P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Clinical Article

https://doi.org/10.47278/journal.ijvs/2023.065

Early Low-Grade Knee Osteoarthritis in Sheep (*Ovis aries*) after 6-Weeks of Total Unilateral Meniscectomy: A Radiographic Evaluation

Jessica Fiolin[1], Bambang Pontjo Priosoeryanto[2], Tri Isyani[2], Radiana Dhewayani Antarianto[3], Marcel Prasetyo[4], Alida Roswita Harahap[1], Trevino Aristarkus Pakasi[5], Ismail Hadisoebroto Dilogo[6] and Ludwig Andre Pontoh[6]

[1]Doctoral Programme of Medical Science, Faculty of Medicine Universitas Indonesia, Indonesia
[2]School of Veterinary Medical & Biomedical Sciences, IPB University, Bogor, Indonesia.
[3]Department of Histology, Faculty of Medicine Universitas Indonesia, Indonesia
[4]Department of Radiology, Faculty of Medicine Universitas Indonesia, Indonesia
[5]Department of Community Medicine, Faculty of Medicine Universitas Indonesia, Indonesia
[6]Department of Orthopaedic and Traumatology Faculty of Medicine Universitas Indonesia, Indonesia
***Corresponding author:** jessicafiolinortho@gmail.com

Article History: 23-200 Received: 12-May-23 Revised: 08-Jun-23 Accepted: 12-Jun-23

ABSTRACT

Different new potential biologic agents have recently been studied to control knee joint osteoarthritis (KOA). Hence, animal models are one of the most important foundations studies to assess early safety and efficacy before human clinical trials. This study aimed to evaluate the radiographic progression of stifle joint osteoarthritis (OA) after lateral meniscectomy. A total of 14 skeletally matured sheep (*Ovis aries*) were subjected to total lateral meniscectomy and evaluated for radiographic cranio-caudal and mediolateral projections at the 6th and 12th week after surgery. Low grade OA was seen radiologically by osteophyte formation at the 6th week, followed by intra-articular mineralization and subchondral sclerosis at the 12th week after meniscectomy. There was statistically non-significant difference in the radiographic scores between 6th and 12th week after meniscectomy. In conclusion, total lateral meniscectomy in the ovine stifle joint could create low grade OA as early as 6 weeks, confirmed radiologically by osteophyte formation. Further studies are needed to evaluate the correlation between radiological and clinical findings.

Key words: Stifle Joint, *Ovis aries*, Lateral Meniscectomy, Radiographic Score

INTRODUCTION

Meniscus is one of the most important structures to prevent knee joint osteoarthritis (KOA) (Beveridge et al. 2011). A number of previous studies have confirmed the role of meniscus in load transmission, shock absorption, nutrition, lubrication and stability in the knee joint (Innes et al. 2004; Beveridge et al. 2011; Veronesi et al. 2020). Patients without intact meniscus slowly develop KOA, beginning with articular cartilage fibrillation, cleft formation and narrowing of joint space (Kopf et al. 2020). Animal models of meniscectomy have also been shown to have post-traumatic osteoarthritis (OA) confirmed through radiological evaluation (Teeple et al. 2013; Lampropoulou-Adamidou et al. 2014; Kuyinu et al. 2016).

Radiological evaluation has been commonly performed to investigate and diagnose joint diseases in humans and animals since 1950s (Little et al. 1997; Innes et al. 2004). Although radiographic features of OA were not proven to correlate well with clinical condition and temporal changes, conventional radiographic assessment has been, and continues to be, a standard clinical tool in KOA (Morgan 1968). Several grading systems have been used to assess the severity of stifle joint OA, all of which have a common ground of the emphasis of osteophyte development (Gilbertson 1975; Dedrick et al. 1993; Widmer et al. 1994). In veterinary practice, probably due to the inability to assess joint space during a weight-bearing radiograph, but also related to the prominent nature of the osteophyte in the stifle joint OA. According to Morgan (1968), development of global effusion,

Cite This Article as: Fiolin J, Priosoeryanto BP, Isyani T, Antarianto RD, Prasetyo M, Harahap AR, Pakasi TA, Dilogo IH and Pontoh LA, 2023. Early low-grade knee osteoarthritis in sheep (*Ovis aries*) after 6-weeks of total unilateral meniscectomy: A radiographic evaluation. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2023.065

osteophytosis and enthesophytes, intra-articular mineralization and subchondral sclerosis/subchondral cyst formation are the features needed to be evaluated in stifle joint OA.

