

A Novel Approach In Treating Teat Wound Using Autologous Peripheral Blood Mononuclear Cells (PBMC) In A Six Cows – A Clinical Study

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Abstract

A total of six cows with teat wounds with fistula or intact teat cistern were subjected to the present study. After clinical examination, all the animals were sedated with inj. xylazine @ 0.1 mg/kg b.wt. I/V and ring block with 2% Lignocaine hydrochloride, the wound edges were debrided and irrigated with 0.5% povidone-iodine diluted with normal saline. The patency of the teat canal was maintained by placing infant feeding tube size No 10 in-situ. A three-layer suturing pattern was followed. The mucosal layer and muscle layer were closed using simple continuous suture pattern with polyglactin 910 size 3-0. The skin was apposed using disposable stainless steel skin staples. Whole blood was collected and processed to extract Peripheral Blood Mononuclear Cells (PBMC) which were injected subcutaneously around the surgical site. Therapeutic phonophoresis @ 1watt/cm² was performed for 5 minutes around the peri-wound area on the day of surgery and the 10th postoperative day. All the animals were administered with intramuscular Inj. Streptopenicillin @ 10mg/kg b. wt and inj. Meloxicam @ 0.5 mg/kg b. wt, postoperatively. Inj. Metrinidazole 10ml with 5 ml of Inj. Gentamicin was given as an intramammary infusion for 5 days. Postoperatively the surgical site was protected with an adhesive bandage (Dynafix). Wound dressing was done on the 7th and staples were removed on the 10th postoperative day. Morphological and Ultrasonographic evaluation of healing was carried out on the 10th postoperative day. PBMC favoured early uncomplicated scarless wound healing which can prevent mastitis in milking cows, and well accepted by the animals.

Keywords: Peripheral Blood Mononuclear Cells, Teat wound

Introduction

Teats in cows were prone to injury due to their anatomical location, increase in the size of the udder and teat during lactation, faulty milking practices, suckling calves, accidental stamping of the teat, etc. (Tiwary *et al.*, 2005). The common etiological factors for teat injury are thorns, barbed wires, and treading (Premsairam *et al.*, 2018). Teat injuries are classified as the full thickness with exposed teat cistern and partial thickness (Nicholas, 2008). All teat wounds should be considered contaminated and carry the risk of mastitis due to the nature of the trauma (Aruljothi and Balagopalan, 2018). Management of teat injuries requires surgery and certainly of major type. Restoration of the teat lumen following teat surgery is still challenging. Early treatment of teat laceration is required to prevent infection and subsequent economic losses to the farmer. The prognosis of teat lacerations is guarded with a high incidence of wound dehiscence and fistula formation (Nicholas and Anderson, 2007). To promote early wound healing biomaterials, play an important role in reconstructive surgery (Aruljothi and Balagopalan, 2018). Peripheral Blood Mononuclear Cells (PBMC) release cytokines like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and interleukin IL-10; therefore, PBMC influence fibroblast activities in the remodelling phase of the healing process (Nami *et al.*, 2016). The present study was carried out for the management of external teat wounds in cows with the application of PBMC 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 the Department of Veterinary Surgery and Radiology, Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry from March to September 2022 were selected for the present study. The animal particulars and history regarding the age of the wound, and etiological factors were recorded (**Table 1**). Clinical examination on the day of the presentation included teat morphometry, and characteristics of the teat wound (**Table 2**). A bacteriological examination of the wound and milk was carried out. The milk from the affected teat was collected and studied for its color, consistency, pH, California Mastitis Test (CMT) and Somatic Cell Count (SCC). Milkability was assessed on the day of presentation and the 10th postoperative day (**Table 3**).

Table 1: Animal particulars (n= 6)

Animal no	Breed	Age	Body weight	Floor type	Feeding pattern	Stage of lactation	Etiology	Teat affected	Shape	length (cm)
1	CBJ	6	353	Muddy	Stall fed	1	Barbed wire	RHT	Cylindrical	5
2	CBJ	9	270	Muddy	Grazing	2	Thorn	RFT	Cylindrical	7
3	CBJ	7	288	Concrete	Grazing	1	Barbed wire	RFT	Cylindrical	6
4	CBJ	5	303	Muddy	Grazing	1	Barbed wire	RHT	Cylindrical	6
5	CBJ	1	342	Muddy	Grazing	1	Thorn	LFT	Cylindrical	7
6	CBJ	8	312	Concrete	Grazing	2	Thorn	LHT	Cylindrical	8

Ultrasonographical examination of the affected teat was performed by B-mode diagnostic ultrasound scanner using a 7.5 MHZ linear probe by water bath method. The teat was dipped in a thin transparent polyethylene cup filled with water. The transducer was placed in vertical/horizontal planes on the outer wall of the cup. Nature of the lesion and other pathological structural changes if any were documented on day one and 10th post-treatment day.

