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A research in the lamb for the compatibility of the amniotic membrane in the urinary system(*)

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Summary

In order to establish the compatibility of sheep amniotic membrane as a tissue implant in three-month-old lambs this experimental work has been carried out for their ureteric reconstruction. The amniotic membrane (TAM) was implanted to the ureters of seven lambs as a patch graft, whilst a follow-up was performed in the postoperative seven months course by haematological, blood chemistry and radiological measures periodically. Two lambs died due to septicaemia; one due to incisional inflammation and another due to ureteral infection stenosis and hydronephrosis. At seven months postoperatively the remaining five lambs were sacrificed. Postmortem macro and microscopical study in all lambs revealed no graft rejections. In conclusion, it was considered worthwhile to recommend the use of allogenous amniotic grafting in the correction of urinary system anomalies, stenoses and traumas.

Key words: Amniotic membrane, free graft, ureteric reconstruction

Introduction

The use of foetal membranes in surgery has been on the agenda since the beginning of this century. Davis[1], was the first to mention the use of foetal membranes in skin grafting, in 1910 Stern [2] and Sabella [3] with their independent publications on the use of amniotic membranes for burnt and ulcerated skin closures followed this pioneering work in 1913. Owing to the better understanding of its advantages in transplantation, the amniotic membrane (TAM) was used in a variety of surgical procedures, such as in, establishing a newly formed vagina's lining [4], repair of conjunctival defects [5], hindering the formation of adhesions in meningrocerebral [6] and abdominal surgery [7,8,9], managing omphalocele and gastroshisis lesions [10], treating ulcers in peripheral vascular diseases [11], arthroplasties [12], etc.

There has been no report in the literature concerning the use of TAM in the urinary system for its various pathologies. This report, is therefore planned to define the outcome of its use and place in the urinary system's anomalies, stenoses and traumas.

Materials and Methods

Seven healthy, three-month-old "Akkaraman" lambs were quarantined for a period of one-and-a half months and made sure they were free of diseases and had usual immunization. An equal number of gravid Akkaraman sheep were observed during their last three months till their delivery, after which their placentae were taken, TAM separated from the chorion, washed with normal saline and stored in jars, containing 10 % polyvidone iodine (Batticon, H. Tromsdorff Arznemittel fabrik 5110 Alsdorf bei Aacher, Germany) and 90 % normal saline, at 5 degress Celcius, in sterile conditions. At the experimental operation every four-and-a half monthold lamb was placed in a left lateral decubitus fashion on the operation table and their left basilic vein cannulated for a 5 % dextrose drip. Their right lomber regions were depiliated and an antisepsis using polyvidone iodine applied after 2.5 % pentothal anaesthesia (Fig 1). A right transverse incision was used to view and prepare the right kidney and upper one third of the ureter. A 3 cm longitudinal incision at the anterior wall of the ureter, 2 cm distal to the ureteropelvic junction led to a fusiform defect which was repaired over a silastic catheter (Fr 8) within the lumen, introduced via renal pelvis and surpassing the incisional defect 5 cm distally, using TAM and con-

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tinious 6/0 atraumatic polypropylene suture material (Fig 2). The catheter was brought out at skin level through a stab wound at least 10 cm away from the incisional site and the incision closed in accordance with its anatomical layers (Fig 3). There were no anaesthetic problems in induction, during the operation or reanimation. Netilmycin 5 mg/kg/day i.m. for five days was given postoperatively for prophlactic reasons. The urinary catheter and the skin sutures were removed on the 25th postoperative day.

Haemoglobin, haematocrit, WBC, BUN, creatinine, Na and K values were established preoperatively and 30, 60, 90 days postoperatively.

At the seventh postoperative month the lambs were intentionally bled to death under anaesthesia and both kidneys and ureters were removed for their macro and micro analysis.

Result

Only one lamb's incisional wound had an infection, which led to her death in the 24th postoperative day causing fulminant septicaemia. Urinary fistulae developed in two, one at the immediate postoperative period and drying in two months and the other starting three months postoperatively and drying out in 15 days due to an urinoma caused by the removal of the stent. There were no significant high WBC apart from these three lambs. Blood test values encountered with these lambs did not differ from to other healthy lambs not operated and used as control (Tables 1&2).

Table I. Mean values of haematological tests for six lambs at the 30th, 60th and 90th postoperative days.

	Routine Blood Tests (Mean values)	
Lamb	WBCx 1000	HGB gr/dl
1	23.0	12.0
2	08.1	11.1
3	13.8	10.9
4	10.4	11.1
5	24.7	13.5
6	09.9	13.0
Control 1	08.3	13.4
Control 2	06.7	12.6
Normal	07-10	13

Table II. Mean values of biochemical blood tests for six lams at the 30th, 60th and 90th postoperative days.

