SHORT COMMUNICATION

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Ozonized autohemotherapy, a novel treatment for teat laceration with fistula in cows: a clinical study

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The present study evaluated the effect of ozonized autohemotherapy (O3-AHT) on early, scarless teat wound healing in cows. A total of six lactating cows presented with teat wounds associated with fistula formation were included. Following thorough clinical examination, all animals were sedated with inj. xylazine (0.1 mg/kg body weight, i.v.), and a ring block was performed using 2% lignocaine hydrochloride. Patency of the teat canal was maintained by placing a size No. 10 infant feeding tube in situ. Under aseptic conditions, the mucosal and muscular layers were closed using a simple continuous suture pattern with polyglactin 910 (size 3-0). Subsequently, 2 mL of whole blood collected from each animal was subjected to ozonation and administered subcutaneously at the surgical site. Skin closure was achieved using disposable stainless steel staples, followed by protection with an adhesive bandage (Dynafix). Postoperatively, all animals received antibiotics, anti-inflammatory therapy, and intramammary treatment. Skin staples were removed on the 7thpostoperative day, and wound healing was assessed through morphological and ultrasonographic evaluation. The findings suggest that O3-AHT is a promising complementary therapy to promote early tissue repair and regeneration in bovine teat injuries.

Keywords: Cows, Ozonated autohemotherapy (O3-AHT), Teat fistula, Teat laceration

Teat lacerations associated with fistula formation are commonly encountered under field conditions. Early and timely intervention is crucial for restoring normal milk production and preventing mastitis. However, achieving early and scarless wound healing remains a significant challenge in field practice. Although various biomaterials have been employed for the management of teat wounds, optimal healing outcomes are still difficult to achieve.

Ozonated autohemotherapy is an emerging therapeutic modality in human medicine. Ozone (O_3) is a triatomic form of oxygen with a cyclic structure that has been reported to possess antioxidant and disinfectant properties and the potential to enhance the healing of chronic wounds in humans (Martinez *et al.,* 2005). Autohemotherapy has been shown to activate the mononuclear phagocytic system and exert immunomodulatory effects (Borges *et al*., 2014). Ozonized autohemotherapy (O3-AHT) is a novel complementary therapeutic approach that has the potential to promote early and scarless wound healing (Bambo *et al.,* 2012).

The present study evaluated the effect of ozonized autohemotherapy on early, scarless teat wound healing in cows.

In the present study, a total of six cows diagnosed with deep, extensive lacerated teat wounds, between March and August 2024, were included. On the day of presentation, detailed information including animal particulars, wound age, etiological factors, wound type, teat morphometry, and wound characteristics was recorded for all cases. Bacteriological examination of both wound swabs and milk samples was performed. Milk from the affected teat was evaluated for colour, consistency, pH, California Mastitis Test (CMT), and somatic cell count (SCC). Milkability was assessed on the day of presentation and on the 7thpostoperative day. Ultrasonographic examination of the affected teat was carried out using a 7.5 MHz linear transducer with a water bath technique on the day of presentation.

Whole blood (2 mL) was collected in an EDTA-coated 2 mL syringe. Ozone was generated using an ozone generator (Auro Zone ozonator; 2 g/hr capacity). Approximately 30 µg/mL of ozone was produced within 3–4 minutes of generation time. The freshly generated ozone was immediately mixed with the collected blood in a 5 mL syringe under aseptic conditions.The ozonized blood was then administered subcutaneously at the sutured muscular site. Postoperative evaluation was carried out on the 7thpostoperative day.

The animals were sedated with inj. xylazine (Xylaxin; Indian Immunologicals; 0.1 mg/kg body weight, i.v.) and positioned in lateral recumbency. The surgical site was prepared under strict aseptic conditions. Local analgesia was achieved by a ring block using 2% lignocaine hydrochloride solution (Themicaine 2%; Themis Medicare). A teat siphon was inserted to maintain teat patency. The wounds were debrided and irrigated with 0.5% povidone iodine solution diluted in normal saline (Fig. 1). A three-layer closure was performed; the mucosal and muscular layers were sutured using a simple continuous pattern with polyglactin 910 (size 3-0) (Fig. 2). Ozonized blood (30 µg/mL), prepared from the same animal, was

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Fig. 1: The wounds were debrided and irrigated with 0.5% povidone iodine solution.

