ORIGINAL ARTICLE

The Effect of Intra-Articular Adipose Mesenchymal Stem Cell-Derived Exosomes in the Treatment of Low-Grade Osteoarthritis in Animal Model: Radiological and Histological Evaluation

Jessica Fiolin[1], Ismail Hadisoebroto Dilogo[2], Ludwig Andre Pontoh[2], Radiana Dhewayani Antarianto[3], Marcel Prasetyo[4], Alida Harahap[1], Trevino Aristakus Pakasi[5], Bambang Pontjo Priosoeryanto[6], Tri Isyani Tungga Dewi[6], Angela Jennifer Tantry[7]

[1] Doctoral Programme of Medical Science, Faculty of Medicine, Universitas Indonesia, Jl Salemba Raya no. 4, Jakarta,Indonesia
[2] Doctoral Programme of Medical Science, Faculty of Medicine, Universitas Indonesia, Jl Salemba Raya no. 4, Jakarta,Indonesia
[3] Department of Histology, Faculty of Medicine Universitas Indonesia, Jl. Salemba Raya no. 4, Jakarta, Indonesia
[4] Department of Radiology, Dr. Cipto Mangunkusumo General Hospital, Universitas Indonesia, Jl. Diponegoro no. 71, Jakarta Pusat, 10430, Indonesia
[5] Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No. 16, Cikini, Jakarta Pusat, 10320, Indonesia
[6] School of Veterinary Medicine & Biomedical Science, IPB University, Bogor Agatis Dramaga Campus IPB Bogor, 16680, Indonesia
[7] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia

ABSTRACT

Introduction: Recent studies have shown the regenerative potency of extracellular vesicle Mesenchymal Stem Cells (EV-MSCs) due to their non-cellular, low immunogenic profile and chondrogenic potency. Osteoarthritis of the knee (KOA) is one of the most prevalent degenerative diseases caused by progressive cartilage degeneration; to date, there is no cure. This study aims to evaluate the effect of intra-articular exosome adipose-derived mesenchymal stem cell (AD)-MSC injection in an ovine low-grade OA model radiologically and histologically. **Materials and Methods:** A total of 14 male Ovis aries sheep with low-grade OA were divided into seven treatment groups: control (NaCl), 1x exosome, 2x exosome, 3x exosome, 1x hyaluronic acid (HA), 2x HA, and 3x HA injection groups. Each group that received multiple injections was injected at a one-week interval. After six weeks of intervention, a radiographic examination was performed, and the ovine was sacrificed. After the sacrifice, histological analysis was performed. **Results**: Radiographic evaluation showed the slowest progression of OA in the 3x exosome group, followed by 2x exosome, 1x exosome, 3x HA, 2x HA, 1x HA, and control group consecutively with delta progression (0.5; 1; 1; 1; 1.5; 1.5; 2). Meanwhile, histological analysis showed that the 3x exosome group retained proteoglycan content in the ovine osteoarthritis model cartilage matrix. **Conclusion:** 3x exosome injection with a one-week interval showed the slowest progression and highest matrix retainment in the ovine low-grade OA model. Further studies with larger samples should be performed to evaluate the statistical result of these differences.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP8):43-48.doi:10.47836/mjmhs.21.s8.7

Keywords: Intra-articular exosome, Adipose-derived mesenchymal stem cell (AD-MSC), Ovis aries, osteoarthritis, radiologic

Corresponding Author:
Jessica Fiolin, M.D. (JF)
Email: jessica_fiolin@yahoo.co.uk
Tel :+6282118123750

INTRODUCTION

Knee osteoarthritis (KOA) is one the most prevalent degenerative joint diseases that causes a vast socio-economic burden worldwide (1, 2). However, to date, there have been no satisfying treatments that can reverse the progressive cartilage degeneration in osteoarthritis (OA). Current advances in regenerative ortho-biologic therapy have been researching the chondrogenic potency of extracellular vesicle Mesenchymal Stem cells (EV-MSCs) (3-6).