Along with the advances in medical technology and knowledge, regenerative therapy has been one of the most studied fields in medicine. More and more biologic therapies are aimed to prevent and control the OA earlier when it is in the low-grade condition (Dilogo et al. 2020; Zhu et al. 2021). However, most studies showed the OA in the advanced condition and little information is available regarding the time needed for the development of OA, especially the low-grade ovine stifle joint OA (O'Brien et al. 2012; Holland et al. 2013; Moya-Angeler et al. 2016). Therefore, this study aimed to evaluate the radiographic progression of a low-grade OA creation in stifle joint after a total lateral meniscectomy procedure in sheep (*Ovis aries*).

MATERIALS AND METHODS

A total of 14 sheep (*Ovis aries*) were included in this study. Clinical, laboratory and radiographic assessments were performed in these animals before the meniscectomy to exclude primary osteoarthritis and previous pathology in the joint and bones. The selection criteria for experimental animals included male ovine, aged 3 years, body weight 25-30kg, skeletally mature without history of trauma and congenital anomaly of the lower extremity. Animals with infection at surgical site, deformity of lower extremity and cartilage damages upon meniscectomy were excluded. All selected animals were subjected to total lateral meniscectomy in the right hind limb at the same time under anesthesia. The wound was closely monitored daily, and the sutures were removed 14 days after meniscectomy.

Radiographic examinations were performed at the 6th and 12th week after meniscectomy through a high-resolution film screen combination, using POX-100BT (POSKOM, Gyeonggi, Korea) and viewed using VetDROC by Insan Teknotama Bersahaja application. One Veterinarian and one orthopedic surgeon evaluated and scored the results. The ethical approval of this research was obtained from our institution with protocol number 22-08-0984 and ethical clearance number KET-932/UN2.F1/ETIK/PPM.00.02/2022 and an Animal Care and Use Committee (ACUC) School of Veterinary Medicine and Biomedical Sciences IPB University number 023/KEH/SKE/IX/2022.

Total Lateral Meniscectomy

Before surgery, all the experimental animals were clinically evaluated for general health, vaccination status and were acclimatized for 2 weeks in the laboratory. The operation site was shaved and conventional radiograph was taken to exclude prior pathology in the lower extremity. Total lateral meniscectomy was performed on the right hind limb of each animal. Prophylactic intramuscular (IM) amoxicillin at the dose of 10mg/kg body weight (BW) was given, while 0.22mg/kg BW of sulfas atropine was administered subcutaneously as premedication. Induction of anesthesia was started by giving 11mg/kg BW of Ketamine (IM), with xylazine 0.22mg/kg BW was given as a muscle relaxant. Intravenous normal saline maintenance was administered at the front limb during the surgery.

Septic and aseptic procedures were done, followed by standard surgical draping. Incision was performed using lateral parapatellar approach, 5cm proximal to the kneecap and 5cm distal to the upper end of tibia. After retraction of subcuticular tissue, vastus lateral muscle and the joint capsule were incised. The lateral meniscus was removed by sharply cutting the cranial and caudal supporting meniscal horns and meniscofemoral ligaments (Fig. 1). The operation field was then irrigated with normal saline and the surgical wound was sutured layer by layer.

After surgery, the animals were kept at the stable for 10-14 days until the wound healed, while sutures were removed before the start of the rehabilitation process. They were allowed to walk on the asphalt ground for 150m every day for 2 weeks before radiographic evaluation was performed at the 6th and 12th week after meniscectomy.

