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RESEARCH ARTICLE

Viability of Split Thickness Autogenous Skin Transplantation in Canine Distal Limb Reconstruction – An Experimental Evaluation

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ABSTRACT

Distal limb reconstruction is complicated by the paucity of local tissues and the frequent association of orthopedic injury with cutaneous loss. Though, second-intention healing or skin stretching techniques are used for wounds involving less than a 30% circumference of the limb, however, skin grafts are recommended for reconstruction of larger superficial wounds. The present study was designed to clinically evaluate the viability of split thickness autogenous skin transplantation (STAST) in dogs. Standardized surgical defects of variable size *i.e.* 3×3, 4×4 and 5×5 sq cm were made on the left middle radial area (forearm) of 15 mongrel dogs assigned to Group A, B and C, respectively having 5 dogs each. Split thickness autogenous skin grafts were harvested from mid thorax and placed in these defects through several simple interrupted sutures. Results indicated a success rate of 80% with no clinical difference in the survival rate of three different sizes of grafts used. Hence, STAST can successfully be used for canine distal limb reconstruction.

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INTRODUCTION

Skin defects on the limbs of animals present a problem from the standpoint that there is not an abundance of skin on the limbs to reconstruct with, as there is on the trunk of an animal (Fowler, 2006). Although second-intention healing or skin stretching techniques are used for wounds involving less than a 30% circumference of the limb however, skin grafts are valuable armamentarium for reconstruction of larger superficial wounds after establishing a bed of granulation tissue or for immediate reconstruction of clean wounds overlying healthy muscle (Bristol, 2005). Grafts may either be full thickness (composed of epidermis and entire dermis), or split thickness (composed of epidermis and varying thickness of dermis) (Isago *et al.*, 2003) and their survival depends upon the blood supply from the recipient site (Adams and Ramsey, 2005; Ercan *et al.*, 2005). A graft 'take' is a successful skin transplantation in which the transplant heals in its new location; grafts 'do not take' on bone, tendon, cartilage, nerve or areas of movement i.e. joints (Windsor *et al.*, 2009).

Skin grafts used in animals are virtually autogenous and can be applied easily in the clinical cases with almost minimal chances of rejection owing to their immunologic identity (Nanchahal *et al.*, 2002). Skin allografts and xenografts have though been described and used for specific application in wound reconstruction but have little role in routine management of open wounds in small animals (Andrea *et al.*, 2005).

In Pakistan, there is no published literature regarding the success rates of various skin transplantations in dogs, as yet. The study was hence, designed to clinically assess the viability of split thickness autogenous skin transplantation (STAST) in mongrel dogs. The article encompasses the preparation of the wound for grafting, the harvesting of the graft, graft placement, postoperative care and the results of the application of this technique in detail and will hopefully be helpful for pet practitioners in Pakistan.

MATERIALS AND METHODS

Study site and experimental animals: The present study was conducted on 15 male/female mongrel dogs (from 1

to 3 years old) assigned to Group A, B and C on the basis of surgical defects of variable size to be inflicted *i.e.* 3×3, 4×4 and 5×5 sq cm, respectively. They were maintained at the research facilities of Surgery Section, Department of Clinical Medicine and Surgery, University of Veterinary & Animal Sciences, Lahore, 10 days prior to the initiation of the project for acclimatization. All the animals were thoroughly examined physically and routine clinical diagnosis was carried out in order to detect any abnormality. Prophylactically, all the animals were sprayed with Fipronil (Frontline® spray, Merial France Pvt. Ltd.) for external parasites and vaccinated against rabies (Rabisin®, Merial France Pvt. Ltd.) prior to the study.

Patient preparation and anesthesia: All the subjects were kept off feed 6 hrs prior to the surgery. The site selected for operation *i.e.* middle radial area (forearm) of each of the dogs was surgically prepared. Surgical operation was carried out in lateral recumbency under preanesthetic dose of xylazine hydrochloride (Xylaz®, Farvet Pvt. Ltd.) at dose rate of 0.1mg/kg IM with the induction being achieved through the IV solution of Sodium Pentothal (Pentothal Sodium®, Abbott Laboratories, Pakistan Ltd.) at the dose rate of 15mg/kg (Muhammad *et al.*, 2009).

Wound preparation for grafting: In the animals of group A, B and C, after induction of anesthesia, a full thickness skin segment measuring 3×3, 4×4 and 5×5 sq cm, respectively was excised from lateral aspect of mid radial area of forelimbs (Fig. 1) slowly and meticulously avoiding the muscular damage. The wounds/defects thus produced were left as such for four days to allow granulation tissue to develop (Fig. 2). Meanwhile, daily dressing was maintained with sterile gauze and cotton bandage using pyodine. On 4th day, after clinical assessment and confirmation of granulation tissue on the limbs, a complete procedure of harvesting and grafting was accomplished for which the wounds were debrided to remove excess of granulation tissue and made afresh for acceptance of graft (Shahar, 2001; Adams and Ramsey, 2005).

