

**A COMPARATIVE EXPERIMENTAL STUDY OF THE USE
OF TUNICA VAGINALIS AND PERICARDIUM AS ALLOGRAFTS
FOR HERNIOPLASTY IN SHEEP**

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(Received December 26, 2004; Accepted April 28, 2005)

ABSTRACT

In this study umbilical hernias were created experimentally in eight sheep. At the 30th postoperative day, hernia developed that had the typical characteristic features of reducible hernia. The animals were divided into two groups and hernioplasty was done using freshly prepared ovine tunica vaginalis in one group and ovine pericardium in the second group. Following hernioplasty, the animals were inspected clinically. Biopsies were taken from the grafts and surrounding tissue at 15, 30, 60, and 90 days. Clinical examination of the experimental animals and the patch grafts showed no apparent abnormalities except for the occurrence of simple multifocal wound infection. Histopathological examination of the patch grafts showed inflammatory reactions that ranged from polymorphonuclear cell infiltration of the graft up to chronic foreign-body granuloma. Dense fibrous tissue was seen invading the graft particularly through perforations of the suturing needle and fissures in the graft. These histopathological changes were not seen at the 60th postoperative day except for the fibrous capsulation of remnants of suture material in the graft. No major differences were seen in the microscopic picture of hernias grafted with tunica vaginalis and those grafted with the pericardium. This study described a model for experimental induction of umbilical hernia in sheep and introduced for the first time in the literature the use of tunica vaginalis as an allograft for hernioplasty in sheep. Further studies are needed on the use of tunica vaginalis patch graft in repairing other types of tissue defects such as in duraplasty.

**دراسة مقارنة تجريبية لاستخدام الغلالة الغمدية والتامور كرقعات مغايرة
لاصلاح الفتوق في الضئان**

نه زاد حسين قادر , بهجت طيفور عباس , حافظ ابراهيم السعدي
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جامعة الموصل , الموصل , العراق

الخلاصة

تضمن هذا البحث احداث فتوق سرية تجريبيا في ثمانية خراف. ظهرت الفتوق عند
اليوم الثلاثين بعد اجراء العمليات الجراحية وكانت من النوع الزرود وامتلكت كافة الاماخذ
المميزة للفتوق الرادة. و بعدها تم تقسيم الحيوانات الى مجموعتين , واصلحت الفتوق في

جمع خزعات من الغرسات والانسجة المحيطة بها خلال 15 , 30 , 60 و 90 يوم بعد اجراء العمليات الجراحية. واطهر الفحص السريري للحيوانات التجريبية والرقعات عدم وجود اعتلالات ظاهرة باستثناء حدوث بؤر متعددة من خمج الجرح. وأوضح الفحص النسيجي المرضي للرقعات تفاعلات التهابية تراوحت من ارتشاح الخلايا متعددة اشكال النواة بداخل الرقعة الى الالتهاب الورمي الحبيبي المزمن نوع الجسم الغريب. كما لوحظ امتداد نسيج ليفي كثيف بداخل الرقعات وخصوصا في الثقوب التي خلفتها ابرة الخياطة وفي الشروخ الموجودة في الرقعات. ولم تلاحظ هذه التغيرات النسجية المرضية عند اليوم 60 بعد اجراء العملية الجراحية باستثناء احاطة بقايا الخيوط الجراحية في الرقعة بنسيج ليفي كثيف. واسم تلاحظ فروق هامة في الصورة المجهرية للفتوق المرقعة بالغلالة الغمدية وتلك المرقعة بالسامور. ختاماً , فقد وصف هذا البحث نموذجاً لاحداث الفتوق تجريبياً في الضأن وأدخل ولأول مرة في النشريات العلمية استخدام الغلالة الغمدية كرقعة مغايرة لاصلاح الفتوق في الضأن. وتبقى الحاجة قائمة لاجراء بحوث اضافية حول استخدام الغلالة الغمدية في اصلاح الانواع الاخرى من عيوب الانسجة كما في حالة اصلاح الجافية.

