after 195 days of surgery (Fig. 11). The collected tissue samples were subjected to biomechanical and histomorphological studies.

a) Macroscopic/Gross changes

The gross changes at the surgical site were recorded at slaughter. Remnants of silk suture could be identified at the site. Encapsulation of the suture material and fibrous thickening was observed. There was no adhesion of viscera at the site of hernioplasty.

b) Biomechanical studies

The collected tissue samples were subjected to tensile strength and elongation at break evaluation using Instron 4501 tensile testing system (Table 5). The tensile strength (kg/cm^2) was 30.25 ± 0.83. The elongation at break (per cent) was 75.78 ± 1.91. Both tensile strength and elongation at break were higher than the values of normal and Group I animals.

c) Histopathology

The healing was complete. Fully matured connective tissue with plenty of collagen could be observed at the site. Neovascularisation was evidenced by the presence of number of capillaries (Fig. 13) and large vessels (Fig. 14). The muscle fibres were found to be intact and arranged in bundles with occasional invasion by proliferating connective tissue. No remnants of graft material could be seen at the site. The histopathological changes observed were suggestive of complete uptake of graft material and healing without any adverse effect.

Fig.9   Recurrence of hernia, 24 days after herniorrhaphy
        (Group I)

Fig. 10 Animals 30 days after herniorrhaphy (Group I)

Fig.11 Animal 195 days after hernioplasty (Group II).

Fig. 12 Photomicrograph of specimen of tissue from the site of
herniorrhaphy (180 days after surgery) showing collagen
fibres predominating in the proliferating connective
tissue (H&E x 100).

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Fig.13 Photomicrograph of specimen of tissue from the site of hernioplasty (195 days after surgery) showing neovascularisation and connective tissue proliferation (H&E x 100).

Fig. 14 Photomicrograph of specimen of tissue from the site of hernioplasty (195 days after surgery) showing neovascularisation. Large blood vessel seen (H&E x 100).
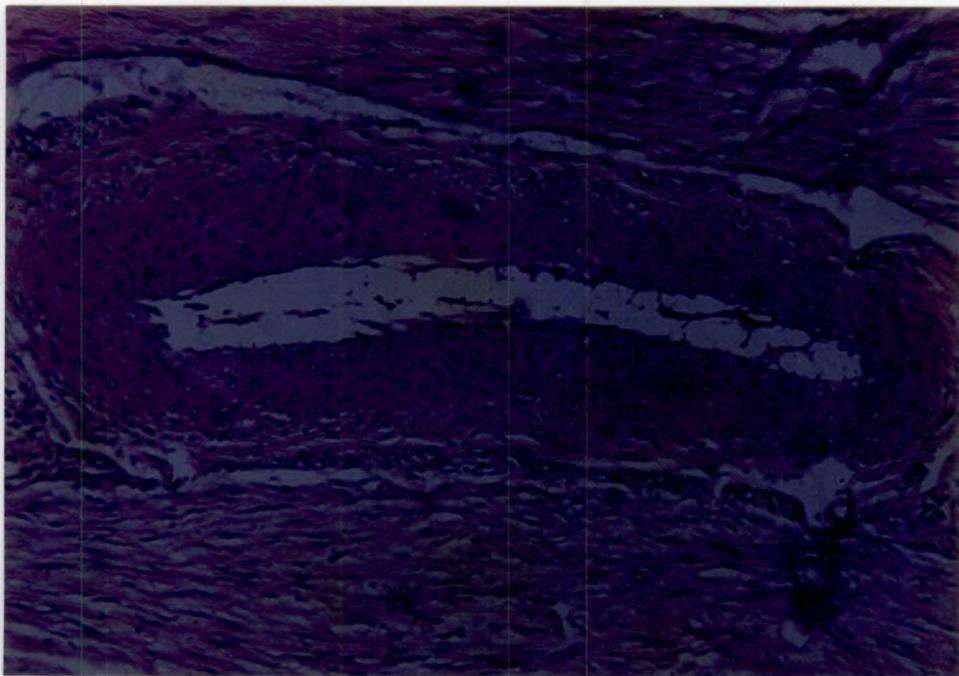
Discussion

5. DISCUSSION

Abdominal wall defects such as those leading to hernia need prosthetic support for the effective closure when the defect is large. Primary wound closure usually fails because the sutures are placed under tension and may tear through the tissues or the tissue become weak and yield to pressure (Kanade and Kumar, 1985).

