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Efficacy of Multi-ion Doped Plasma Spray Nano-hydroxyapatite Coated Titanium Implants in Long Bone Fracture Repair in Dogs- Radiographical and Bone Markers Evaluation

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ABSTRACT

Background: Fracture of long bones is a common orthopaedic condition noticed in dogs and its primary goal is to completely restore the function of the injured limb as early as possible. Osteo-conductivity of hydroxyapatite can be improved further by decreasing the particle size to nanometre range and incorporation of inorganic materials in hydroxyapatite can enhance osteoblast cell material interactions. Strontium, zinc, silver and fluorine are known to play an important role in the bone formation and also affect bone material characteristics such as crystallinity, degradation behaviour and mechanical properties. When doped with plasma spray nanohydroxyapatite, these multi-ions cause no harm to the physical environment during the degradation process of hydroxyapatite as these are nontoxic and play significant role in bone metabolism, growth and nourishment. Bone markers have tremendous potential as a rapid and sensitive method for assessing the response of the skeleton to medical or surgical interventions providing valuable information regarding bone turn over in animals. Hence, the current study was undertaken to evaluate the potential of multi-ion doped nano-hydroxyapatite coated intramedullary titanium implants in long bone fracture repair in dogs compared to the conventional intramedullary titanium implants through radiographical studies and evaluation of bone markers.

Methods: Radiographical evaluation, sandwich ELISA kits developed by bioassay technology laboratory.

Result: Plasma spray nano-hydroxyapatite coated titanium intramedullary implants have shown excellent osteo-conductivity when doped with multi-ions of strontium, zinc, silver and fluorine facilitating rapid osteoblastic activity and rapid bone turnover at the fracture site and complete fracture healing by 3rd week post-operatively as evidenced by radiographic scores and a peak BALP (Canine Bone Alkaline Phosphatase) values and early limb usage. Bone reabsorption and bone tissue remodelling due to osteoclastic action at the fracture site was quicker when the multi-ion doped nano-hydroxyapatite coated titanium intramedullary implants were used which is evidenced by the radiographic scores and highest CTX (Canine C-telopeptide of Type 1 Collagen) values indicating completion of fracture healing and near completion of bone tissue remodelling by 9th post-operative week in long bone fracture repair in dogs.

Key words: Bone markers -BALP, CTX, Long bone fractures in dogs, Multi-ion doped plasma spray nano-hydroxyapatite coated titanium implants, Radiographical evaluation.

INTRODUCTION

Numerous biochemical assays are available for measuring serum concentrations of markers of bone metabolism to evaluate healing process in fracture bones. Bone markers have been studied in general with regard to age and breed related differences in dogs (Breur *et al*., 2004), their clinical applications in osteomyelitis (Southwood *et al*., 2003) in rabbits, osteosarcomas and osteoarthritis (Garzoto *et al.,* 2000 and Lucas *et al*., 2008), delayed bone union and non-union in tubular bones (Komnenou *et al*., 2005) and experimental bone fracture healing (Paskalev *et al*., 2005). Bone markers have been used in veterinary orthopaedics to assess skeletal maturation, effects of hormones on bone metabolism and in the diagnosis of bone pathology (Allen *et al*., 2000 and Allen, 2003). On the other hand, biochemical markers of bone cell activity are widely used in the research on metabolic diseases like osteoporosis (Greenblatt *et al.,* 2017) and in diseases like Paget's disease (Alvarez *et al.,* 2001) in humans.

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Bone alkaline phosphatase (BALP) and carboxy-terminal telopeptide of type 1 collagen (CTX) are the emerging bone markers in orthopaedics that can aid in the earlier assessment of treatment effects in fracture repairs

compared to measurement of bone density (Allen *et al.,* 2000). Fracture complications can be avoided by an early measurement of bone turnover markers like BALP and CTX that assess accurately the bone healing stages. BALP is an extracellular enzyme produced by osteoblasts. Serum BALP concentration is a specific and sensitive indicator of osteoblast activity and bone formation and a high serum concentration of BALP is indicative of high level of osteoblastic activity (Lin *et al.,* 2016). CTX contains important inter molecular cross-linking agents of type 1 collagen and residues of cross-linking agents. The serum CTX concentration is an ideal marker of bone resorption (Seibel *et al.,* 2001 and Lin *et al.,* 2016). The current study was undertaken to clinically evaluate the efficacy of multi-ion doped plasma spray nano-hydroxyapatite coated titanium implants in long bone fracture repair in dogs by radiographical evaluation supported by concurrent evaluation of BALP and CTX.