Radiographic Evaluation by Conventional Radiography

Radiographic evaluations were performed before, 6 weeks after and 12 weeks after the meniscectomy. For each animal, mediolateral and craniocaudal projections of the stifle joints using the hindlimb stifle joint protocol were performed. For the craniocaudal projection, the animal was held in the 'sitting' position to obtain a seemingly 'weight-bearing' position. Meanwhile for mediolateral projection, the animal was positioned in lateral decubitus with the contralateral limb held away from the film. All of the results were processed through VetDROC application with Insan Teknotama Bersahaja® using craniocaudal and mediolateral hindlimb stifle protocol (Fig. 2).

A total of five parameters were observed from conventional radiography according to Innes et al. (2004); these included global score for overall disease severity (0-3), joint effusion (0-2), osteophytosis (0-3), intra-articular mineralization (0-2) and tibial subchondral sclerosis (0-1), as shown in Fig. 3. Each of these parameters was evaluated by two observers and the mean score was recorded.

RESULTS

A total of 14 sheep weighing from 25-30kg were subjected to mediolateral and craniocaudal hind stifle joint radiograph before meniscectomy to show that these animals were free from any prior pathology on the cartilage. There was non-significant difference in the total radiograph score between 6 weeks and 12 weeks after meniscectomy (5.18$\pm$0.39 vs 5.88$\pm$0.78; P=0.662). There were also non-significant differences in the mean progression of radiographic OA score after 12 weeks of meniscectomy between animals of 28-30kg and above 30kg (5.86$\pm$0.69 vs 5.78$\pm$0.83; P=0.842). Mean scores of each radiographic OA parameter are given in Table 1.

Although there were non-significant differences in the total OA score radiographically between 6th and 12th weeks, however the mechanism of post-traumatic OA was elaborated by evaluating the progression of these