Autogenous grafting is the safest but cannot be employed always in animal patients owing to the difficulty in harvesting the tissue and implantation. Rejection of autografts is rare as they elicit least antigenic reaction.

Collagen is being used increasingly in clinical surgery, because cross-linking of collagen fibrils decrease the antigenicity. Cross-linking also result in increased stability and tensile strength. Minimal tissue reaction after implantation is a significant factor favouring the use of collagen as a biomaterial (Simpson, 1983). Collagen stimulate neofibrogenesis favouring wound healing and it also act as an excellent substrate for cell attachment and cell ingrowth (Sastry, 1989).

Preparation of graft

In the present study the porcine oesophageal collagen was cross-linked with glutaraldehyde for use as prosthetic material. Glutaraldehyde cross-linking significantly reduces biodegradation but preserves the anatomic integrity, leaflet strength and flexibility. Glutaraldehyde reacts relatively quickly and reacts with larger number of amino groups resulting in tightly cross-linked network (Jayakrishnan and Jameela, 1996).

The processed oesophageal allografts were sterilised by gamma radiation (2.0 megarads). Chvapil *et al.* (1973) suggested that collagen products sterilised by gamma radiation could be used for medical applications. The sterilised collagen sheets were stored in isopropanol. There was no shrinkage or deterioration of quality of the material after storage for about 12 months.

The size of the collagen graft (11.0 x 4.0 cm) prepared was sufficient to cover the tissue defect during correction of hernia. The handling qualities of the prepared graft were satisfactory. The flexibility of the graft material was good. The collagen graft had good suture holding power and could be sutured as an onlay graft after the primary closure of the hernial ring.

Pre surgical considerations

Twelve clinical cases of umbilical hernia in pigs aged two to three months were treated under this study. Eight of the animals were females and the remaining four were males. Warwick (1926) and Huston *et al.* (1978) reported that the incidence of umbilical hernia was more common in females than in males. In the present study also it was observed that the percentage of incidence of umbilical hernia was more in female piglings than in male piglings.

Triflupromazine hydrochloride was administered at the rate of 1.5 mg/kg body weight I/M. The combination of triflupromazine hydrochloride I/M with local infiltration anaesthesia was found satisfactory for the surgical procedures.

Surgical techniques
Herniorrhaphy

All the six animals of Group I were subjected to herniorrhaphy. In five animals the hernial contents were reducible and herniorrhaphy was performed. In one animal irreducibility was observed and simple reduction of hernial contents could not be achieved. The ileum was incised to remove the intestinal contents. Devitalization was not

observed and hence resection of intestinal segment was not performed. The enterotomy incision was closed, the contents reduced and herniorrhaphy was done. Markel *et al.* (1987) reported that enterocutaneous fistula could develop in strangulated umbilical hernia of horses. It was further observed that jejunum and ileum were more commonly strangulated with hernial ring. It was emphasised that hernia should be frequently monitored and opined that strangulation might develop as late as 30 months after birth. Out of 12 clinical cases of umbilical hernia in this study only one was irreducible and it was rendered reducible in surgery.