Fig. 2: The mucosal layer and muscle layer were closed using simple continuous suture with polyglactin 910 size 3-0.

injected subcutaneously at the sutured site. Skin closure was achieved using disposable skin staples (Acos; Sunmedix) in all animals (Fig. 3). Postoperatively, the surgical site was protected with an adhesive bandage (Dynafix).

A sterile No. 10 infant feeding tube was placed within the teat lumen and secured in situ, and was connected to a 2 mL disposable syringe for milk drainage and administration of antibiotics. Postoperatively, inj. streptopenicillin (10 mg/kg body weight) and inj. meloxicam (0.5 mg/kg) were administered intramuscularly for 5 and 3 days, respectively. Additionally, 10 mL of inj. metronidazole combined with 5 mL of inj. gentamicin was administered as an intramammary infusion for 5 days. Skin staples were removed on the 7th postopera-tive day.

Fig. 3: (A) The skin was closed by disposable skin staples; (B) On 10th postoperative day, the surgical site showing no discharge and wound dehiscence.

In the present study, all animals were crossbred Jersey cows aged between 3 and 6 years, with body weights ranging from 280 to 340 kg. The occurrence of teat wounds was associated with inadequate bedding and non-concrete flooring conditions (Gurunathan *et al*., 2021). All animals (100%) were maintained under grazing systems. The age of the wounds ranged from 1 to 2 days.The etiological factors included thorn injuries in four cows (67%) and barbed wire injuries in two cows (33%) (Arul Jothi *et al.*, 2012). A single teat was affected in all cases. The fore teat was involved in four cows (67%), while the hind teat was affected in two cows (33%).

Full-thickness wounds involving all three layers (teat mucosa to skin) were observed in all cows. The direction of the wound was vertical in all cases, which may be related to the anatomical orientation of the teat and the nature of traumatic forces (Premsairam *et al.*, 2018).

Bacteriological examination of teat wound swabs revealed Staphylococci in four cows and Escherichia coli in two cows. Ultrasonographic examination on the day of presentation revealed hyperechogenicity with loss of normal echotexture of the skin, muscular, and mucosal layers in all animals. A three-layer suturing technique was employed, which was effective in achieving complete closure of the teat cistern (Balagopalan and Arul Jyothi, 2016).

Autohemotherapy combined with ozone has been reported to be more effective than autohemotherapy

alone. Medical ozone exhibits bactericidal properties, enhances phagocytic activity, increases cellular oxygen-carrying capacity at the wound site, and promotes local tissue repair (Sagai and Bocci, 2011).

Skin closure was achieved using disposable skin staples, which are inert, minimally tissue-reactive, and provide superior tissue holding capacity and tensile strength (Premsairam *et al*., 2018; Gurunathan *et al*., 2021). In the present study, healing time was reduced, and skin staples were removed on the 7thpostoperative day in all animals (Fig 4). This accelerated healing may be attributed to ozonized autohemotherapy, which promotes early tissue repair without complications such as wound dehiscence or infection (Wu *et al*., 2013), compared to conventional teat wound healing, which typically requires 10–15 days (Arul Jothi *et al*., 2012).

Ultrasonographic evaluation on the 7th postoperative day revealed restoration of normal echotexture and hyperechogenicity of the teat wall, indicating complete wound healing with minimal scar formation. A single application of this therapy reduced the need for repeated postoperative dressings and minimized animal handling and transport stress. Moreover, ozonized autohemotherapy is easy to prepare, cost-effective, and well tolerated by all animals.

It was concluded that the use of ozonated autohemotherapy promotes early, scarless wound healing and helps prevent complications such as mastitis.

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