Preparation of Autologous Peripheral Blood Mononuclear Cells (PBMC)

To isolate Peripheral Blood Mononuclear Cells (PBMC), blood samples were collected in EDTA tubes. PBMCs were isolated from blood by density gradient centrifugation using HiSep™ LSM 1077 (Himedia, Mumbai, India). The obtained PBMCs were washed with phosphate-buffered saline (Himedia, Mumbai, India) and counted using trypan blue dye using a haemocytometer (Bhattacharjee *et al.*, 2017 and Vanaki *et al.*, 2018).

Table 2: Characteristics of wound on day of presentation

Animal no	Duration of injury (<12 / >12 hrs)	Location (full length)	Direction	Involvement of teat wall layers	Colour	Nature of wound edges	Nature of exudate	Foreign body
1	<12	Base to Tip	Vertical	All layers	Red	Defined	No	Nil
2	>12	Base to Tip	Vertical	Intact teat cistern	Red	Loosely adhered	Sero-sanguineous	Debris
3	>12	Base to Tip	Vertical	Intact teat cistern	Pale pink	Undefined	No	Nil
4	>12	Base to Tip	Circumferential	Intact teat cistern	Pale pink	Defined	No	Nil
5	>12	Base to Tip	Vertical	All layers	White	Loosely adhered	Sero-sanguineous	Debris
6	>12	Base to Tip	Circumferential	Intact teat cistern	Pale pink	Defined	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 (2 cases) (Grade – 1)	Negative (Grade – 0)
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

Sl. No	10 th postoperative day	
1	Complete scar healing	6
2	Healing with scar	Nil
3	Incomplete epithelisation with exposed suture	Nil
4	Ultrasonographic examination (intact skin with normal echo texture of muscular layer with anechoic cavities of blood vessels)	100%
5	Milkability	Increased
6	Quality of milk	No significant change in colour, consistency, pH, CMT and SCC

Anesthesia and Surgical procedure

The animals were sedated with Inj. Xylazine (Xylaxin, Indian Immunologicals) @ 0.1mg/kg was 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) (**Fig 1**). A Teat siphon was inserted to maintain the teat patency. The wounds were debrided and irrigated with 0.5% povidone-iodine solution diluted in normal saline (**Fig 2**). The three-layer suturing pattern was followed, and the mucosal layer and muscle layer were closed using a simple continuous suture pattern with polyglactin 910 size 3-0 (**Fig 3**). Autologous PBMC was injected subcutaneously around the surgical site (**Fig 4**) and therapeutic phonophoresis @ 1 watt/cm² was performed for 5 minutes around the surgical site. The skin was closed by disposable skin staples, (Acos, Sunmedix) (**Fig 5**) in all the animals. Postoperatively the surgical site was protected with an adhesive bandage (Dynafix) (**Fig 6**). A sterile infant feeding tube size No. 10 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 @ 10mg/kg b. wt and inj. Meloxicam @ 0.5 mg/kg b. wt was

administered intramuscularly for 5 and 3 days, respectively. Inj. Metrinidazole 10ml with 5 ml of Inj. Gentamicin was given as an intra-mammary infusion for 5 days. On the 7th postoperative day, therapeutic ultrasound was given @ 1Watt/cm² for 5 mins around the surgical site. Skin staples were removed on the 10th postoperative day. The teat wound healing was assessed by morphology and ultrasonography by water bath method using 7.5 MHZ linear probe.

