Plate-rod Technique with Engraftment of Platelet Rich Plasma Seeded Hydroxyapatite for Fracture Management of Tibia in a Blackbuck (Antelope cervicapra)

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Abstract
An adult one year old female Black Buck, weighing about 20kg, maintained in Arignar Anna Zoological Park, Vandalur, Chennai was referred to Madras Veterinary College with a complaint of non-weight bearing lameness on the left hind limb. Physical examination and radiogram revealed comminuted fracture of left tibia with cranially displaced bone fragments. Plate-rod technique of biologic osteosynthesis was performed with adjunct therapy of engraftment of autologous platelet rich plasma seeded hydroxyapatite which provided better clinical outcome without post-operative complication and early ambulation. Plate-rod technique of biologic osteosynthesis in combination with engraftment of autologous platelet rich plasma seeded hydroxyapatite for the management of tibial fracture improved the clinical outcome in a Black buck.

Key words: Antelope, Black buck, Hydroxyapatite, Ketamine, Platelet rich plasma, Plate-Rod, Tibia, Xylazine

Introduction
Black buck is the only existing representative of genus antelope in India and is declared as an endangered species and brought under Schedule-I of Wildlife Act of India, 1947. In free-ranging ruminants among the non-infectious diseases, fracture constitutes a major reason for mortality (Mboera and Kitalyr, 1992). Tibial fracture is predominant in small ruminants among the long bone fractures (Gahlod et al., 2012). Fracture treatment in wild and free-ranging animals pose a challenge for veterinary surgeon as the
temperament, habit and habitat differ from domestic animals, post-operative care and management is difficult as repeated immobilization is not possible (George et al., 1986). Fracture of fore limb, hind limb and pelvis is relatively infrequent in antelopes (Gahlod et al., 2012). In free-ranging animals, the basic principles of fracture healing are similar to other animals; but the outcome of healing vary with site, type and age of fracture, fracture immobilization technique and factors associated with patient itself. Although numerous options are available for fracture treatment, surgical options are preferred, where various categories of plates and intramedullary pins are used and plates are combined with intramedullary rods to resist rotational forces (Ayyappan et al., 2011). As tibia is one of the major weight bearing long bones it requires a fracture immobilization technique with rigid fixation to resist rotational forces and better osteosynthesis (Andrianov et al., 2007).

Hydroxyapatite (HA) is a bone graft extender known for osteoconduction and exhibits excellent biocompatibility with soft tissues such as skin, muscle and gums; making it an ideal candidate for orthopaedic and dental implants or components of implants. Synthetic hydroxyapatite has been widely used in repair of hard tissues and its common uses include bone repair, bone augmentation, coating of implants and as fillers in bone or teeth. Similarly, autologous platelet rich plasma (PRP) has been used as a source of osteoinductive factor. A combination of autologous PRP and hydroxyapatite is expected to create a composite with both osteoconductive and osteoinductive properties (Oryan et al., 2012).

The present case report described the surgical management of midshaft comminuted fracture in an Black buck treated with plate-rod technique for biologic osteosynthesis and engraftment of autologous PRP and hydroxyapatite composite to provide osteoconduction and osteoinduction as adjunct therapy with the objective of minimizing post-operative handling and early ambulation.

**Materials and Methods**

**Chemical immobilization and preoperative planning**

An adult one year old female Black Buck, weighing about 20 kg, maintained in Arignar Anna Zoological Park, Vandallur was refereed to Madras Veterinary College with a complaint of non-weight bearing lameness on the left hind limb. The animal was chemically restrained using xylazine and ketamine at the dose rate of 1.0 and 2.5 mg/kg body weight respectively using blow gun (William et al., 2014). The eyes were blind folded and transported to the Zoo Veterinary Hospital. Physical examination and palpation of left hind limb revealed crepitation indicating midshaft fracture of left tibia with swelling at the site of fracture. Medio-lateral and cranio-caudal radiograms of left hind limb distal to stifle revealed comminuted
fracture of tibia with cranially displaced bone fragments (Unger et al., 1990) (Figure 1). Blood and serum samples were collected during initial examination for preoperative checkup.

Preoperative plan was prepared as per the small animal preoperative planning guide developed by the AO/ASIF group using plain radiograms. Preoperative medio-lateral radiogram of the contralateral limb was measured for selection of the diameter and length of the plate and the cranio-caudal view to determine the diameter and length of the screws. The weight of the Black buck was taken into consideration for plate selection (Piermattei and Flo, 1997 and Ayyappan et al., 2011).