INTRODUCTION

Several types of connective tissues, muscles, and synthetic materials have been used experimentally and clinically in reconstructing congenital or acquired soft tissue defects. Among the dense connective tissue grafts used for this purpose were fascia lata, pericardium, dura mater, and diaphragm(1-8). In human medicine , most muscles of the human body have been evaluated for potential use as flaps (9,10). In veterinary medicine , the most commonly used muscles were the rectus femoris, cranial sartorius, cervical part of the trapezius , semitendinosus, and latissimus dorsi (11-14). Synthetic meshes have been also used and they include non-absorbable meshes (stainless steel, tantalum, teflon, silicon, monofilament –polyfilament nylon, polyepoxy compound, polypropylene, polytetrafluoroethylene, polyethylene, tetraphthalate, and polyester) and absorbable mesh (polyglycolic acid, polyglactin 910, and the Bulgarian antimicrobial polyamide), (15-25).

The purpose of this study was to evaluate the use of tunica vaginalis in repairing experimentally induced abdominal hernia in sheep and to compare the use of this type of graft with the use of pericardium for the same purpose. From a review of the literature it became evident that the use of tunica vaginalis for hernioplasty has never been attempted both in humans or animals.

MATERIALS AND METHODS

1. Preparation of the patch grafts:

a) Preparation of ovine tunica vaginalis:

The tunica vaginalis were freshly harvested from 2-3 years old healthy rams immediately after slaughter under veterinary supervision. The tunica vaginalis were rinsed in a sterile physiological saline solution and their external loose connective tissue were stripped mechanically. The remaining fibrous sheet was re-rinsed by repeated washing in sterile physiological saline solution and stored at 4°C in glassware jar bathed in sterile physiological saline solution and ready for hernioplasty on the same day of collection and preparation (Fig.1).

b) Preparation of ovine pericardium:

The pericardia were freshly harvested from 6-12 months old healthy sheep, immediately after slaughter under veterinary supervision. The pericardia were washed in sterile physiological saline solution. They were stripped mechanically of their external loose connective tissue coat including adipose tissue, vessels, and nerves. The remaining fibrous membrane was re-rinsed by repeated washing in sterile physiological saline solution and stored at 4°C in glassware jar bathed in sterile physiological saline solution ready for hernioplasty on the same day of collection and preparation (Fig.2).

2. Animals:

Eight 6-12 months old sheep weighing 17-21 kg were used for experimental induction of umbilical hernia. The sheep were apparently healthy, ear tagged and housed under the same feed and management conditions.

3. Experimental induction of umbilical hernia:

Umbilical hernias were created experimentally in all eight sheep. Each animal was fastened for 24 hrs from food and 12 hrs from water prior to surgical operation. The skin at the mid-abdominal region from the xiphoid to the pupic symphysis was prepared for aseptic surgery. General anaesthesia was induced by intravenous administration of mixture of xylazine¹ (0.05 mg/ kg) and ketamin HCL² (2 mg/kg), ten minutes following premedication with atropine sulfate ³(0.2 mg/kg) given intramuscularly.

The animal was placed in dorsal recumbency and a 10cm long mid-line abdominal skin incision was made at the center of the umbilical region. The skin was bluntly dissected from the abdominal connective tissue. The linea alba was incised for about 6-8 cm and finally the peritoneum was opened and dissected from the rectus abdominus muscles. Two elliptical incisions were made at the abdominal muscles around the umbilicus and a circular piece of 4-6 cm diameter was removed from the abdominal muscle fibers. Following proper hemostasis, the peritoneal edges were sutured to the circular rim of the dissected abdominal muscle and fascia following a simple continuous mattress suture pattern using size 0 chromic catgut, so as to produce an artificial ring. The skin was closed with an interrupted suture pattern with size 1 silk suture. The wounds were dressed and supported for 10 days following the surgical operation by a cloth-made abdominal belt. The skin stitches were removed at the 10th post-operative day and animals were followed for one month for the development of umbilical hernias.

4. Hernioplasty:

¹ Scton 2% Xylazine, Calier S.A., Spain.

² Tekam 50, Ketamin, Al-Hakma Drug Compang, Jordan.

³ Atropine Sulphate 1 mg, S.D.I., Samara, Iraq.

Following development of the umbilical hernia, which occurred on the 30th day post-hernioplasty, The experimental animals were randomly divided into two groups.