MATERIALS AND METHODS

After a detailed clinical, orthopaedic, neurological and radiological examination, twenty-four dogs with long bone fractures were selected from 78 dogs presented to the Department of Surgery and Radiology and Veterinary Clinical Complex of Sri Venkateswara Veterinary University, Tirupati, for a period of two years *i.e.*, from November 2017 to November 2019. The selected dogs were free from concurrent fracture of flat bones, pelvis fractures, spinal fractures, neurological, metabolic or infectious diseases. The data regarding the incidence of long bone fractures in dogs were collected and variables like age, breed, sex, bone, limb involved, weight of the animal, etiology of fracture, location, type of fracture and clinical symptoms were recorded and analysed. The 24 dogs were divided into four groups with six dogs in each group and fracture repair was carried out in all the dogs. Open reduction and internal immobilization were done with uncoated titanium intramedullary pins (group I), or with plasma spray nano-hydroxyapatite coated titanium intramedullary pins (group II), or with plasma spray multi-ion (5% zinc, 2.5% strontium and 2.5% fluorine) doped nano-hydroxyapatite coated titanium intramedullary pins (group III) or with plasma spray multi-ion (5% zinc, 5% strontium and 2.5% silver) doped nano-hydroxyapatite coated titanium intramedullary pins.

Lateral and cranio-caudal radiographs of the fractured bone were taken at 90^{o} angle (Shales, 2008) for confirmatory diagnosis, classification of fractures, selection of implants and approach. In all the dogs, fractures were classified according to the fracture configuration using Winquist-Hansen (W-H) and AO (ASIF) classification. Preparation, characterization and standardization of multi-ion doped plasma spray nano-hydroxyapatite titanium intramedullary pins were done at Central Glass and Ceramic Research Institute (CGCRI), Kolkata. Steinmann pins of thickness sufficient to fill up 70 - 80 per cent of the marrow cavity at the isthmus were used. Cerclage wire of appropriate size was used for ancillary fixation in necessary cases.

Atropine-Xylazine @ 0.02 mg and 1 mg/kg body weight, IM as pre-anaesthetic medication and after an interval of 30 minutes, Ketamine-Diazepam at the dose rate of 8 mg/kg body weight and 0.3 mg /kg body weight respectively were administered IV for induction. Following induction of anaesthesia, dogs were intubated with endotracheal tubes of suitable size. General anaesthesia was maintained with 1.5 to 2 per cent Isoflurane.

Lameness grade was assigned to all the dogs during the pre and post-treatment period based on the weight bearing nature during stance and while walking (Vasseur *et al.,* 1995). Radiographs of repaired fractures were taken post-operatively on 0th day, immediately after 3rd, 6th and 9th week to assess the fracture healing by radiographic scoring system (Hayashi *et al.,* 2008) (Table 1). Blood samples were collected in vacutainer tubes post-operatively on 0th day, immediately after 3rd, 6th and 9th week to evaluate the levels of serum calcium, serum phosphorus and serum alkaline phosphatase, BALP and CTX. Serum concentrations of BALP and CTX were estimated using ELISA kits developed by Bioassay Technology Laboratory. Functional limb outcome during post-operative period was assessed in all the dogs by periodical clinical examinations. The data collected were statistically analysed using SPSS-ANOVA Post Hoc tests. Implants were removed on 9th post-operative week after evaluating the radiographs for fracture healing.

RESULTS AND DISCUSSION

Out of 78 long bone fractures in dogs, femoral fractures accounted for 69.23% followed by radius (14.10%), humerus (10.25%) and tibia (6.41%). Singh *et al.* (2017) and Uddin *et al.* (2017) also documented similar observations. Femur was found to be the most commonly affected long bone which might be due to the counteracting pulls of flexor and extensor muscles and also due to more exposure of hind quarters directly to the major force of impact. Similar findings were recorded by Dvorak *et al.* (2000). In the present study, left limb was most commonly involved in fracture, as observed by Rani *et al.* (2004) and Raghunath *et al.* (2007).

Fractures were classified based on AO/ASIF classification and Winquist - Hansen (W-H) classification. In the present study majority of long bone fractures were of grade I (66.66%) followed by grade II, IV, V and III. Gondalia and Siddiqui (2015) reported higher incidence of grade II fractures in dogs. AO/ASIF classification of fractures revealed that majority of long bone fractures were simple (75.64%) followed by compound (17.95%) and complex type (6.41%). Diaphyseal fracture was the most observed (71.80%) fracture type. Dvorak *et al.,* 2000 and Kumar *et al.,* 2007 also documented similar observation.