Hernioplasty

All the six animals of Group II were subjected to hernioplasty. In all the animals the hernial ring was large. Total closure of the hernial ring edges could not be achieved by simple suturing. The edges of hernial ring were sutured by simple interrupted sutures and size of hernial ring was reduced. Processed oesophageal allograft was placed as an onlay graft and satisfactory closure was obtained. Rajendran *et al.* (1974) described a similar technique of hernioplasty with polyethylene mesh in a clinical case of umbilical hernia in a pig. Philip (1973) and Velden and Klein (1994) opined that suturing marlex mesh, as onlay graft was simpler, easier and time saving. Shoukry *et al.* (1997)

reported that peritoneal adhesions would develop when polyester fabric mesh was used as inlay graft for correction of hernia.

Clinical observations

All the animals were active and alert within 24 hours postoperatively. The animal that underwent enterotomy and herniorrhaphy was dull up to fourth day, which could be due to additional stress caused by surgical procedure and change in the diet. The quantity of feed given postoperatively was reduced to minimise the abdominal pressure and possible rupture of suture line. Pain, Swelling and inflammatory oedema was observed in all animals of Group I and Group II up to seventh postoperative day. The inflammatory reaction subsided by 15th postoperative day in Group I animals and by 21st postoperative day in Group II animals. The longer duration of inflammation observed in Group II animal could possibly be due to the graft implantation. Recurrence of hernia was noticed only in one animal of Group I. The apposition sutures placed at the hernial ring broke, resulting in recurrence of hernia. Recurrence of hernia or complications was not observed in any of the animals of Group II. There were no sign of infection or rejection reaction against graft material for a period of about six months after surgery.

Physiological parameters

A marginal increase in rectal temperature was observed in all the animals of Group I and Group II on the first and second day postoperatively. The temperature gradually decreased to reach normal value on the seventh postoperative day. There was a significant difference (P< 0.05) in rectal temperature between Group I and Group II animals on the fifth postoperative day. The increase in rectal temperature observed initially could be attributed to the post surgical tissue response. Similar observations were noticed by Balagopalan (1998). A marginal increase in pulse rate and respiration rate in the early postoperative period was noticed in all the animals of Group I and Group II. There was no significant variation between Group I and Group II animals postoperatively.

Haemogram

In all the animals of Group I and Group II, reduction in haemoglobin concentration was noticed up to seventh postoperative day. The haemoglobin concentration reached near normal level on 21st postoperative day. There was no significant difference in haemoglobin concentration between Group I and Group II animals. A marginal increase in packed cell volume and total leucocyte count was noticed on the first and third postoperative day in all the animals of

Group I and Group II. There was no significant difference in packed cell volume and total leucocyte count between Group I and Group II animals. Similar observations are recorded by Nandi *et al.* (1994) in goats following cystoplasty with pericardial graft.

Increase in neutrophil count was noticed up to seventh postoperative day in Group I and Group II animals. Significantly higher neutrophil count was noticed in the Group II animals on the seventh postoperative day. This could be due to persistence of inflammatory reaction secondary to implantation of graft material. Moderate decrease in lymphocyte count was noticed on the first and third postoperative day in both Group I and Group II animals. Significantly lower lymphocyte count was noticed in the Group II animals on seventh postoperative day. This could possibly explain that there was no cellular reaction against the implanted graft material and the graft material could be considered safe for implantation. The monocyte and eosinophil count showed marked fluctuation in the postoperative observations.

The variations observed in haemogram may be due to the cellular reactions to surgical trauma during healing process (Gourley and Vasseur, 1985).

Serum biochemical changes

Decreased total serum protein was noticed up to 21st postoperative day in Group I animals whereas the level increased after 14th postoperative day in Group II animals. There was no significant difference in serum protein level between Group I and Group II animals. The initial decrease observed in total serum protein content may be attributed to the relative protein deficit associated with surgical stress (Carlson, 1997).

The serum sodium and potassium levels were within the normal range in both Group I and Group II animals, and there were no significant variations in the serum sodium and potassium levels between the Group I and Group II animals. The changes in the serum sodium and potassium level after surgery were minimal and insignificant.