Tunica

vaginalis patch grafts were used in hernioplasty of one group of animals and the pericardium for the other.

a) Hernioplasty with tunica vaginalis patch graft:

Anesthesia and preoperative techniques were similar to those followed in the induction of hernia. A mid-line surgical skin incision was made above the umbilicus. A single incision over the hernial sac extending slightly beyond its cranial and caudal edges. By blind dissection the skin was separated from the hernial sac where adhesion or incarceration of the contents to the sac was seen. An open reduction was performed to isolate the attachments. The redundant skin overlying the hernial sac was removed along with the hernial sac which was palpated to be sure that it is empty. The fibrous attachments of the sac to the fascia were divided to expose clean fascia on all sides from the periphery to the edge of the ring. The peritoneum was treated to left intact, otherwise closed by a row of simple continuous mattress sutures of No. 0 chromic catgut. A suitable size of the patch graft was sutured to the hernial ring, with the visceral side of the tunica vaginalis facing the abdominal cavity (Fig.4). The material used for suturing the patch graft was No.0 chromic catgut by simple interrupted mattress. The skin was closed by a No. 1 braided silk by simple interrupted mattress.

b) Hernioplasty with pericardium patch graft:

Anesthesia and preoperative preparations were similar to those followed in the induction of hernia. The hernia was corrected by the same procedure described for the tunica vaginalis except for the use of pericardium sac a patch. The cardiac side of the pericardium was placed facing the abdominal cavity (Fig.5).

5- Tissue collection:

Tissue specimens were collected from the patch grafts and surrounding tissue at 15, 30, 60, and 90 days following hernioplasty. The process was performed aseptically following sedation and local infiltration analgesia. Tissue specimens were fixed in 10% formalin solution for 48hrs, trimmed to suitable sizes, washed, dehydrated, cleared in xylol, embedded in paraffin wax, sectioned at 5-6 μ thickness, stained with hematoxylin and eosin, and examined with a light microscope (26).

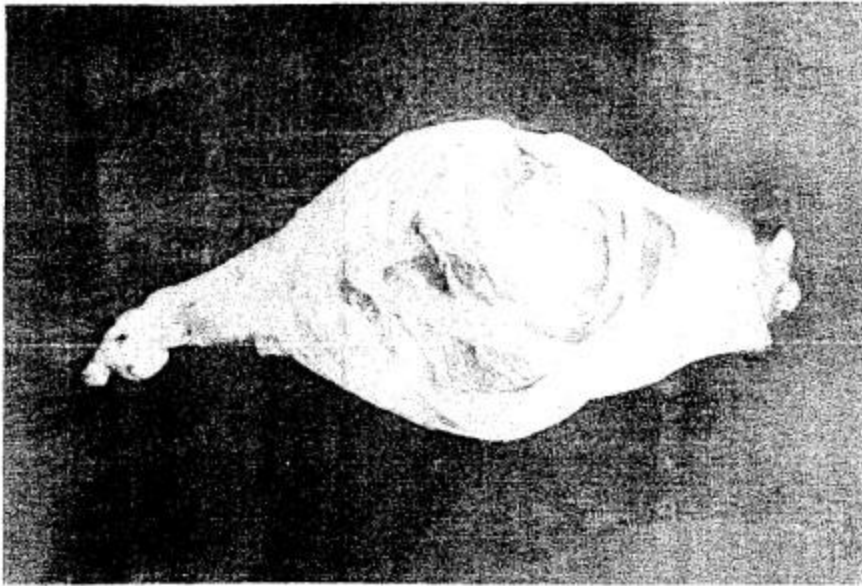


Fig .1 - A fresh tunica vaginalis from the testes of a ram ready to be used in the hernioplasty.