Lameness was graded on the basis of gait and weight bearing in all the animals at 0th day, 3rd, 7th, 14th, 21st, 30th, 45th and 60th post-operative days. Lameness was not observed in all the dogs on 60th post-operative day. Dogs subjected to open reduction and internal immobilization with group-IV pins had the earliest limb usage without lameness by 21st post-operative day.

Uncoated titanium implants were stable in all the dogs throughout the study in group I dogs. Stable reduction and stable implants were observed in all the animals by 3rd week. Radiographic evaluation score immediately after 6th and 9th post-operative week was 4 and 5 in all the dogs (Fig 1).

Fracture reduction was stable in all the group II dogs during the entire period of study. After 3rd post-operative week, most of the dogs had radiographic evaluation score of 3 and on 6th post-operative week, the score was 4. By 9th post-operative week, the score was 5 in 3 dogs and the remaining 3 dogs had a score of 6.

Fracture reduction was stable in all the group III dogs during the entire period of study. Radiographic evaluation of all the dogs in the group after 3rd, 6th and 9th post-operative week revealed score of 4, 4 and 6 respectively.

Fracture reduction was stable in all the group IV dogs during the entire period of study. Radiographic evaluation of dogs on 3rd post-operative week revealed score 5 in most of the dogs and the score was 5 and 6 on 6th and 9th post-operative week in all the dogs.

BALP values increased significantly up to 6th post-operative week in group I dogs followed by a significant decrease by 9th week. In group II, III and IV dogs, BALP values increased up to 3rd post-operative week and decreased later on (Table 2). The increase in BALP values up to 3rd post-operative week and subsequent decrease, after 3rd to 9th post-operative week was rapid in dogs of group III and group IV and this might be due to the use of multi-ion doped nano-hydroxyapatite coated titanium intramedullary pins for fracture repair in these group of dogs. The rapid increase in BALP values by 3rd post-operative week caused due to the intramedullary pins in group III and group IV dogs was suggestive of a rapid bone turnover at the fracture site facilitating faster fracture healing and early limb usage which on observation was also corroborated by the radiographic scores, lameness grading and serum biochemical studies. This was in agreement with the findings of Sousa *et al*. (2015) who reported higher BALP concentrations during early fracture healing in dogs. Southwood *et al*. (2003) also reported increased serum BALP concentration at 8 weeks in femur defects in a rabbit model.

The mean±SE values of CTX in group I and IV dogs were significantly different on 0th day, after 3rd, 6th and 9th week post operatively (Table 2). In group II dogs, the CTX values remained unchanged up to 3rd post-operative week but were significantly different after 6th and 9th post-operative weeks. The CTX values of group III dogs were not significantly different up to 6th post-operative week but were significantly different on 9th post-operative week. The CTX values were at peak levels on 9th post-operative week in all the groups and this was rapid and higher in group III and highest in group IV dogs indicating bone reabsorption and bone tissue remodelling was rapid due to osteoclastic activity at the fracture site in these group of dogs. The highest CTX values in group IV followed by group III dogs from 3rd week to 9th week is indicating the completion of fracture healing and near completion of bone tissue remodelling facilitated by the multi-ion doped nano-hydroxyapatite coated titanium intramedullary implants. Completion of fracture healing was evidenced by 3rd week in the radiographic scores and serum biochemical studies in these three groups. Lucas *et al.* (2008) observed significant increase in serum CTX concentration in dogs with osteosarcoma. However, Herrman *et al.* (2002) reported no significant difference in concentrations of CTX in normal and delayed healing of fractures in human. Moghaddam *et al.* (2011) reported lower serum

Table 1: Radiographic scoring system

Score	Radiographic signs
0	Presence of recent fracture with no bone formation.
1	Irregularity at fragment lines of fracture site.
2	Initial/discrete periosteal proliferation
3	Exuberant/organized periosteal proliferation
4	Exuberant osseous callus in evolution with presence of periosteal proliferation.
5	Exuberant osseous callus in evolution with discrete radiolucent line at gap between the fracture fragments.