	Blood Chemistry (Mean values)			
Values / Lamb	BUN mg/dl	Crea. mg/dl	Na mmol	K mmol
1	20	0.8	145	4.7
2	23	1.1	151	4.9
3	20	1.0	137	5.5
4	27	0.7	142	6.1
5	21	1.3	144	5.1
6	29	1.6	145	4.5
Control 1	21	1.0	145	4.6
Control 2	28	1.2	151	5.3
Normal	25	1.0	145	5.0

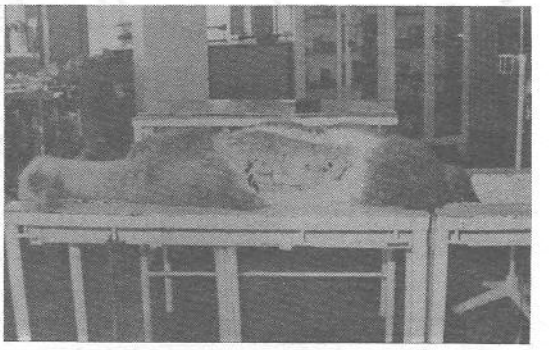

Figure 1. Preoperative view of lamb with depiliated right lomber region (lamb no.1).

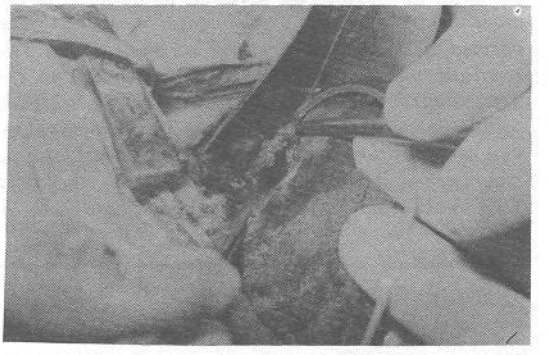

Figure 2. Repair of the right ureter using TAM and 6/0 polyprolene (lamb no.3).

Figure 3. Postoperative view of the reapproximated operative incision and the ureteral catheter at stab wound (lamb no. 5).

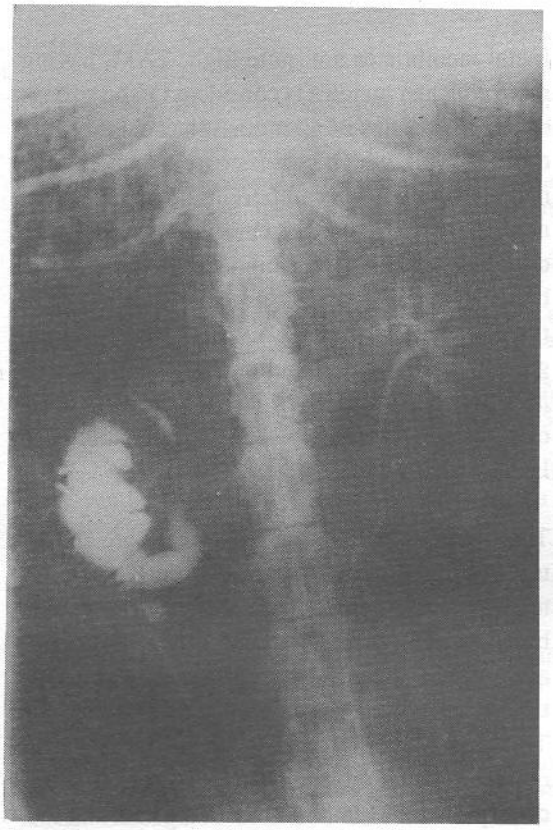

Figure 4. Hydronephrotic right kidney at descending pyelography (lamb no.1).

KUB, IVP and ascending pyelographic examinations six months postoperatively showed only two slight distentions just proximal to the repair area involving the proximal ureter and the calyceal system (Fig 4). Yet both of these ureters could be visualized with a normal appearence distal to the repair, showing adequate renal and ureteric function. The remaining kidneys and ureters were accepted normal both in radiological and postmortem studies (Fig 5).

The lamb with late ureteral fistula died at the postoperative 190th day due to ureteral stenosis. A postmortem examination showed leak of urine around the catheter, an intact graft, an infected urinoma, gross edema and bilateral hydronephrosis. The postmortem examination of all the lambs sacrificed, showed a foreignbody granulation tissue around the suture material in two on the operated side, while quite normal kidneys and ureters on the contralateral side.

Normal renal and ureteric appearance with intatct graft and thick fibrous tissue around suture material and overgrowth of the mucosa and musculature at the graft site was encountered in the remaining three (Fig 6).