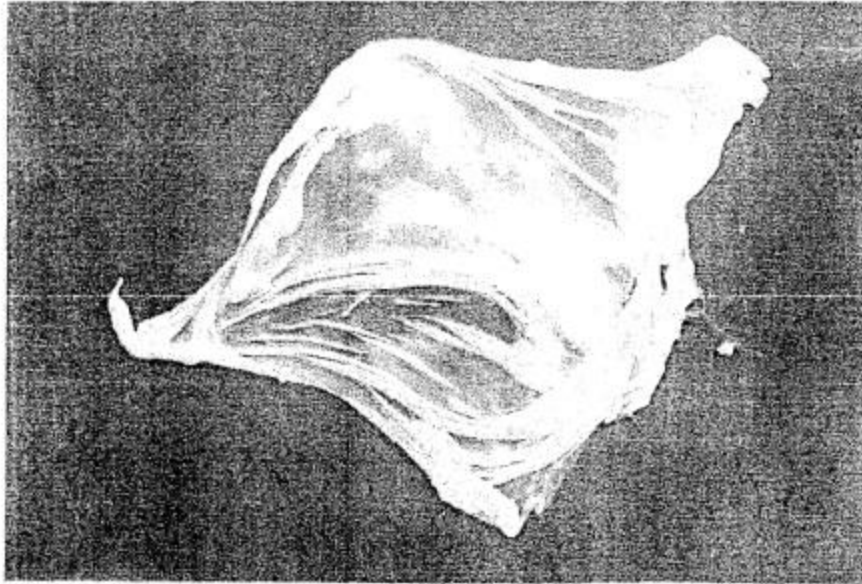


Fig.2- A fresh pericardium from sheep ready to be used in hernioplasty.

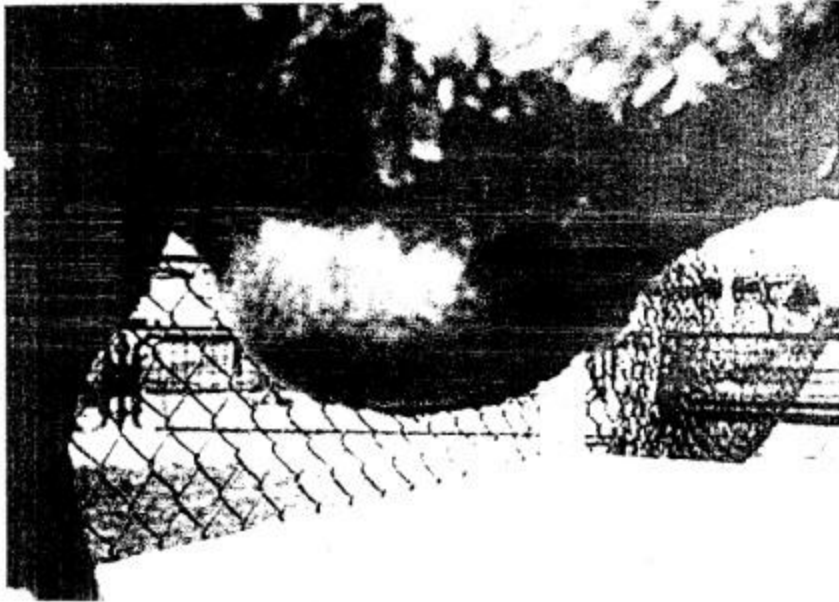


Fig. 3- A ram with experimentally induced umbilical hernia 30 days postoperation. The diameter of the ring was 13-15 cm.

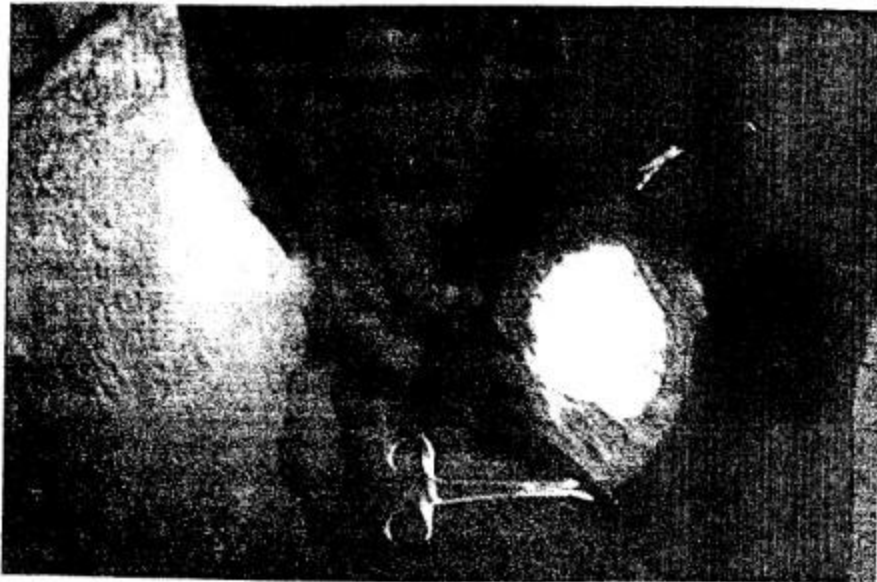


Fig. 4- Tunica vaginalis grafted over the peritoneum in a ram.