Table 2: Mean±SE values of bone markers at different time intervals during the study

Parameter	Group	Time interval			
		0 day	3 weeks	6 weeks	9 weeks
BALP	Group I	0.43± 0.03^{a}	5.63±0.07^{b}	6.77±0.03^{c}	6.07±0.02^{d}
	Group II	0.44±0.02^{a}	6.61±0.02^{b}	5.65±0.01^{c}	4.13±0.01^{d}
	Group III	0.60±0.01^{a}	6.83±0.01^{b}	6.24±0.01^{c}	3.06±0.01^{d}
	Group IV	0.62±0.02^{a}	7.63±0.01^{b}	7.23±0.01^{c}	3.23±0.01^{d}
CTX	Group I	0.23±0.00^{a}	0.26±0.02^{b}	0.35±0.01^{c}	0.77±0.01^{d}
	Group II	0.25±0.01^{a}	0.26±0.01^{a}	0.36±0.01^{b}	0.77±0.01^{c}
	Group III	0.33±0.01^{a}	0.35±0.01^{b}	0.36±0.01^{b}	0.83±0.01^{c}
	Group IV	0.26±0.00^{a}	0.36±0.01^{b}	0.45±0.01^{c}	0.96±0.01^{d}

Means bearing different superscripts different significantly P≤0.05.

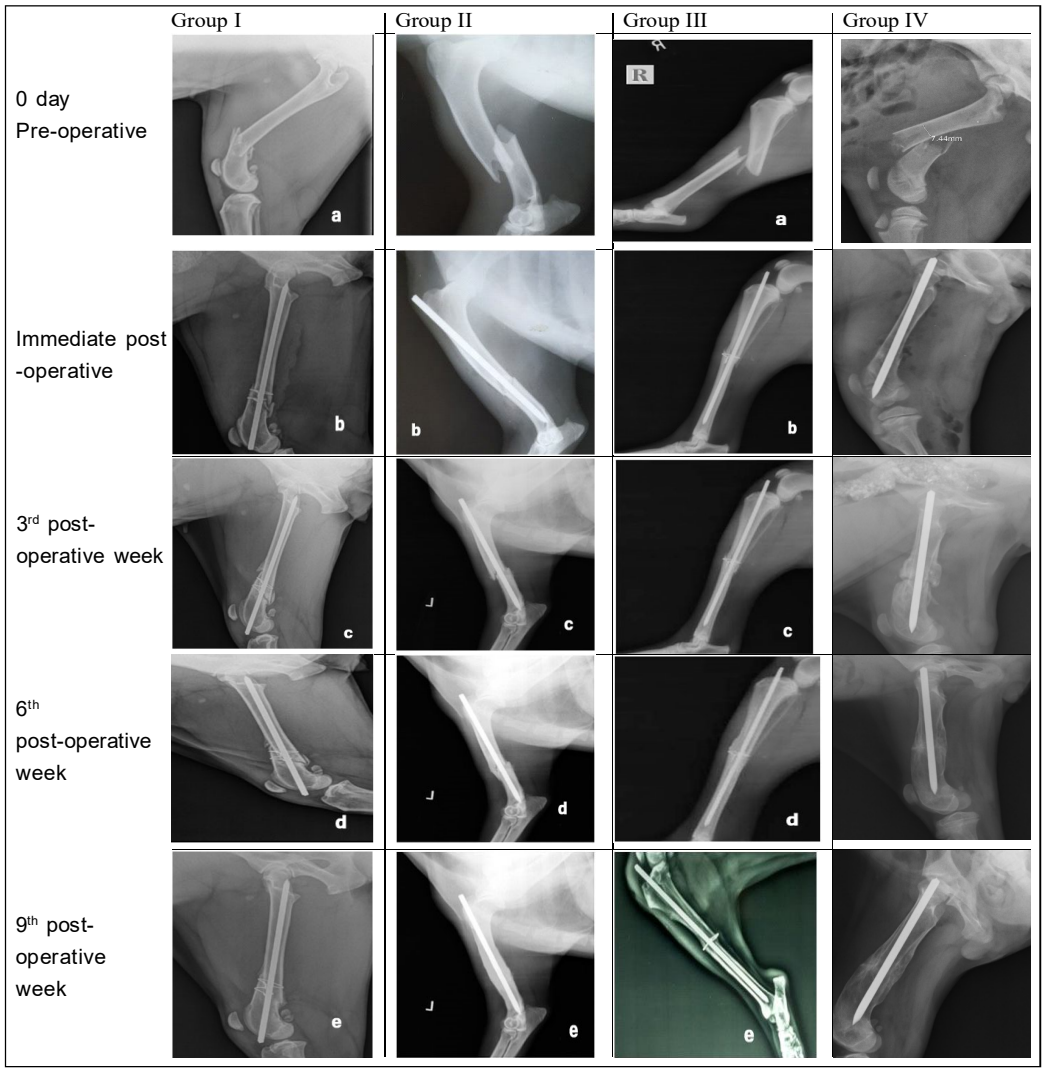

Fig 1: Skiagrams showing progressive radiographic changes in animals of all the groups.

concentration of CTX after surgery in delayed fracture healing in humans. However, Belic *et al.* (2010) reported higher CTX values in heavier dogs.