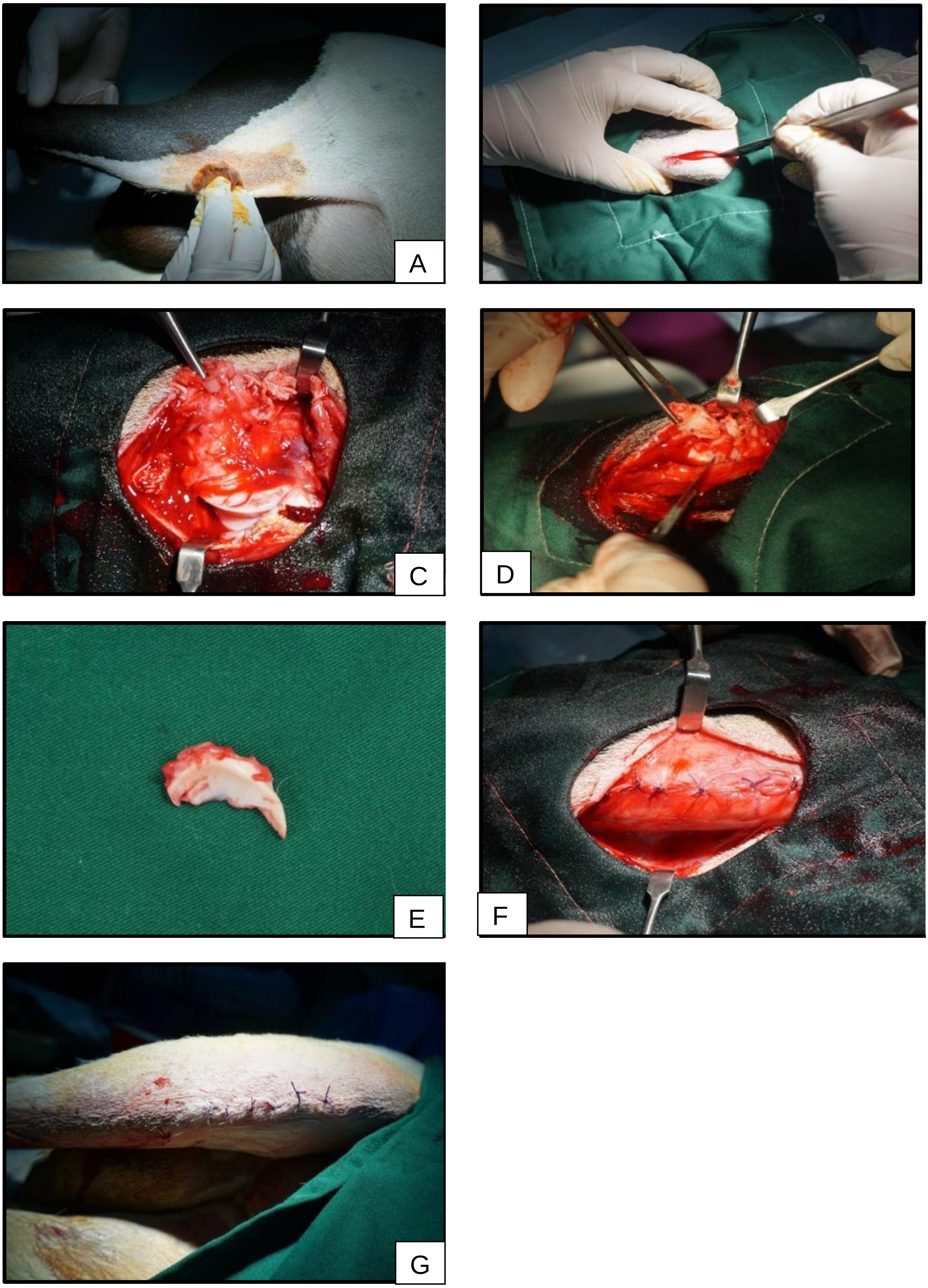

Fig. 1: Total lateral meniscectomy. A) Septic and antiseptic procedure, B) skin incision proximal and distal to the stifle joint, C) exposure of the stifle joint and anterior meniscus horn, D) sharp excision of the anterior meniscus horn, E) meniscus lateral excised, F) soft tissue closure and G) skin closure.