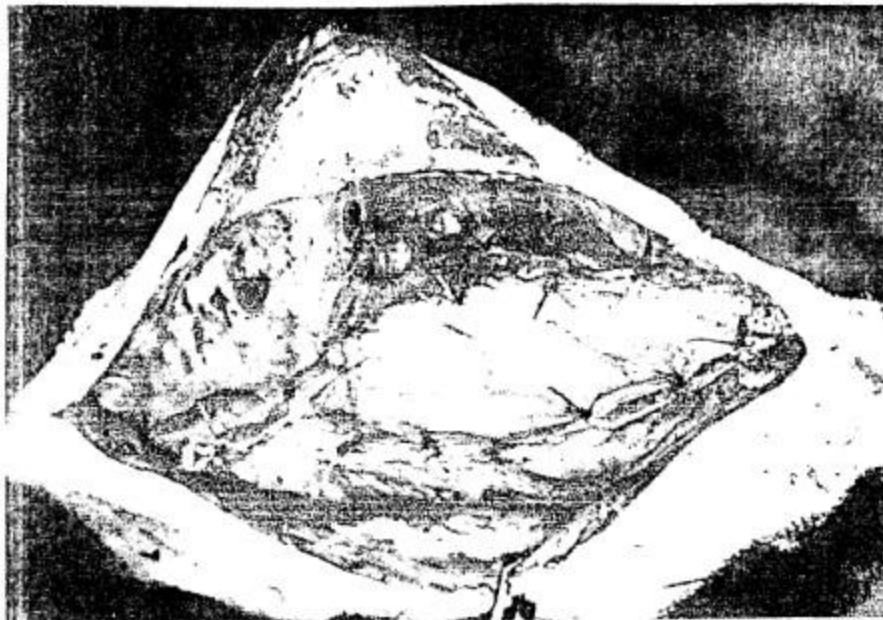


Fig. 5- Ovine pericardium grafted over the peritoneum in a ram .

RESULTS

A- Clinical Observations:

- 1) Hemiooplasty with tunica vaginalis : Anorexia was noted during the second postoperative day and then disappeared after the 3rd postoperative day. A mild redness and swelling of the site of operation were seen and disappeared within 4-5 days following the surgical operation . Multiple minute areas of infection were grossly seen at the site of operation and disappeared following the use of local antiseptic solution. During the first week following surgery, the average body temperature was 39.1 - 40°C and the pulse rate was 95-104 beats / minute and the respiratory rate was 27-36 times / minute .
- 2) Hemiooplasty with pericardium: Starting the second postoperative day, the animals suffered form anorexia and stood uncomfortable. The average body temperature for the first 7 postoperative days was 39.1 - 40°C and the pulse rate was 95-100 beats per minute and the respiratory rate was 27-36 times / minute. A severe redness and edema of the site of operation was seen and the whole site was painful to manual palpation. These symptoms disappeared within 7-10 days after surgical operation. Multiple areas of infection were noted at the site of operation in two of the four cases and were treated locally . Furthermore, hernia reoccurred in one of the four cases at the end of the 3rd post-operative week despite the use of two folds of the pericardium. Additionally, severe adhesion was seen during biopsy collection from the site of operation.

B- Histopathologic observations:

1) Hernioplasty with tunica vaginalis: At 15 day postoperation, the patch graft was interwoven closely with the subcutaneous tissue and it was difficult to differentiate between the two types of tissues. Thus, well vascularized fibrous tissue consisting of few fibrocytes and dense bundles of collagen fibers was visualized bridging between the two types of tissues (Fig.6). Perivascular cuffing of some of the capillaries in the fibrous tissue was seen. At the 30th postoperative day, diffuse mononuclear cell (plasma cells, lymphocytes, and macrophages) infiltration were seen in the fibrous tissue particularly at sites of suturing (Fig.7). Remnants of suture material were visualized in some of the sections and these were surrounded by connective tissue. Furthermore, granulomatous secretion (macrophages and giant cells mainly) was seen around these remnants (Fig.8). The fibrous tissue extended from the host tissue into the graft via the perforations produced by the suturing needle. At the 60th postoperative day, mature fibrous tissue was seen surrounding remnants of suture material (Fig.9). A picture similar to this was found at the 90th postoperative day 2-
Hernioplasty with pericardium : At 15 days postoperation, the graft was closely interwoven with the subcutaneous tissue and it contained foci of polymorphonuclear leukocytes mainly eosinophils (Fig.10). At the 30th postoperative day, the graft contained foci of dense aggregations of giant cells in sites of perforations produced by the suturing needle (Fig.11). Additionally, dense infiltration of inflammatory mononuclear cells was seen in the interface between the graft and the subcutaneous tissue (Fig.12). At the 60th postoperative day, a dense well vascularized mature fibrous tissue made up of spindle – shaped fibrocytes and collagen fibers (Fig.13). A similar picture was seen at the 90th postoperative day.



Fig. 6- Cross section of tunica vaginalis patch graft 15 days after grafting in a Ram with umbilical hernia. Note the well vascularized fibrous tissue bridging between the graft and surrounding tissue H & E X 40

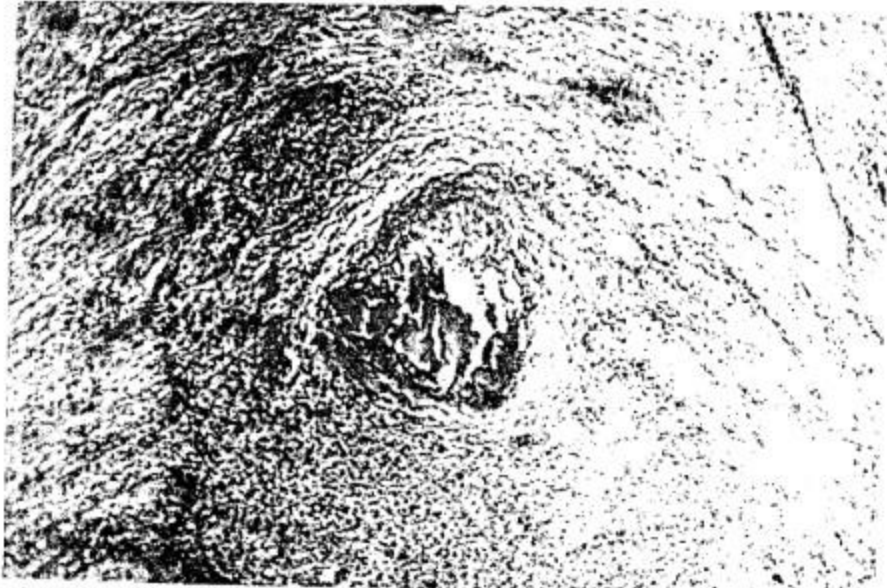


Fig. 7- Cross section of tunica vaginalis patch graft 30 days after grafting in a Ram with a bilical hernia. Diffuse, mononuclear cell infiltration of fibrous tissue could be seen. A remnant of suture material could also be seen. H & E X 40



Fig. 8- Cross section of tunica vaginalis patch graft 30 days after grafting in a Ram with an umbilical hernia. Note the dense aggregation of macrophages and giant cells around a remnant of suture material and the infiltration of granulation tissue into the graft. H & E X 40 .