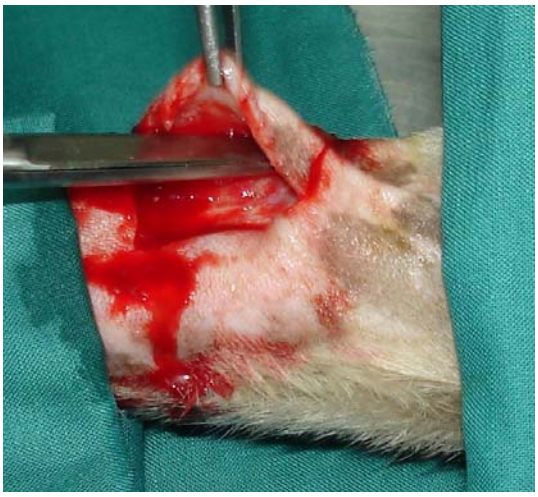

Fig 1: Excision of full thickness skin segment from middle radial area of the dog.

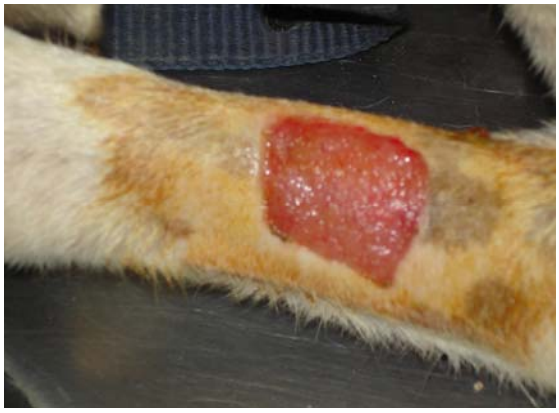

Fig 2: Healthy granulation tissue at the surgically created site in a dog.

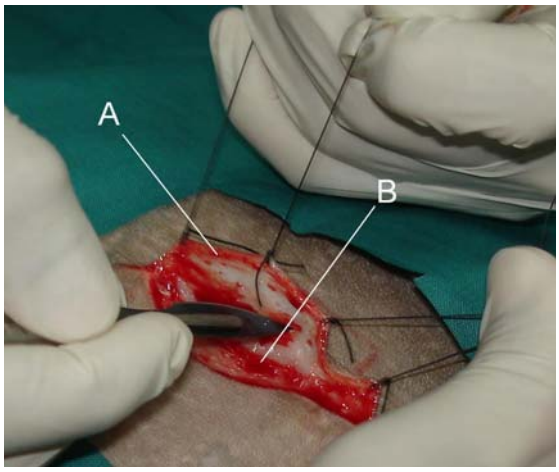

Fig 3: Traction sutures applied in detached portion of skin in order to ease split thickness harvesting procedure through the remainder of the graft; A: Graft; B: Underlying remaining dermis.

Fig 4: Interrupted sutures applied after placement of the split thickness graft on wound bed.

Harvesting of graft: From the left mid thorax of same dog, a split thickness skin graft was harvested meticulously. A partial thickness skin incision *i.e.* not extending through all layers of skin was made on the planned donor area with a scalpel held perpendicular to the skin surface and the angle of blade was changed placing it parallel to skin once desired depth of incision

Table 1: Graft survival rates in dogs implanted with split thickness autogenous skin transplantation

Group	A							B					C		
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Results	+	-	+	+	±	+	+	±	-	±	±	+	±	-	±
Size of defect (sq cm)			3×3					4×4					5×5		
Survival rate			80%					80%					80%		

+ Complete healing ± Partially damaged - Complete Failure

was reached. When a 3-4mm partial thickness of skin portion was detached, two to four traction sutures were placed in detached portion of skin to apply traction on it in order to ease split thickness harvesting procedure through the remainder of the graft (Fig. 3) as described by Corr (2009).

Placement of graft: After complete harvesting of the graft from lateral thorax, it was kept moist with normal saline swabs and placed immediately on recipient bed securing it with several interrupted sutures 3-4mm apart (Fig. 4). After routine dressing, the site was wrapped with a cotton bandage followed by a layer of adhesive plaster. To avoid self mutilation by the animals, an Elizabethan collar was applied and a taste deterrent Bitter Apple® (Grannick's Bitter Apple Co. CT, USA) was sprayed over the bandage.

Post operative (PO) care and assessment of graft viability: The graft was bandaged up to 10 day PO with a total of four bandages applied on day 0, 3, 6 and 9 (Mathes *et al.,* 2010). The sutures were removed 10 days post grafting and dogs were kept for another three weeks for assessment of graft viability. The graft indicating satisfactory vascularization evidenced by color was considered as 'take' and then all 'takes' were put to subjective and objective evaluation as documented by Böttcher *et al.* (2009) and Nguyen *et al.* (2010).