The graft was no longer distinguishable by its

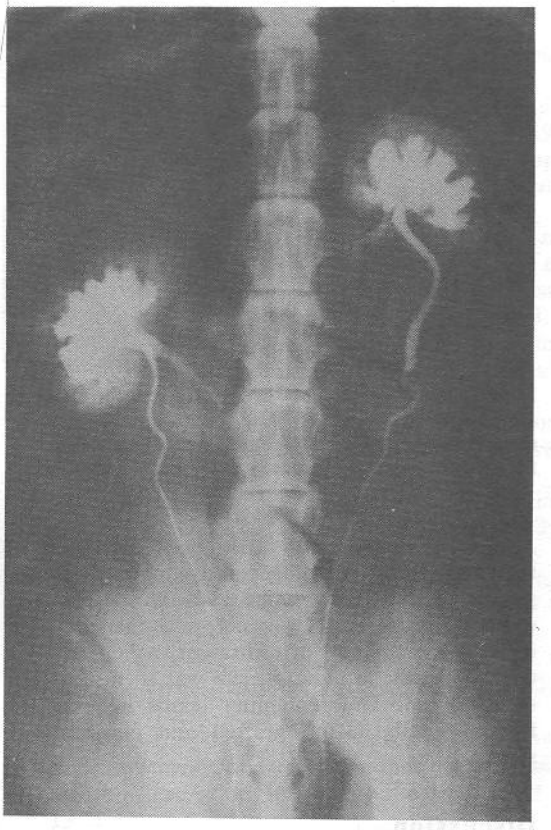

Figure 5. Normal kidneys at ascending pyelography (lamb no.2).

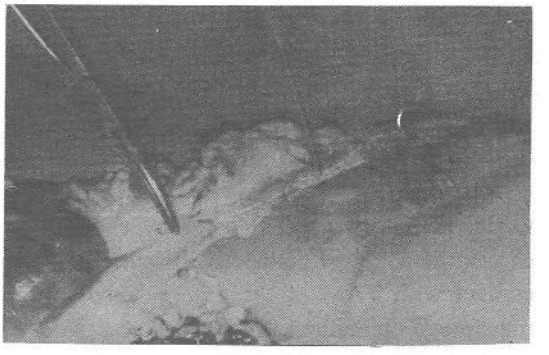

Figure 6. Postmortem view of the healed graft site, between a haemostat and a syringe needle (no stricture formation or hydroureteric changes) (lamb no.4).

gross appearance or microscopically at seven months postoperatively apart from lymphocytic infiltration and propagation of connective tissue elements at the site. The longitudinal incision on the ureter at the operation still showed separated muscle bundles (with sligth regeneration from both sides). The side of the specimen just next to the lumen at the same area was covered with transitional epithellium and connective tissue in between (Fig 7).

Figure 7. Histological sectioning of the ureter at the distal graft site, showing separated muscle bundles at 7 o'clock, covered with transitional epithelium (lamb no.6) (HEx10)

Advanced lymphocytic infiltration, dilatation of the tubuli, structural degenerative changes at the glomeruli were seen in the lamb with long term urinary fistula. Slight parenchymal changes with indefinite lymphocytic infiltration, believed to be due to stenosis at the graft site was seen in another lamb too. The remaining lambs had normal findings both in the grafted and contralateral sites.

Discussion

Foetal membranes consist of two layers; namely the amnion (TAM) and the chorion, which are loosely adhered to one-another. TAM or "the inner layer" the inner surface of which is covered with cuboidal or flat cells turn into the ectoderm of the foetus with epiblastic differentiation and the outer surface with mesenchymal connective tissue. The chorion or "the outer layer" comprises of mesenchymal connective tissue that is in contact with TAM of the external ectoderm's transitional epithelium [13,14].

The advantages of TAM as a biological dressing has been well documented [2,3,11]. It not only relieves pain, lessens fluid, electrolyte and protein loss from the wound surface but also drops down the bacterial count within the wound an protects it from any further bacterial contamination [15]. Human TAM has also been shown to execute glycolysis, enhance hexosamine synthesis, metabolize prostoglandins store triglicerides and cholesterol esters [16].

The tensile strength and pressure resistance of foetal membranes are quite high. TAM, having more collagen bundles is considered to be stronger [17,18]. Besides its strength, it has elastic properties as well, which enables ease at surgcal manupulation. A further important advantage is its near to inert structure. Acute immune rejection does not happen when human amniotic membrane is homologously transplanted since it lacks the epithelial surface HLA, A,B,C and DR antigens and B2 microglobulins [19]. Many experimental and clinical work have shown that most organisms respond only slightly immunologically to TAM [9,14,20].

The results of the present experiment are similar to those in literature. In none of the 7 postmortem examinations could there be any foetal membrane seen in gross and microscopical analysis. It appears that it is mostly reabsorbed and in a short time it is covered by the transitional epithelium. This new-mucosa and connective tissue rich in collagen keeps the lumen intact leading to the concept that the method is advantageous and foetal membranes can be considered as good repair materials. In the long run, the graft incorporates into the surrounding tissues and becomes irrecognizable from the epithelia and the connective tissue elements.

The infections and mortalitiy are believed to be due to either urine leak from the catheter placed at the renal pelvis or to the ascending infection, using the same route. The ureteric diameters encountered ranged from about 3 to 4 mm in the normal healthy lamb. The longitudinal incision placed on the lambs very narrow ureter and the similarly placed graft used for repair is believed to be the disadventage to the present method. It may well be accepted that, TAM may be used in the repair of the urinary system. Very thin monofilament sutures used in the experiment may well suit its purpose. The minimal or rather near to none rejection seen to the suture material may be pointed out to prove the fat. On the whole TAM can be interpreted as an easily available and tissue compatible material in the newborn lambs that can be used in the urinary system as well, causing no ipsilateral distal ureteral or contralateral urinary pathology.

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