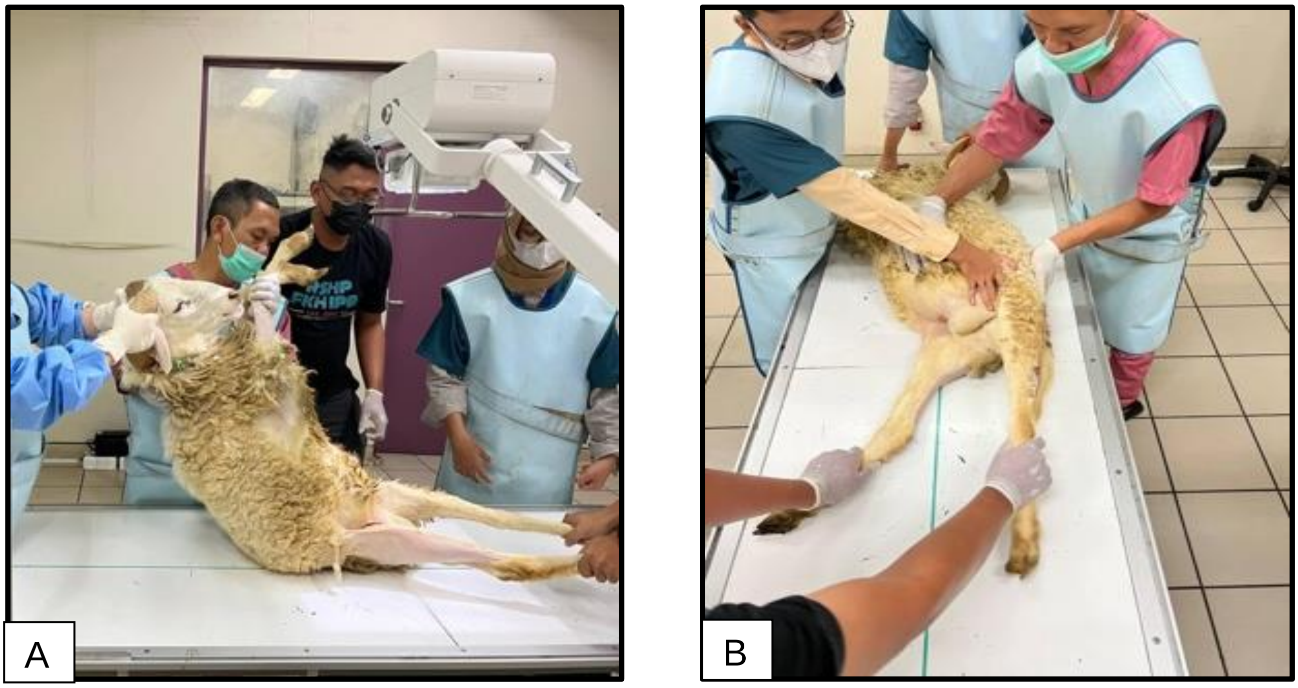

Fig. 2: Conventional radiograph positioning for craniocaudal and mediolateral projection.

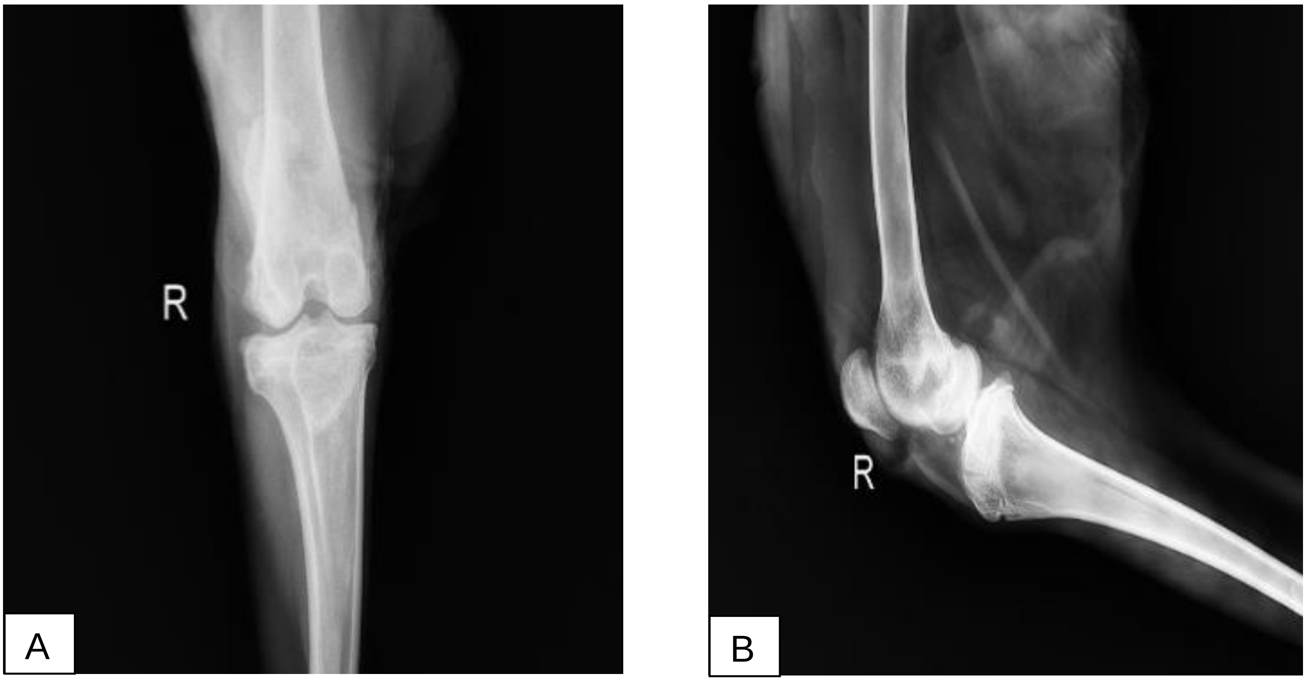

Fig. 3: Radiographic scoring of stifle joint. Arrow shows osteophyte formation, while asterisk shows intra-articular mineralization.