The clinical symptoms and haematological data which shows very little variation from the preoperative value indicate that the surgical technique and the use of processed collagen in the hernioplasty technique was effective and safe for the animals.

Tissue specimen

Specimens collected from three animals from each group were used to evaluate healing process and biomechanical characters. Tissue collected from three normal animals was also studied for comparison.

There was no adhesion of viscera at the surgical site, in any of the animals. Remnants of silk suture could be identified at the site in both the groups of animals. The implanted graft was indistinguishable from the surrounding tissues.

Biomechanical studies revealed increased tensile strength and elongation at break for Group II animal than Group I and normal animals. The tensile strength for Group I animals was greater than the normal animals. The elongation at break for Group I animals was less than the normal animals. Chvapil *et al.* (1973) reported that hernial ring sutured and reinforced with collagen mesh showed greater tensile strength. In the present study, increased tensile strength was observed in the Group II animals. Histomorphological study of the grafted site revealed large number of capillaries and newly formed large vessels indicating neovascularisation with collagen fibres arranged regularly, illustrating normal healing at the surgical site.

The evaluation of healing process in Group II animals, in which processed oesophageal allografts were used, indicated that the graft material was completely accepted with minimal local reaction by the host tissue. Host collagen was seen laid down replacing the graft. There was no untoward reaction during the healing process.

Summary

6. SUMMARY

The present study was conducted on twelve clinical cases of umbilical hernia in pigs of either sex, aged two to three months. The animals were divided into two groups (Group I and Group II) of six animals each, based on the size of hernial ring.

Group I animals with smaller hernial ring were subjected to herniorrhaphy. Group II animals with larger hernial ring were subjected to hernioplasty using processed oesophageal allografts.

Fresh pieces of oesophagus collected from slaughtered pigs were processed and cross-linked with glutaraldehyde to prepare the graft.

Triflupromazine hydrochloride was administered at the rate of 1.5mg/kg body weight I/M and local infiltration anaesthesia at the site using two per cent solution of lignocaine hydrochloride was effected for surgical procedures.

In five animals of Group I where the hernial contents were reducible, simple reduction and overlapping mattress suture for closing hernial ring using silk was done. In one animal where it was irreducible enterotomy was

performed to remove the intestinal contents before reduction and herniorrhaphy.

In all the six animals of Group II, the hernial ring was large and the contents were reducible. After reduction, the edges of hernial ring were sutured using silk by simple interrupted sutures, to reduce the size of hernial ring. Processed oesophageal allografts were placed as an onlay graft, over the suture line and fixed using silk sutures.

All the animals became active and alert within 24 hours postoperatively except the animal, which underwent enterotomy and herniorrhaphy. All the animals were fed with reduced quantity (75 per cent of normal ration) of feed till healing was complete. The surgical site was dressed daily and skin sutures were removed on the seventh postoperative day. In one animal of Group I wound infection resulted in skin wound disruption, which was treated as open wound. Recurrence of hernia was noticed in one animal of Group I, 24 days after surgery.

Marginal increase in rectal temperature, pulse rate and respiration rate were observed during early postoperative period in all the animals and it became normal within seven days after surgery.

A marginal decrease in haemoglobin concentration was noticed during postoperative period in all the animals but reached near normal level by 21st postoperative day. Marginal increase in packed cell volume was observed during early postoperative period followed by a decrease to reach near normal level by 14th postoperative day. Increase in total leucocyte count was seen up to the seventh postoperative day in all the animals of both the groups. Neutrophilia was noticed up to seventh postoperative day in both the group of animals. Lymphocyte count was seen decreased during early postsurgical period followed by increase to reach near normal level by 14th postoperative day. The monocyte, eosinophil and basophil count did not show any significant variation between the groups during postoperative period.

A marginal decrease in total serum protein was noticed during postoperative period but was normal by 21st postoperative day. Serum sodium and potassium level did not show any significant variation and the changes were within the normal range.

