

Original Research Article

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Effect of Activated Autologous Platelet Rich Plasma (PRP) Gel on Teat Wound in Cows

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ABSTRACT

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A total of 6 cows with teat wounds involving teat cistern were subjected for the present study. All the cows were subjected for preoperative evaluation. Under sedation with Inj. Xylazine @ 0.1 mg/kg B.wt. I/V and ring block with 2% Lignocaine hydrochloride, the muscosal and muscular layers were sutured by continuous suture pattern using 3-0 polyglactin 910. Freshly prepared and activated autologous platelet rich plasma (PRP) gel was applied to the sutured muscular layer. The skin was opposed by applying disposable stainless steel skin staples, followed by the application of activated autologous PRP gel to the apposed skin. Postoperative evaluation was carried on 5th day with second application of activated autologous PRP gel to the surgical site and morphological and ultrasonographical evaluation of healing was carried out on 10th postoperative day. PRP favoured early uncomplicated healing in teat wounds.

Introduction

Teats in cows were prone for injury due to their anatomical location, increase in size of the udder and teat during lactation, faulty milking practices, suckling calves, accidental stamping of the teat etc. (Tiway *et al.*, 2005). The common etiological factors for teat injury are thorn, barbed wires and treading (Premsairam *et al.*, 2018). Teat injuries are

classified as full thickness with exposed teat cistern and partial thickness (Azizi *et al.*, 2007). All teat wounds should be considered as contaminated and carry risk of mastitis due to nature of trauma (Mulon *et al.*, 2016). Management of teat injuries requires surgery and certainly of major type. Restoration of teat lumen following teat surgery is still challenging. The prognosis of teat lacerations is guarded with high incidence of wound

dehiscence and fistula formation (Nichols *et al.*, 2007). To promote an early wound healing biomaterials plays an important role in reconstructive surgery. The present study was carried out for the management of external teat wounds in cows with the application of activated autologous platelet rich plasma gel to favour early wound healing in teats to prevent mastitis of the affected teat.

Materials and Methods

A total of six cows diagnosed with teat wounds were presented at Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry during April to September 2019 were selected for the present study.

The animal particulars and history regarding age of the wound, etiological factors were recorded. Clinical examination on the day of presentation included were teat morphometry, characteristics of the teat wound.

Bacteriological examination of the wound and milk was carried out. The milk from the affected teat was collected and studied for its colour, consistency, pH, California mastitis test and somatic cell count. Milkability (table 3) assessed on the day of presentation and 10th postoperative day.

Preparation of autologous platelet rich plasma

Autologous PRP was prepared from the whole blood of the affected animal, 10 ml of blood was collected in EDTA coated syringe and centrifuged at 1000 rpm for 10 minutes and the supernatant was collected in a sterile vial and again centrifuged at 800 rpm for 10 minutes. The plate rich plasma was collected in a sterile vial and activated with 10%

calcium chloride (3-4mg/ml) and allowed to form gel (Cavallo *et al.*, 2016).

Anesthesia and Surgical procedure

The animals were sedated with Inj. Xylazine (Xylaxin, Indian Immunologicals) @ 0.1mg/kg administered intravenously and positioned on lateral recumbency. The surgical site was prepared aseptically. Local analgesia was achieved by ring block using Inj. 2 % lignocaine hydrochloride solution (Themicaïne 2%, Themis Medicare). The wounds were debrided and irrigated with 0.5% povidone iodine solution diluted in normal saline. The mucosal and the muscular layers were sutured separately in simple continuous pattern with polyglactin 910 (No. 3-0). Activated autologous PRP gel was applied to sutured muscular layer (Fig.4). The skin was closed by disposable skin staples, (Acos, Sunmedix) (Fig.3) in all the animals and PRP gel was applied to the surgical site. Postoperatively the surgical site was protected with adhesive bandage (Dynafix).

A sterile prosthetic tube (No.10- Romsons Scientific and Surgicals India) was placed into the teat lumen and fixed in-situ and was connected to a 2ml disposable syringe which was used to drain the milk and administration of antibiotics.

Postoperatively Inj. streptopenicillin at the dose rate of 10mg/kg body weight was administered intramuscularly for 5 days. Systemic and intramammary administration of antibiotics based on the antibiotic sensitivity test was followed from 5th day onwards. On 5th postoperative day, second application of the freshly prepared activated autologous platelet rich plasma gel was applied on the surgical site and protected. Skin staples were removed on 10th postoperative day. The teat wound healing was assessed by morphology and ultrasonography.