Table 1: Mean radiographic scores at 6- and 12-weeks post meniscectomy

Parameter	6 Weeks*	12 Weeks*
Global	2.00±0.00	2.12±0.33
Effusion	2.00±0.00	1.12±0.33
Osteophyte	1.12±0.33	1.88±0.33
Mineral	0.06±0.24	0.06±0.24
Sclerosis	0.00	0.65±0.61
Total	5.18±0.39	5.88±0.78

*Data is presented as mean±SD.

parameters. Initially after the injury, inflammation took place hence higher effusion score was seen at the 6th week compared to the 12th week (2±0.00 vs 1.12±0.33). There was an increasing osteophyte formation as the time passed, but the difference was statistically non-significant (1.12±0.33 vs 1.88±0.33; P=0.378). However, at the later stage, the incidence of sclerosis was observed, which was not seen initially (Fig. 4).

DISCUSSION

The most important finding in this study is that total lateral meniscectomy in stifle joint could create low grade OA as early as 6 weeks after intervention proven radiologically. Knee osteoarthritis is one of the most common degenerative diseases and is the most studied

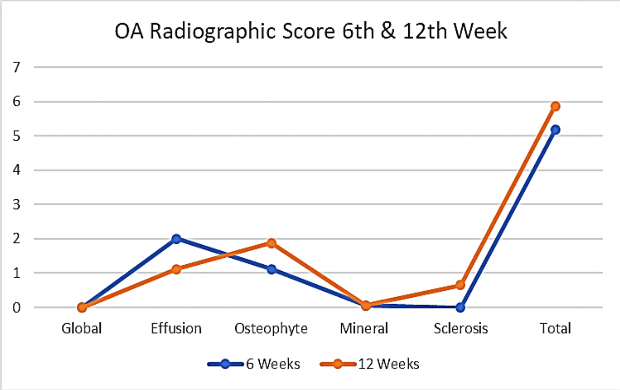

Fig. 4: OA Radiographic score at 6th and 12th week post meniscectomy.

problem (Cui et al. 2020). Recently, many new biologic agents have been investigated, aiming to regenerate the damaged cartilage process at the earlier stages of OA (Dilogo et al. 2020; Shegos and Chaudhry 2022; Rauck et al. 2022). Evaluation of a new biologic agent must first comply with the basic safety and efficacy based on the animal study. The model used must be as analogous as the human knee joint. *Ovis* The stifle joint of sheep (*Ovis aries*) was selected in this study because of its similarity in size and topography with the human knee joint (Burger et al. 2007; Oláh et al. 2019). Previous studies have elaborated the progression of OA in the stifle joint of dogs (Gilbertson 1975; Dedrick et al. 1993; Widmer et al. 1994). However, in a country such as ours with multicultural beliefs, the usage of dogs as an animal model is not recommended due to the local culture. The other commonly used OA models are rabbit (Yoshioka et al. 1996) and horse joints (Mcilwraith et al. 2012) but the size is rather too small in the rabbit and too large in the horse. Monkey's knee joint (Bi et al. 2021) is quite similar to human's knee joint but it also has some ethical issues. Therefore, in this study we selected ovine stifle joint for this study due to the above reasons.

Lateral total meniscectomy was chosen due to its natural function that carries a larger proportion of load in the lateral compartment and a more understood character in the lateral compared to the medial meniscus. According to Little et al. (1997), lateral meniscectomy produces histomorphological and immunohistochemical cartilage changes as in early OA in ovine OA model after six months. This finding correlates with that of our study, where osteophyte formation as early as 6 weeks was seen radiologically in 100% of the ovine model.

Information on radiographic progression of stifle osteoarthritis is lacking, especially for the *Ovis aries* sheep stifle joint (Appleyard et al. 2003; von Lewinski et al. 2006). Several workers tried to elaborate the radiologic parameters on stifle joint OA and suggested that although several radiologic parameters measured can be related to the severity of the disease, none could be related with clinical and temporal conditions (Gilbertson 1975; Dedrick et al. 1993; Widmer et al. 1994). Our findings are supported by those of Innes et al. (2004), who showed osteophyte formation and effusion in the earliest phase (after 6 weeks), while at the later stage effusion went down and intra-articular mineralization and subchondral sclerosis took place. These findings correlate well with the pathophysiology of post-traumatic OA, which starts with inflammation and hematoma and is followed by the compensation remodeling (subchondral sclerosis).