Subjective assessment tests included:
Skin color test: Graft was pressed and released by a finger to observe the speed of color return against normal skin.
Dermal bleeding test: A 3mm incision was made in dermis and bleeding quality was observed.
Hair growth test: Hair growth was visually assessed 21 days post grafting.
Pain sensation test: Needle prick was used to assess the pain sensation.

Objective assessment tests included:
Saline Wheal Test: An intra dermal injection of 0.2ml of 0.8% saline solution was given and absorption was compared with that on another healthy area of the body.
Atropine Absorption Test: An intra-dermal injection of 0.2ml of 10% atropine sulphate (Brookes Pharma Labs Pakistan Ltd) was given and its absorption into general circulation was judged by tachycardia, dryness of mouth and papillary dilatation.

RESULTS AND DISCUSSION

Graft survival rates: The survival rates of different sizes of grafts in groups A, B and C are presented in Table 1. The dog No. 5 in group A, 8 and 10 in group B, and 11, 13 and 15 in group C showed partial failure whereas the dogs No. 2, 9 and 14 in groups A, B and C, respectively showed complete failure of grafts. The remaining dogs exhibited a firm attachment of the graft at recipient site and complete success.

In the dogs with complete success, the 1st bandage change on third day revealed an edematous graft as expected to a normal tissue response (Bowler *et al.,* 2001). The STAS graft is devoid of blood vessels when harvested from donor site and applied on the wound. The transudate is produced on the recipient bed in such circumstances and is absorbed by plasmatic imbibition and ultimately leads to edema (Michelle and Tristan, 2008). At the 2nd bandage change on sixth day, the grafts were bluish black in color. The healing process associated with random vascular anastomosis of vessels with veins rather than with arteries explains this bluish black tinge as reported by Jennifer *et al.* (2006). At the 3rd bandage change on ninth day, the graft was seen to be revascularized by ingrowth of new vessels from the bed into the graft. Newly formed vessels are dilated for some time and later on mature as the differentiation process continues until a new system of vessel network is developed (Ercan *et al.,* 2005; Robert *et al.,* 2005). The firm anchorage of graft to the recipient bed indicated graft survival as documented by other reports (Rhonda and Smeak, 2003; Adams and Ramsey, 2005; Bigham *et al.,* 2011). In a nutshell, the animals showing complete graft success had followed a routine normal pattern of wound healing without any untoward consequence. They developed complete attachments with the subcutaneous tissues and could be moved just like normal skin.

Contrarily, in animals showing partial failure of the graft, the 1st bandage change showed normal pattern as in those with successful grafts. However, the graft did not assume the bluish black color consistent with the former successful grafts at 2nd bandage. The graft was rather pale with seroma formation between it and its bed and hence could not establish a firm attachment with the recipient bed. This was attributed to faulty loose bandage. Though, the serum was drained through an incision however, a partial failure was observed in graft survival. This is in consistent with the work previously documented (Adams and Ramsey, 2005; Owen and Hakim, 2009; Hodge *et al.,* 2011). Similarly, in animals showing complete failure of the graft, a differing pattern from that in successful grafts was seen in different days of bandage change. In general, these dogs were stray animals and not in habit of being tied up. The struggle to escape, barking, tearing off the bandage, exhaustion and self mutilation were some of the reasons which led to a torn away graft with complete failure. Various restraining techniques such as Elizabethan collar, muzzle or taste deterrent were of little value in these behavior stricken dogs. Bandage was completely torn off and graft was detached from its bed. It was left as such to heal by second intention. Hence, the failures, either partial or complete, were related to self mutilation

owing to the dog's temperament and improper bandaging but not to the healing potential of graft with the bed. The successful grafts were considered 'take' and all 'takes' were subjectively and objectively evaluated.

Subjective assessment tests: The skin color test revealed the skin color from light pinkish to pinkish red whereas the dermal bleeding test showed light red to cherry red. The growth of hair was not promising enough and found to be from scarce to mild. Similarly, all the successful grafts showed a poor pain response. These results are in line with those reported by Rhonda and Smeak (2003) and Adams and Ramsey (2005).

Objective assessment tests: The saline wheal test did not show marked difference between the graft and control area regarding absorption time of the solution. Similarly, a marked increase in heart beats per minute and pulse rate, dryness of oral mucosa and papillary dilatation were observed after intra dermal injection of 0.2ml of atropine sulphate in the graft which clearly indicated the survival and vascularization of graft on its bed. Adams and Ramsey (2005) and Bachmann *et al*. (2008), have reported similar findings earlier.

Conclusions: The results of the study indicate no clinical difference in the survival rate of three different sizes of split thickness autogenous grafts implanted. Hence, they can easily be used in canine distal limb reconstructive surgeries. The failures encountered in all three groups were related to self mutilation and improper bandaging but not to the healing potential of the grafts. The study will plausibly be helpful for pet practitioners in Pakistan in dealing with the canine distal limb mutilations.

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