**Preparation of platelet rich plasma (PRP)**

Fresh blood (5 ml) was collected from jugular vein of the Black buck before surgery and mixed with 3.8 per cent sodium citrate anticoagulant and centrifuged in Falcon tubes at 3000 rpm for 10 minutes for promoting the separation of the plasma from red blood cells. The centrifugation resulted in three basic components in the tube. The red blood cells at the bottom of the tube, platelet rich plasma (PRP) at the middle of the tube and the platelet poor plasma (PPP) at the top of the tube. The one milliliter of plasma above the puffy coat (seen above red blood cells) was collected after pipetting out the superficial content as marked on the tube (Nagata et al., 2010 and Ferdousy et al., 2013). The platelet count before processing was 3.26 lakhs per cu.mm and was concentrated to 6.08 lakhs per cu.mm after centrifugation.

**Anaesthesia and surgical procedure**

Anaesthesia was induced with xylazine 1.0 mg/kg and ketamine 2.5 mg/kg body weight intravenously and maintained with incremental doses of ketamine. The animal was placed on left lateral recumbency. Surgical site was prepared aseptically and cranio-lateral incision was made over the tibia at fracture site. The extensor digitorum lateralis and deep digital flexor muscles were separated and the peroneal nerve was preserved (Tralman et al., 2010). The fracture fragments were exposed and the free floating broken bone pieces were removed (Figure-2). Fracture site was stabilized with 3.5 mm intra medullary pin (IMP) which was introduced in retro-grade manner and additional stabilization was provided using dynamic compression plate (DCP) 3.5mm, 7 hole plate, applied on medial aspect following the standard procedure (Ayyappan et al., 2011) (Figure-3). PRP was seeded on to hydroxyapatite and was engrafted at the fracture site. The muscles were sutured with PGA 1 in simple continuous manner. Sub-cuticular sutures were applied to prevent dead space. Skin incision was closed using No.1 braided silk. The operative site was covered with povidone iodine gauze and bandaged. Injections ceftriaxone 20 mg/kg and tramadol 2mg/kg body weight were administered intravenously. External co-optation was provided with fiber glass gutter splint (Figure-4).
Post-operatively the animal was maintained with restricted movements in a separate cell and the wound was examined after one week. Animal had an uneventful recovery and was able to bear weight 3 weeks later.

**Results and Discussion**

The dose of xylazine and ketamine 1.00 mg/kg and 2.5 mg/kg body weight induced anaesthesia and muscle relaxation for the entire surgical procedure without any complications. Heart rate per minute, respiratory rate per minute and rectal temperature recorded during anaesthesia were 65, 21 and 38.2°C after immobilization, 60, 18 and 38.0°C during maintenance and 68, 23 and 38.4°C before recovery and were within normal clinical limits (Sontakke et al. 2009). The duration of anaesthetic maintenance was 42 minutes. Xylazine-ketamine immobilization and anaesthesia was practiced in antelopes (Jessup et al., 1983; Galka et al., 1999 and William et al., 2014) as a safe technique. Incremental dose of ketamine at the rate of 2mg/kg body weight (Gahlod et al., 2012 and Monteith et al., 2012) was administered to maintain anaesthesia for 42 minutes. The preoperative haematological parameters were also within the normal limits. The PRP preparation technique of centrifugation at 3000 rpm for 10 minutes was found to be ideal as the concentration had increased to approximately double fold from 3.26 lakhs to 6.08 lakhs per cu.mm (Camargo et al., 2013).

Tibial fractures account for the third most common type of fracture after femur and radius and ulna (Seaman and Simpson, 2004) and comprise 21.0 per cent (Unger et al., 1990) of all long bone fractures. Tibial diaphyseal fractures account for 75.0 per cent to 81.0 per cent of all tibial fractures (Boone et al., 1986).