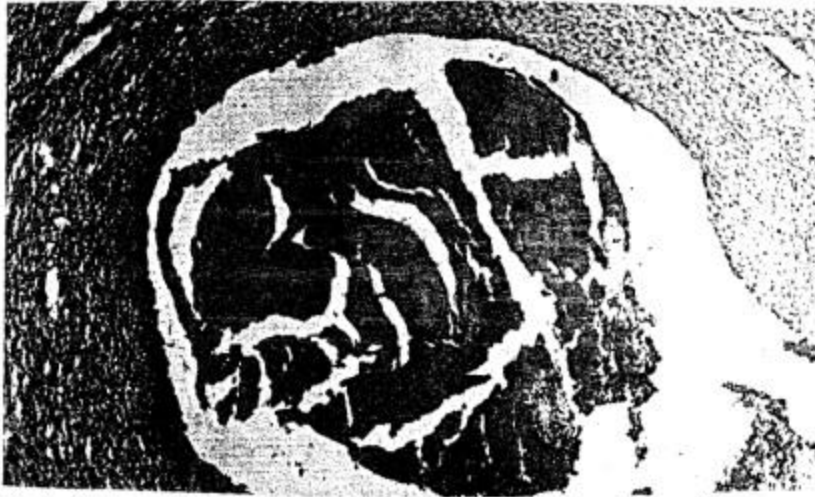


Fig. 9- Cross section of tunica vaginalis patch graft 60 days after grafting in a Ram with umbilical hernia. A remnant of suture material surrounded by dense fibrous tissue could be visualized. H & E X 40.

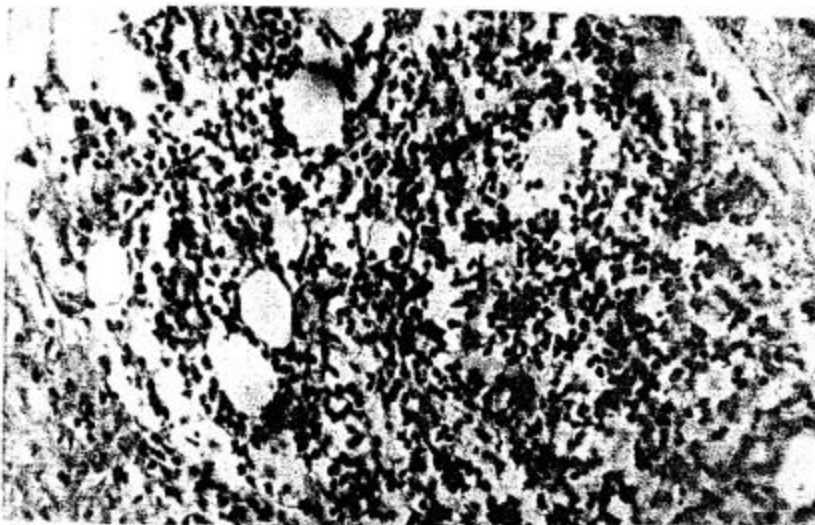


Fig. 10-Cross section of pericardium patch graft 15 days after grafting in a Ram with umbilical hernia. Note a focus of eosinophilic infiltration in the graft. H & E X 60

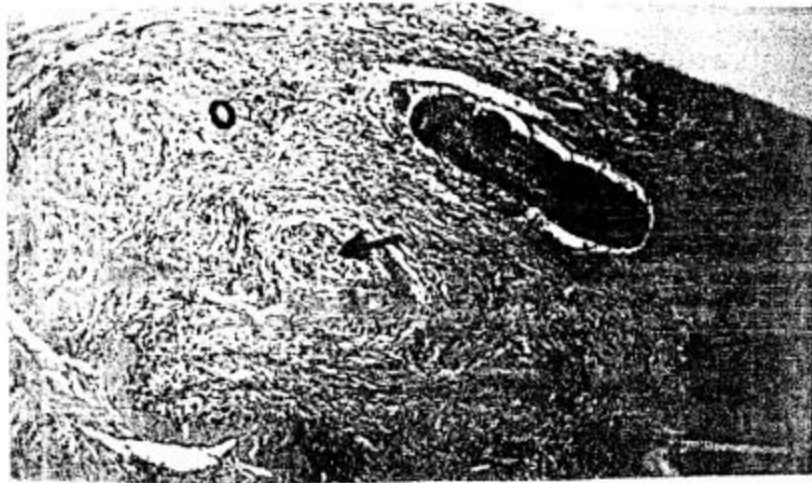


Fig. 11 – Cross section of pericardium patch graft 30 days after grafting in a Ram with umbilical hernia. Note the fibrous tissue extending into the graft and the presence of clumps of giant cells at sites of perforations by the suturing needle. H & E X 40