Results and Discussion

In the present study all the animals were cross bred jersey cows and aged between 3 to 8 years. Body weight ranged between 260-317 kg and five animals (83%) were kept on muddy floor and one (17%) was kept on concrete floor (Table.1). Occurrence of injuries on the udder were predisposed by lack of proper bedding and non-concrete floor (Aleri *et al.*, 2011). Five animals (83%) were left for grazing and one (17%) was stall fed. All the animals were in the first stage of lactation (Molaei and Ebrahimi, 2013). The age of the wound ranged between 1-8 days. The etiological factors were thorn injury in four cows (66%), treading and barbed wires in one cow each (17%) (Table.1). In the present study, most animals were maintained in grazing, they were more prone for injuries caused by thorns and barbed wires. Treading is the common cause for teat injury in stall fed cows due to over crowdedness (Nicholas *et al.*, 2016 and Premsairam *et al.*, 2018).

Single teat was affected in all the animals. Right fore teat was found to be affected in four cows (66%) and right hind teat in two cows (34%). Teat laceration in the fore teats were higher than that of hind teats and wounds were often caused when the animals are in grazing, tie stall or free stall barns (Nichols *et al.*, 2016). The affected teats were funnel shaped and cylindrical in three cows each (50%). The length (cm) of the affected teat ranged between 5 to 11 cm, longer teats are prone for injuries (Grommers *et al.*, 1971). The characteristics of the wound were duration of the wound, was more than 12 hours (chronic) in five cows (83%) and less than 12 hours (acute) in one cow (17%) (Nicholas *et al.*, 2008). Wound involving fulllength (base to teat tip) (Fig.1) in three cows (50%), base of the teat, mid teat and teat tip in one cow each (50%) were noticed. The direction of the wound was circumferential in

one cow (17%) and vertical in five cows (83%). Vertical wounds were predominant and could be due to the anatomical position of the teat (Premsairam *et al.*, 2018). In all six cows, all the layers of teat wall was involved viz; skin, muscular and muscular layers. Full thickness teat wound could be due to etiological factors like sharp thorn and barbed wires (Nichols *et al.*, 2016) (Table.2).

The organism identified from the teat wounds on bacteriological examination were *Staphylococci* in three cows, *Escherichia coli* in two cows and *Pseudomonas* in one cow. Isolates identified on bacteriological examination of the milk samples was *Staphylococcus*, sensitive for ceftriaxone, amikacin, enrofloxacin and gentamicin and resistant to ciprofloxacin, penicillin, amoxicillin-clavunate and tetracycline in the order of sensitivity.

Ultrasonographical examination revealed hypoechogenicity and loss of normal echotexture of the skin, muscular and mucosal layers with fistula at the base to tip in three cows (Fig.2), at base of the teat in one cow, at mid teat and teat tip in one cow each were noticed. Ultrasonography is a noninvasive technique that can be used for examining the bovine udder and teat to diagnose the pathological alterations such as congenital changes, inflammation, mucosal lesions, tissue proliferation, foreign bodies, milk stones, haematoma and abscess (Szencziová, and Strapák 2012).

The main advantages of PRP were its cost effectiveness, autologous nature, non invasive collection process and rapid preparation Iacopetti *et al.*, (2011).

The anaesthetic protocol by sedation with Inj. Xylazine @ 0.1mg/kg body weight and 2% of Inj. Lignocaine Hydrochloride injected at the base of the teat in ring fashion was found

satisfactory (Balagopalan *et al.*, 2016). The wound was debrided and irrigated with 0.5% povidone iodine solution (Aruljothi *et al.*, 2012). Three layer suturing was followed in this study was effective in complete closure of the teat cistern (Balagopalan *et al.*, 2016) (Fig.3). Application of PRP gel showed good adherence to the wound and found non-reactive to the surrounding tissue (Iacopetti *et al.*, 2011).

Closure of skin by disposable skin staples. Skin staples were found to be inert, with less tissue reactive, better tissue holding capacity and better tensile strength (Premsairam *et al.*, 2018). It is very useful in teat wound healing to favour early healing without any wound dehiscence.

Based on antibiotic sensitivity test, Inj. ceftriaxone @ 10 mg/kg body weight intramuscular and 500 mg intramammary, Inj. Enrofloxacin @ 5 mg/kg body weight intramuscular and 250 mg intramammary administered found to be effective in preventing mastitis..

On 5th day, the surgical site was clean in all animals (100%), no discharge and wound dehiscence noticed, with dry surgical site which indicated the absence of wound infection which is due to the antimicrobial effect of autologous platelet rich plasma which reduced the infection at the wound site and promoted early healing by release of growth factors (Burnouf *et al.*, 2013). Intact staples in all the animals.

On 10th day, three animals (50%) showed complete scarless healing and apparently

100% epithelialization (Fig.6). Two animals (33%) showed thin scar with focal incomplete epithelialization and one (17%) had incomplete healing with exposed suture knot without fistula (Table.4).