The primary aim of fracture treatment is to achieve the fastest possible healing and enable the patient to function normally by allowing early walking (Aron, 1998); which is enabled by anatomical agreement between the joints above and below the fractured bone and functioning of the extremity (Piermatei and Flo, 1997). Kumar (2006) reported successful management of metacarpal fracture in a male Blackbuck using padded bamboo splints and the author preferred the technique for simple fractures of long bones. Suresh et al. (2007) managed fracture of tibia in a Black buck with bone plating and external cooptation with plaster of paris cast. Gahlod et al. (2012) successfully treated midshaft tibial fracture in a Black buck with intramedullary nailing and full circlage wiring with plaster cast external cooptation. The basic principles of fracture healing in Antelopes are similar to those of other species; but factors associated with fracture, temperament of the animal and limitations in post-operative management affect the clinical outcome. The choice of fracture repair technique has impact on the success of clinical outcome. Plate-rod
technique is developed on AO/ASIF principles for the surgical management of unstable fractures of long bones and reported excellent clinical outcome in dogs (Andrianov et al., 2007 and Ayyappan et al., 2011) and sheep (Tralman et al., 2010). The dynamic compression plate used in this study was a special implant developed by AO group for compression and stabilization of the fracture. When screws were inserted on either side of fracture gap, the bone and the plate moves longitudinally relative to one another with the plate under tension and the bone under compression thereby the fracture gap was narrowed. Three major factors were taken into consideration for plate application. The plate was applied on the tension side of the bone to convert tensile forces of eccentrically loaded bone to compressive forces, contouring the plate to original shape and curve of the bone to bring about adequate fracture alignment and ideally minimum of six cortices should be engaged by screw threads on either side of the fracture. Eccentric drill guide provided displacement of plate relative to bone. As the screws were tightened, interfragmentary strain was created on the adjoining bone fragments (Sakhvadze Sh., 2009). Tibia is ‘S’ or sigmoid in shape. The tension surface of the proximal tibia is cranial but plates were generally placed in the medial side of the tibia because of the ease of application due to less muscular coverage; though it was best to place the plate on the tension side of the bone or on the convex surface of a bone (Seaman and Simpson, 2004; Andrianov et al., 2007 and Ayyappan et al., 2011). The plate application on medial surface in the present case report was found to be effective to counteract the fracture forces like compression, tension, bending, torsion and shearing forces.

The type of fracture was comminuted fracture of tibia with cranially displaced bone fragments which required special consideration to augment healing and minimize post-operative handling and complication. Three core elements required for bone healing and growth are osteoconduction, osteoinduction and osteogenesis. Providing a graft or physical structure with surface, whose biocompatibility supports migration of mesenchymal stem cells is osteoconduction. The ability of the graft to allow recruitment of mesenchymal stem cells and providing microenvironment for survival of the cells is osteoinduction. Osteogenesis is synthesis of new bone by the cells within the graft by transdifferentiation (De Long et al., 2007). Hydroxyapatite along with autologous PRP or bone marrow aspirate induces osteoconductive and osteoinductive actions (Maesstretti et al., 2007; Birch and D’Souza, 2009 and Kurien et al., 2013). During maturation phase of bone healing osteoblast secrets growth factors, which are also present in platelets (Everts et al., 2006 and Nikolidakis et al., 2006). The platelets act as an exogenous agent to provide growth factors stimulating the activity of the osteoblasts (Thiede et al., 1993). In the fracture site platelets release platelet derived growth factors (PDGF) and transforming growth factors (TGF). TGF-1 and TGF-2 are two isoforms; between which TGF-1 has the greatest potential to repair bone because osteoblasts and chondrocytes are rich with TGF-1 receptors. TGF helps in the
differentiation of mesenchymal stem cells into osteoblasts and osteogenic cells. Similarly cell proliferation is increased by the mitogenic action of PDGF (Everts et al., 2006). Normal weight bearing was noticed in the present case report on the third post-operative week whereas, Gahlod et al. (2012) reported gradual weight bearing only after 6 weeks following fracture immobilization with intramedullary pinning and external cooptation in a Black buck.

Bone plating with cortical screws provided effective stabilization of fractured segments and additional stabilization provided by IMP, which was introduced in retro-grade manner. Hydroxyapatite seeded with autologous PRP acted as an adjunct to plate-rod osteosynthesis by the osteoconductive and osteoinductive properties in promoting osteogenesis and early healing of tibial fracture in the Black buck. External cooptation with fiber glass gutter splint was tolerated by the animal and provided ancillary support in the immobilization of fracture. Postoperatively the animal showed gradual improvement in lameness to normal weight bearing over the period of study. No post-operative complication was noticed.

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