Conclusion

Based on the findings of the present study it can be concluded that total lateral meniscectomy in the ovine stifle joint could create low grade OA as early as 6 weeks, which was confirmed radiologically by osteophyte formation. Further studies are needed to evaluate the correlation between radiological and clinical findings.

Authors' Contribution

All authors contributed equally in the execution of the study and preparation of the manuscript.

Funding

The study was funded by the Riset dan Inovasi Indonesia Maju 2022.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could cause a potential conflict of interest.

REFERENCES

Appleyard RC, Burkhardt D, Ghosh P, Read R, Cake M, Swain MV and Murrell GAC, 2003. Topographical analysis of the structural, biochemical and dynamic biomechanical properties of cartilage in an ovine model of osteoarthritis. Osteoarthritis Cartilage 11(1): 65–77. https://doi.org/10.1053/joca.2002.0867

Beveridge JE, Shrive NG and Frank CB, 2011. Meniscectomy causes significant *in vivo* kinematic changes and mechanically induced focal chondral lesions in a sheep model. Journal of Orthopaedic Research 29(9): 1397–1405. https://doi.org/10.1002/jor.21395

Bi X, Li T, Li M, Xiang S, Li J, Ling B, Wu Z and Chen Z, 2021. A new method to develop the primate model of knee osteoarthritis with focal cartilage defect. Frontiers in Bioengineering and Biotechnology 9: 727643 https://doi.org/10.3389/fbioe.2021.727643

Burger C, Mueller M, Wlodarczyk P, Goost H, Tolba RH, Rangger C, Kabir K and Weber O, 2007. The sheep as a knee osteoarthritis model: Early cartilage changes after meniscus injury and repair. Laboratory Animals 41(4): 420–431. https://doi.org/10.1258/002367707782314

Cui A, Li H, Wang D, Zhong J, Chen Y and Lu H, 2020. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. The Lancet 29: 100587. https://doi.org/10.1016/j.eclinm.2020.100587

Dedrick DK, Goldstein SA, Brandt KD, O'Connor BL, Goulet RW and Albrecht M, 1993. A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. Arthritis and Rheumatology 36(10): 1460–1467. https://doi.org/10.1002/art.1780361019

Dilogo IH, Canintika AF, Hanitya AL, Pawitan JA, Liem IK and Pandelaki J, 2020. Umbilical cord-derived mesenchymal stem cells for treating osteoarthritis of the knee: A single-arm, open-label study. European Journal of Orthopaedic