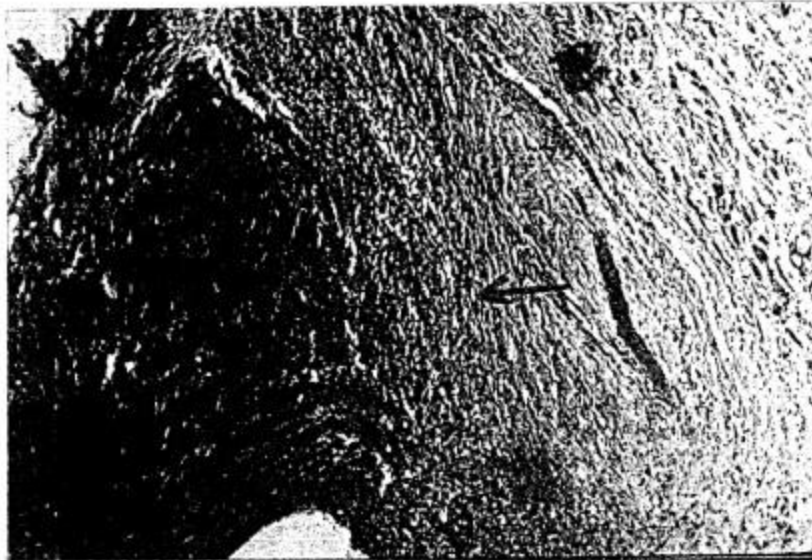


Fig.12- Cross section of pericardium patch graft 30 days after grafting in Ram with umbilical hernia. Note the heavy infiltration of mononuclear cells into the fibrous tissue between the graft and surrounding tissue .
H & E X 40.



Fig. 13- Cross section of pericardium patch graft 60 days after grafting in a Ram with umbilical hernia . Note the presence of mature fibrous tissue consisting of large number of fibrocytes and collagen fibers. H & E X 60.

DISCUSSION

Results of the present study have indicated that hernia could be created experimentally in rams within 30 days. The induced hernia was comparable to naturally -occurring one and consisted of a sac, ring, and content. The ring was first detected through manual palpation at the 7th post-operation day. This period coincided with the phase of fibroplasia of the reparative response which is usually completed at 21 days following the injury (27). The diameter of the experimentally created hernias ranged 6-10 cm except in one case in which the diameter was 13-15 cm. This discrepancy in diameters of the rings could be attributed to variations in food intake, degree of dilatation of the rumen, and the physical status (strength) of muscles of the abdominal wall .

Redness and edema of the operative site in all of the experimental animals could be attributed to trauma inflicted on the tissues during surgery. Following trauma, inflammation occurs leading to hyperemia and edema (27). These gross changes disappeared gradually starting on the 5th postoperative day.

The clinical signs and the local inflammatory changes (edema and redness of the site of operation) disappeared within 4-5 days in animals grafted with tunica vaginalis and within 7-10 days in animals grafted with pericardium. The difference could be explained on the basis that tunica vaginalis is more acceptable as grafts (28).

In animals grafted with pericardium, some areas of infection and adhesions were seen grossly. These changes could be attributed to the use of chromic catgut in suturing the various layers of the peritoneum. In support of this proposition , these changes have not been reported when silk was used by others to repair experimentally created diaphragmatic hernia in dogs (16). Furthermore, similar spots of infections, were also seen at the site of operation in animals grafted with tunica vaginalis.

Histologically, the reaction of the graft was basically similar in cases grafted with tunica vaginalis and those grafted with pericardium .Growth of the graft into the

surrounding tissue occurred through the formation of granulation tissue that grow in clefts and perforations produced by the suturing needle. Foci of mononuclear cell infiltration were seen infiltrating the tunica vaginalis graft at the 30th postoperative day indicating progression of the inflammatory reaction to chronicity. At this period, clumps of foreign – body giant cells were seen in the graft particularly at sites of presence of suture material. At 60 and 90 postoperation days, remnants of suture material were seen in the graft encysted by dense connective tissue capsule. In cases grafted with the pericardium, foci of polymorphonuclear cells (particularly eosinophils) were seen in the graft at 15 days. This finding indicated that the reaction was still of the acute type. At the 30th postoperative day, dense mononuclear cell infiltration was seen in the interface between the graft and surrounding tissue. This finding has indicated that the reaction became chronic. At 60 and 90 postoperation days, highly cellular fibrous tissue was seen in the interface between the graft and the surrounding tissue.

From the clinical and histopathological results of this study it could be concluded that the tunica vaginalis could be used with good results in hernioplasty in ruminants. This investigation could be considered the first report on the use of tunica vaginalis as allograft for hernioplasty in domestic animals.

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