CONCLUSION

Long bone fracture repair using multi-ion doped nano-hydroxyapatite coated titanium implants in dogs was evaluated by radiographical studies and by bone markers evaluation. Majority of dogs subjected to open reduction and internal immobilization with multi-ion doped nano hydroxyapatite coated titanium intramedullary pinning, showed early limb usage with no post-operative complications like pin migration and pin track infection. However, using multi-ion doped *i.e.*, either 5% zinc, 5% strontium and 2.5% silver ion doped (group IV) or 5% zinc, 2.5% strontium and 2.5% fluorine ion doped (group III) nano hydroxyapatite coated titanium intramedullary implants for long bone fracture repair in dogs resulted in excellent functional limb out come with the earliest limb usage by 3rd post-operative week as evidenced by radiographical evaluation scores and serum concentration levels of BALP and CTX values. Radiographical evaluation scores improved from 5 in 3rd / 6th post-operative week to a score of 6 by 9th post-operative week. BALP values increased rapidly up to 3rd post-operative week in group III and group IV dogs suggesting rapid bone turnover at the fracture site facilitating faster fracture healing and early limb usage. CTX values were at peak levels on 9th post-operative week in all the groups and this was rapid and higher in group III and highest in group IV dogs indicating osteoclastic activity at the fracture site resulting in completion of fracture healing and near completion of bone tissue remodelling facilitated by the multi-ion doped nano-hydroxyapatite coated titanium intramedullary implants with no post-operative complications. Radiographical evaluation clubbed with concurrent evaluation of serum bone marker concentrations could aid in the earlier assessment of treatment effects in fracture repairs and hence fracture complications could be avoided as BALP and CTX values can precisely assess the bone healing stage during fracture repair.

REFERENCES

Allen, L.C., Allen, M.J., Breur, G.J., Hoffmann, W.E. and Richardson, D.C. (2000). A comparison of two techniques for the determination of serum bone-specific alkaline phosphatase activity in dogs. Research in Veterinary Science. 68: 231-23.

Allen, M.J. (2003). Biochemical markers of bone metabolism in animals: Uses and limitations. Veterinary Clinical Pathology. 32(3): 101-113.

Alvarez, L., Guanabens, N., Peris, P., Vidal, S., Ros I., Monegal, A., Bedini, J.L., Deulofeu, R., Pons, F., Munoz-Gomez, J. and Ballesta, A.M. (2001). Usefulness of biochemical markers of bone turnover in assessing response to the treatment of Paget's disease. Bone. 29(5): 447-452.

Belic, M., Svetina, A., Kušec, V., Rakoèevic, S., Grizelj, J., Robić, M. and Turk, R. (2010). Bone alkaline phosphatase, osteocalcin and c-terminal telopeptide as bone turnover markers in canine bitches. Veterinarski Arhiv. 80(6): 705-713.

Breur, G.J., Allen, M.J., Carlson, S.J. and Richardson, D.C. (2004). Markers of bone metabolism in dog breeds of different size. Research in Veterinary Science. 76(1): 53-55.

Dvorak, M., Necas, A. and Zatloukal, J. (2000). Complication of long bone fracture healing in dogs: Functional and radiological criteria for their assessment. Acta Veterinaria Brno. 69: 107.

Garzoto, C.K., Berg, J., Hoffman, W.E. and Rand, W.M. (2000). Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. Journal of Veterinary Internal Medicine. 14: 587-592.

Gondalia,V. and Siddiqui, S.S. (2015). Management of open fracture of tibia diaphysis (Gustilo-Anderson Classification Type-II and above). Journal of Evolution of Medical and Dental Sciences. 4(40): 7032-7041.

Greenblatt, M.B., Tsai, J.N. and Wein, M.C. (2017). Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. Clinical Chemistry. 63(2): 464-474.

Hayashi, A.M., Matera, J.M., Sterman, F.A., Muramoto, C. and Cortopassi, S.R.G. (2008). Evaluation of electro acupuncture in bone healing of radius-ulna fracture in dogs. Brazilian Journal of Veterinary Research and Animal Science. 45(5): 339-347.