Results and Discussion

In the present study, all the animals were cross-bred jersey cows aged between 1 to 9 years. Body weight ranged between 270-353 kg and four animals (66.66%) were kept on a muddy floor and two (33.33%) were kept on a concrete floor (**Table 1**). Occurrences of injuries on the udder were predisposed by a lack of proper bedding and a non-concrete floor (Gurunathan *et al.*, 2021). Five animals (83%) were left for grazing and one (17%) was stall-fed. The age of the wound ranged between 1-3 days. The etiological factors were thorn injury in three cows (50%) and barbed wire in three cows (50%) (**Table.1**). In the present study, most animals were maintained in grazing, they were more prone to injuries caused by thorns and barbed wires (Aruljothi *et al.*, 2012). Treading is the common cause of teat injury in stall-fed cows due to overcrowdedness (Gurunathan *et al.*, 2021, Premsairam *et al.*, 2018 and Nicholas *et al.*, 2016). Single teat was affected in all the animals. Right fore teat was found to be affected in two cows (33.33%), right hind teat in two cows (33.33%), left fore teat in one cow (16.66%), and left hind teat in one cow (16.66%). Teat lacerations in the fore teats were equal to that of hind teats and wounds were often caused when the animals are in grazing, tie stall, or free stall barns (Nicholas *et al.*, 2016). The shape of the teat was cylindrical in all the animals. The length (cm) of the affected teat ranged between 5 to 8 cm, longer teats are prone to injuries. The characteristics of the wound on the day of presentation were based on duration in five cows (83%) more than 12 hours and in one cow (17%) less than 12 hours (Nicholas *et al.*, 2008). Wounds involving full length (base to teat tip) (**Fig.7**) in all the cows were noticed. The direction of the wound was circumferential in two cows (34%) and vertical in four cows (66%). Vertical wounds were predominant and could be due to the anatomical position of the teat (Premsairam *et al.*, 2018). In two cows, the teat cistern was exposed and in four cows, an intact teat cistern was noticed (**Fig 8**). Full-thickness teat wounds could be due to etiological factors like a sharp thorn and barbed wires (Aruljothi *et al.*, 2012 and Nicholas *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. On the day of presentation, ultrasonographic examination revealed hyperechogenicity and lack of normal echo texture of the skin, muscular and mucosal layers in all six animals; whereas, the presence of discontinuity indicates fistula above tip in two cows (**Fig.9**). Ultrasonography is a non-invasive 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 (Aruljothi and Balagopalan, 2021 and Szencziová, and Strapak, 2012). 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 a ring fashion was found satisfactory (Balagopalan and Aruljothi, 2016). The wound was debrided and irrigated with 0.5% povidone-iodine solution (Aruljothi *et al.*, 2012). Three-layer suturing followed in this study was effective in the complete closure of the teat cistern (Balagopalan *et al.*, 2016). Autologous PBMC was injected subcutaneously around the surgical site in all six animals. Application of PBMC showed good adherence to the wound and was found nonreactive to the surrounding tissue. PBMC induces cell proliferation, migration, and production of proteases in epithelial cells, fibroblasts, and monocytes which in turn regulates angiogenesis, wound healing, and tissue remodelling (Nami *et al.*, 2016). The main advantages of PBMC were its cost-effectiveness, autologous nature, non-invasive collection process, and rapid preparation (Bhattacharjee *et al.*, 2017 and Vanaki *et al.*, 2018). Therapeutic phonophoresis @ 1 watt/cm² was performed for 5 minutes around the surgical site on the day of surgery and the 10th postoperative day. Phonophoresis is a technique by which therapeutic ultrasound is used to introduce pharmacologic agents and able to kill bacteria by activating the sonosensitizers to produce reactive oxygen species, which are reported to be toxic to microbes during wound healing (Fares *et al.*, 2017). Closure of skin was achieved 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 and Gurunathan *et al.*, 2021). It is very useful in teat wound healing to favour early healing without any wound dehiscence. On the 10th postoperative day, the surgical staples were removed in all the animals, and no discharge and wound dehiscence were noticed indicating the absence of wound infection which is due to the antimicrobial effect of autologous PBMC which reduced the infection at the wound site and promoted early healing by the release of growth factors (Burnouf *et al.*, 2013) (**Fig 10**). An ultrasonographic evaluation revealed heperechogenecity of the teat wall indicating normal echotexture of the skin and confirmed wound healing (**Fig 11**). Early wound healing in teat wound is very essential to prevent mastitis in cows. Infiltration of autologous PBMC enhanced early healing of the suture site and prevents complications by

hastening the healing without infection and not affecting the quality of milk and milkability postoperatively. Autologous PBMC can be easily prepared and well accepted by animals. Phonophoresis favours better drug penetration at the wound site. The skin staples also favoured healing without complication by providing good tissue holding capacity, reduced dressing, and meagre tissue reaction and easy to remove.

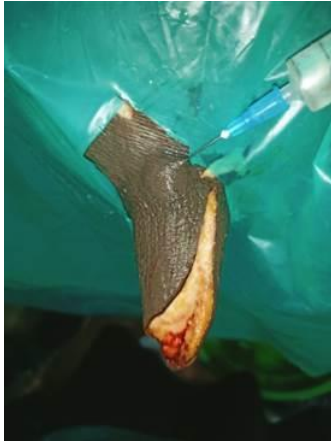


Fig 1: Local analgesia was achieved by ring block using Inj. 2 % lignocaine hydrochloride solution



Fig 2: The wounds were debrided and irrigated with 0.5% povidone-iodine solution diluted in normal saline



Fig 3: Three-layer suturing pattern was followed, the mucosal layer and muscle layer were closed using a simple continuous suture pattern with polyglactin 910 size 3-0



Fig 4: Autologous PBMC was injected subcutaneously around the surgical site



Fig 5: The skin was closed by disposable skin staples



Fig 6: Postoperatively, the surgical site was protected with adhesive bandage (Dynafix)



Fig 7: Wound involving full length (base to teat tip)



Fig 8: Exposed teat cistern



Fig 9: On the day of presentation, ultrasonographic examination revealed hyperechogenicity and lack of normal echo texture of the skin, muscular and mucosal layers.



Fig 10: On 10th postoperative day, the surgical site was assessed in all animals, no discharge and wound dehiscence



Fig 11: On 10th postoperative day, ultrasonographic evaluation revealed hyperechogenicity of the teat wall indicating normal echotexture of the skin confirmed wound healing

Conclusion

The use of autologous PBMC is a pilot study and a novel method followed in the treatment of teat wound healing in milking cows to favour early scarless wound healing. Uncomplicated wound healing prevents mastitis which is a common problem encountered in the dairy industry.

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Contribution by Authors

Equal contribution

Conflict of Interests

There is no conflict of interest.

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