On ultrasonographical evaluation, all six cases (100%) shown bright echoic line of intact teat skin indicative of intact skin with normal echotexture. The muscular layer appeared as hyperechoic with anechoic cavities of blood vessels indicative of complete healing without discontinuity of skin and teat wall and anechogenicity of teat cistern indicate presence of milk. Intact mucosal layer appeared as thin bright line (Franz *et al.*, 2009, Balagopalan and Aruljothi, 2016; Mulon, 2016) (Table.4). The complete closure of the wound was noticed in all six cases which was due to the platelet concentrates that contains fibrin, fibronectin and vitronectin which acted as cell adhesion molecules. PRP gel applied wounds showed faster and complete healing without scar (Marx *et al.*, 2004). Platelets have shown increased healing by promoting endothelial growth factor (EGF), which contributed for earlier and faster epithelialization with less pain and less scar tissue formation in most of the cases (Lee *et al.*, 2008).

Application of activated autologous platelet rich plasma gel was found to be effective in healing of all types of teat wounds with complete early healing without scar tissue without affecting the quality of milk and milkability postoperatively. The skin staples also favoured healing without complication by providing good tissue holding capacity, reduced dressing and meagre tissue reaction.

Table.1 Animal particulars (n=6)

Animal No.	Breed	Age (years)	Body weight (kg)	Floor type	Feeding pattern	Stage of lactation	Etiology	Teat affected	Shape	Length (cm)
IA	CBJ	5	305	Muddy	Grazing	1	Thorn	RFT	Funnel	6
IB	CBJ	8	276	Muddy	Grazing	1	Thorn	RFT	Cylindrical	11
IC	CBJ	5	290	Muddy	Grazing	1	Thorn	RFT	Cylindrical	5
ID	CBJ	4	260	Muddy	Grazing	1	Treading	RHT	Funnel	9
IE	CBJ	5	302	Muddy	Grazing	1	Thorn	RFT	Cylindrical	6
IF	CBJ	6	317	Concrete	Stall fed	1	Treading	RFT	Funnel	10

Table.2 Characteristics of wound on day of presentation

Animal No.	Duration of injury (<12/>12hrs)	Location	Direction	Involvement of teat wall layers	Colour	Nature of wound edges	Nature of exudate	foreign body
IA	>12	Base to Tip	Vertical	All layers	Red	Defined	No	Nil
IB	<12	Base	Circumferential	All layers	Pale pink	Undefined	Sero-sanguineous	Nil
IC	>12	Tip	Vertical	All layers	white	Fibrotic	No	Nil
ID	>12	Base to Tip	Vertical	All layers	Red	Loosely adhered	Sero-sanguineous	Nil
IE	>12	Base to Tip	Vertical	All layers	black brown	Undefined	Sero-sanguineous	Debris
IF	>12	Mid	Vertical	All layers	Pale pink	Firmly adhered	No	Nil

Table.3 Qualitative examination of milk

Sl.No	Parameters	Quality of milk	
		Day of presentation	10 th postoperative day
1	Colour	Normal	Normal
2	Consistency	Normal	Normal
3	pH (Mean)	6.91	6.83
4	California mastitis test	Positive (IC,IF)	Negative
5	Somatic cell count (10 ³ /ml)	309	311
6	Milkability (kg/quarter) (Mean±S.E.)	Dribbling of milk	1.33±0.29

Table.4 Postoperative evaluation

10 th postoperative day		
4	Complete Scar less healing	3
5	Healing with scar	2 (Thin scar)
6	Incomplete epithelialization with exposed suture	1
7	Ultrasonographical Examination (Intact skin with normal echotexture of muscular layer with anechoic cavities of blood vessels)	100%
8	Milkability	Increased
9	Quality of milk	No significant change in color, consistency, pH, CMT and SCC

Fig.1 Full length wound involving all layers of teat wall (IE)



Fig.2 Discontinuity of skin and lack of normal echo texture of the muscular and mucosal layers with anechoic fistulous tract at base (IB).



Fig.3 Closure of mucosal and muscular layers with 3-0 polyglactin 910 by continuous suture pattern



Fig.4 Application of activated autologous PRP gel on sutured muscular layer



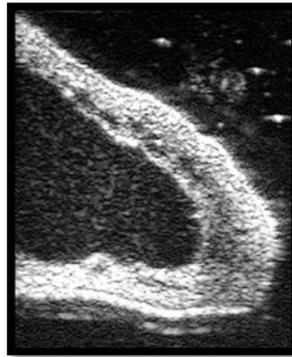
Fig.5 Skin apposed with disposable skin staples (Acos, Sunmedix)



Fig.6 10th Postoperative day - complete scarless healing with 100% epithelialization (ID).



Fig.7 Bright echoic line of intact skin and hyperechoic muscular and mucosal layers containing anechoic cavities of blood vessels indicative of healing (IA).



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