Surgery and Traumatology 30: 799–807. https://doi.org/10.1007/s00590-020-02630-5
Gilbertson EM, 1975. Development of periarticular osteophytes in experimentally induced osteoarthritis in the dog: A study using microradiographic, microangiographic, and fluorescent bone-labelling techniques. Annals of the Rheumatic Diseases 34(1): 12–25. http://dx.doi.org/10.1136/ard.34.1.12
Holland JC, Brennan O, Kennedy OD, Mahony NJ, Rackard S, O'Brien FJ and Lee TC, 2013. Examination of osteoarthritis and subchondral bone alterations within the stifle joint of an ovariectomised ovine model. Journal of Anatomy 222(6): 588-597. https://doi.org/10.1111/joa.12051
Innes JF, Costello M, Barr FJ, Rudorf H and Barr ARS, 2004. Radiographic progression of osteoarthritis of the canine stifle joint: a prospective study. Veterinary Radiology and Ultrasound 45(2): 143-148. https://doi.org/10.1111/j.1740-8261.2004.04024.x
Kopf S, Sava MP, Stärke C and Becker R, 2020. The menisci and articular cartilage: a life-long fascination. EFORT Open Reviews 5(10): 652-662. https://doi.org/10.1302/2058-5241.5.200016
Kuyinu, EL, Narayanan G, Nair LS and Laurencin CT, 2016. Animal models of osteoarthritis: Classification, update, and measurement of outcomes. Journal of Orthopaedic Surgery and Research 11: 19. https://doi.org/10.1186/s13018-016-0346-5.
Lampropoulou-Adamidou K, Lelovas P, Karadimas EV, Liakou C, Triantafillopoulos IK, Dontas I and Papaioannou NA, 2014. Useful animal models for the research of osteoarthritis. European Journal of Orthopaedic Surgery and Traumatology 24(3): 263–271. https://doi.org/10.1007/s00590-013-1205-2
Little C, Smith S, Ghosh P and Bellenger C, 1997. Histomorphological and immunohistochemical evaluation of joint changes in a model of osteoarthritis induced by lateral meniscectomy in sheep. Journal of Rheumatology 24(11): 2199–2209.
Mcilwraith W, Frisbie DD and Kawcak CE, 2012. The horse as a model of naturally occurring osteoarthritis. Bone and Joint Research 1(11): 297-309. https://doi.org/10.1302/2046-3758.111.2000132
Morgan JP, 1968. Radiographic diagnosis of bone and joint diseases in the horse. The Cornell Veterinarian 58: 28-47.
Moya-Angeler J, Gonzales-Nieto J, Monforte JS, Altonaga JR, Vaquero J and Forriol F, 2016. Surgical induced models of joint degeneration in the ovine stifle: Magnetic resonance imaging and histological assessment. The Knee 23(2): 214-220. https://doi.org/10.1016/j.knee.2015.11.017
O'Brien EJO, Beveridge JE, Huebner KD, Heard BJ, Tapper JE, Shrive NG and Frank CB, 2012. Osteoarthritis develops in the operated joint of ovine model following ACL reconstruction with immediate anatomic reattachmentof the native ACL. Journal of Orthopaedic Research 31: 35-43. https://doi.org/10.1002/jor.22187
Oláh T, Reinhard J, Gao L, Haberkamp S, Goebel LKH, Cucchiarini M and Madry H, 2019. Topographic modeling of early human osteoarthritis in sheep. Science Translational Medicine 4(11): 508. https://doi.org/10.1126/scitranslmed.aax6775
Rauck RC, Eliasberg CD, Rodeo S and Rodeo SA, 2022. Orthobiologics for the management of early arthritis in the middle-aged athletes. Sports Medicine and Arthroscopy Review 30(2): e9–e16. https://doi.org/10.1097/JSA.0000000000000337
Shegos CJ and Chaudhry AF, 2022. A narrative review of mesenchymal stem cells effect on osteoarthritis. Annals of Joint 21: 16. https://doi.org/10.21037/aoj-21-16
Teeple E, Jay GD, Elsaid KA and Fleming BC, 2013. Animal models of osteoarthritis: challenges of model selection and analysis. American Association of Pharmaceutical Sciences Journal 15(2): 438-446. https://doi.org/10.1208/s12248-013-9454-x
Veronesi F, Vandenbulcke F, Ashmore K, Di-Matteo B, Nicoli Aldini N, Martini L, Fini M and Kon E, 2020. Meniscectomy-induced osteoarthritis in the sheep model for the investigation of therapeutic strategies: A systematic review. International Orthopaedics 44(4): 779–793. https://doi.org/10.1007/s00264-020-04493-1
Von Lewinski G, Stukenborg-Colsman C, Ostermeier S and Hurschler C, 2006. Experimental measurement of tibiofemoral contact area in a meniscectomized ovine model using a resistive pressure measuring sensor. Annals of Biomedical Engineering 34(10): 1607–1614. https://doi.org/10.1007/s10439-006-9200-y
Widmer WR, Buckwalter KA, Braunstein EM, Hill MA, O'Connor BL and Visco DM, 1994. Radiographic and magnetic resonance imaging of the stifle joint in experimental osteoarthritis of dogs. Veterinary Radiology and Ultrasound 35(5): 371–384. https://doi.org/10.1111/j.1740-8261.1994.tb02057.x
Yoshioka M, Coutts RD, Amiel D and Hacker SA, 1996. Characterization of a model of osteoarthritis in the rabbit knee. Osteoarthritis and Cartilage 4(2): 87–98. https://doi.org/10.1016/s1063-4584(05)80318-8
Zhu C, Wu W and Qu X, 2021. Mesenchymal stem cells in osteoarthritis therapy: A review. American Journal of Translational Research 13(2): 448–461.