Tissue samples were collected from surgical site from three animals each of Group I and Group II on 180 and 195 days respectively for biomechanical studies and histomorphological changes. The tensile strength and elongation at break were higher for Group II animals than for Group I. The histomorphological study indicated complete

healing and replacement of the graft material without any untoward effect.

From the results of the present study, it could be concluded that,

1. Porcine oesophageal tissue could be processed to prepare collagen sheets with good shelf life.

2. The collagen could be used to provide satisfactory support as onlay graft in surgical closure of umbilical hernial ring in pigs.

3. The glutaraldehyde cross-linked porcine collagen did not elicit any untoward local reaction and the graft was accepted.

4. Processed porcine oesophageal collagen grafts can be used successfully in the repair of large abdominal /umbilical hernial defects in pigs.

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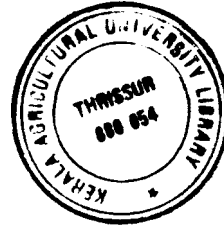
PROCESSED OESOPHAGEAL ALLOGRAFTS FOR HERNIOPLASTY IN PIGS

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ABSTRACT OF THESIS
Submitted in partial fulfillment of the
requirement for the degree

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ABSTRACT

The present study was conducted with the objectives of:

    i.   Evaluating the suitability of processed collagen based oesophageal allografts as a biological tissue substitute for hernioplasty in pigs and

    ii.   Comparing the healing in herniorrhaphy and hernioplasty, in pigs.

Twelve clinical cases of umbilical hernia in pigs of either sex, aged two to three months were used in the study. The animals were divided into two groups (Group I and Group II) of six animals each based on the size of hernial ring. Group I animals were subjected to herniorrhaphy and Group II animals were subjected to hernioplasty.

Fresh pieces of oesophagus collected from slaughtered pigs were processed and cross-linked with glutaraldehyde to prepare the graft.

Triflupromazine hydrochloride was administered at the rate of 1.5 mg/kg body weight I/M to all the animals and local infiltration anaesthesia using lignocaine

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hydrochloride at the site was employed for the surgical procedures.

In five animals of Group I where the hernial contents were reducible, simple reduction and overlapping mattress sutures with silk were employed for closing the hernial ring. In one animal where it was irreducible, enterotomy was performed to remove the intestinal contents before reduction and herniorrhaphy.

In all the six animals of Group II, the hernial ring was large, and the contents were reducible. After reduction, the edges of hernial ring were sutured using silk by simple interrupted sutures, to reduce the size of hernial ring. Processed oesophageal allografts were placed as an only graft over the suture line and fixed using silk sutures.

All the animals became active and alert within 24 hours postoperatively except the one, which underwent enterotomy and herniorrhaphy. The surgical site was dressed daily and skin sutures were removed on the seventh postoperative day. In one animal of Group I wound infection resulted in skin wound disruption, which was surgically treated as open wound. Recurrence of hernia was noticed in one animal of Group I, 24 days after herniorrhaphy.

Marginal increase in rectal temperature, pulse rate and respiration rate were observed during early

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Marginal decrease in haemoglobin concentration was noticed during postoperative period in all the animals but it reached near normal level by 21st postoperative day. Marginal increase in packed cell volume and total leucocyte count was observed up to third postoperative day. Neutrophilia and lymphopenia was noticed during early postsurgical period but reached near normal level by 14th postoperative day.

Marginal decrease in total serum protein was noticed during postoperative period but was normal by 21st postoperative day. Serum sodium and potassium level did not show any significant variation and the changes were within the normal range.

Tissue samples were collected from surgical site from three animals each of Group I and Group II on 180 and 195 days respectively for biomechanical studies, gross and histomorphological changes. The tensile strength was greater in Group II animals than Group I and normal animals. There was no adhesion of viscera at the surgical site. Remnants of silk suture could be identified in all the animals. The histomorphological study indicated complete healing and replacement of the graft material without any untoward effect.