Herrman, M., Klitscher, D., Georg, T., Frank, J., Marzi, L. and Herrman, W. (2002). Different kinetics of bone markers in normal and delayed fracture healing of long bones. Clinical Chemistry. 48: 2263-2266.

Komnenou, A., Karayannopoulou, M., Polizopoulou, Z.S., Constantinidis, T.C. and Dessiris, A. (2005). Correlation of serum alkaline phosphatase activity with the healing process of long bone fractures in dog. Veterinary Clinical Pathology. 34(1): 35-8.

Kumar, K., Mogha,I.V., Aithal, H.P., Kinjavdekar, P., Singh, G.R., Pawde, A.M. and Kushwaha, R. B. (2007). Occurrence and pattern of long bone fractures in growing dogs with normal and osteopenic bones. Journal of Veterinary Medicine. 54: 484-490.

Lin, J.P., Shi, Z.J., Shen, N. J., Wang, J., Li, Z.M. and Xia, J. (2016). Serum N terminal telopeptide of type I collagen as an early marker of fracture non-union in rabbits. Experimental and Therapeutic Medicine. 12: 3595-3601.

Lucas, P.W., Fan, T.M., Garrett, L.D., Griffon, D.J. and Wypij, J.M. (2008). A comparison of five different bone resorption markers in osteosarcoma-bearing dogs, normal dogs and dogs with orthopaedic diseases. Journal of Veterinary Internal Medicine. 22: 1008-1013.

Moghaddam, A., Muller, U., Roth, H.J., Wentzensen, A., Grutzner, P.A. and Zimmermann, G. (2011). TRACP 5b and CTX as osteological markers of delayed fracture healing. Injury. 42: 758-764.

Paskalev, M., Krastev, S. and Filipov, J. (2005). Changes in some serum bone markers after experimental fracture and intramedullary osteosynthesis in dogs. Trakia Journal of Sciences. 3(5): 46-50.

Raghunath, M., Singh, M., Yadav, R.K. and Singh, S.S. (2007). Distribution and classification of canine long bone fractures. Indian Veterinary Journal. 84: 1243-1246.

Rani, R. U., Vairasamy, K. and Kathiresan, D. (2004). A retrospective study of bone fractures in canines. Indian Veterinary Journal. 81: 1048-1050.

Seibel, M.J., Lang, M. and Geilenkeuser, W.J. (2001). Inter-laboratory variation of biochemical markers of bone turnover. Clinical Chemistry. 47: 1443-1440.

Shales, C. (2008). A Fracture management in small animal practice 1 - Triage and stabilization. In practice. 30(6): 314-320.

Singh, C.K., Sarma, K.K., Kalita, D., Tamuly, S., Hussain, J., Deuri, B. and Nath, P.J. (2017) Haemato-biochemical, radiographic and clinical outcome in healing of femoral fracture with retrograde intramedullary pin in conjunction with demineralized bone matrix in dogs. Journal of Experimental Biology and Agricultural Sciences. 5(2): 201-207.

Sousa, C.P., Dias, I.R., Pena, L.M., Camassa, J.A., Lourenco, P.J., Judas, F.M., Gomes, M.E. and Reis, R.L. (2015). Bone turnover markers for early detection of fracture healing disturbances: A review of the scientific literature. Annals of the Brazilian Academy of Sciences. 87(2): 1049-1061.

Southwood, L.L., Frisbie, D.D., Kawcak, C.E. and Mcilwraith, C.W. (2003). Evaluation of serum biochemical markers of bone metabolism for early diagnosis of nonunion and infected nonunion fractures in rabbits. American Journal of Veterinary Research. 64: 727-735.

Uddin, K. Md., Islam, M.M.M., Hassan, Z. Md., Rahman, M., Islam, R. Md., Hoque, F. Md. and Das, B.C. (2017). Internal fixation of fracture of femur of dog using intramedullary pinning. Asian-Australian. Journal of Bioscience and Biotechnology. 2(3): 243-246.

Vasseur, P.B., Johnson, A.L., Buderberg, S.C., Linwln, J.B., Toombs, J.B., Whitebain, J.G. and Lentz, E. L. (1995). Randomized, controlled trials of the efficacy of carprofen, a non- steroidal anti - inflammatory drug in the treatment of osteoarthritis in dogs. Journal of American Veterinary Medical Association. 206: 807-811.