Recently MSC exosome has garnered attention as a biological therapy in OA due to its ability to affect cellular communication7, smaller size molecule compared to MSC, while having less adverse effects and have the advantage of production, distribution and storage easiness compared to MSC. Exosomes are nano-sized extra vesicles 50-150 nm in diameter that contain nucleic acid, functional protein, and bioactive lipids secreted by MSCs. Various MSC sources have been documented in the isolation of exosomes (7, 8). Adipose-derived MSC (ADMSC-derived EVs) can rectify abnormal osteoblast metabolism and promote cartilage and bone regeneration by regulating focal adhesion, extracellular matrix (ECM)-receptor interaction, actin cytoskeleton, cAMP, and PI3-Akt signaling pathways (9).

The effect of exosome injection could be evaluated radiologically and histologically. Several radiological score systems have been developed to assess the progression of OA in animals, such as radiological scoring by Innes et al., 2004 (10). Meanwhile, histological analysis can be used to see cartilage quality change or damage severity after treatment (11). Sheep have similar size, biomechanics, and joint structure to humans. These similarities make sheep an advantageous animal model to study cartilage histological processes in orthopaedic studies (12).

However, to date, no study has evaluated the clinical effect of intraarticular AD-MSC in vivo in a larger animal model with low-grade OA. This study aimed to evaluate the effect of intraarticular exosome AD-MSC in the ovine early OA model radiologically and histologically.

MATERIALS AND METHODS

A total of 14 Ovies aries sheep included in this study performed general examination and radiographic assessment before the study to rule out prior pathology in the lower extremity area. Inclusion criteria involve; (1) male aged three or more years old, weighing 25-30 kg, (2) skeletally mature, and (3) no history of trauma and congenital anomaly at the lower extremities. While exclusion criteria include; (1) death; (2) surgical site infection; (3) deformity of the lower extremity; and (4) cartilage damage upon meniscectomy. Ten days before the meniscectomy, the ovine was acclimatized to the cage and the environment. The meniscectomy was performed totally at the lateral meniscus of the right ovine hind limb at the same time under anesthesia as described in the previous study (13). After the procedure, one veterinarian managed wound care for two weeks before suture removal and rehabilitation. Radiographic evaluations were performed before and after meniscectomy. This study had received ethical clearance protocol 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

Radiologic Evaluation by Conventional Radiology
Radiographic examinations were performed before meniscectomy, six weeks after meniscectomy, and four weeks after the intervention period (10 weeks after meniscectomy using a high-resolution film screen combination using POX-100BT by POSKOM, Gyeonggi, Korea and viewed using VetDROC by Insan Teknotama Bersahaja application. One veterinarian and one orthopedic surgeon evaluated and scored the results two separate times and the total mean score was used for evaluation. Mediolateral and craniocaudal projections of the stifle joints using the hindlimb stifle joint protocol are performed for each sample. For the craniocaudal projection, the ovines was held in the 'sitting' position to obtain a seemingly 'weight-bearing' position. Meanwhile, the ovines were positioned in lateral decubitus for mediolateral projection, with the contralateral limb held away from the film. The results were processed using the VetDROC application by Insan Teknotama Bersahaja using craniocaudal and mediolateral hindlimb stifle protocol. In contrast, the radiologic parameters used were 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) (10). Two observers evaluated each of the parameters and recorded the mean score.

Total Lateral Meniscectomy Procedure
All ovine were clinically evaluated for general health, vaccination status, and acclimatized for two weeks in the laboratory before the procedure. Before the procedure, all ovines were shaved, and a conventional radiograph was taken to exclude prior pathology in the lower extremity. A total lateral meniscectomy was performed at the right hind limb of the ovines. Prophylactic intramuscular (IM) amoxicillin 10 mg /kg body weight (BW) and 0.22 mg /kg BW of sulfas atropine were administered as a premedication subcutaneously. Induction was started by giving 11 mg/kg BW of Ketamine IM with xylazine 0.22 mg /kg BW as a muscle relaxant. Regular intravenous (IV) saline maintenance was administered at the front limb during the surgery.

After the septic and aseptic procedures, one surgeon performed standard surgical draping. The incision was performed using a lateral parapatellar approach 5 cm proximal to the kneecap and 5 cm distal to the upper end of the tibia. After subcuticular tissue was retracted, the 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 (Figure 1). The operation field was then irrigated with normal saline, and the surgical wound was sutured layer by layer.

After the surgery, the ovine was kept at the stable for 10-14 days until the wound was healed and suture removal was performed before the rehabilitation process started. The ovine was exercised to walk on the asphalt ground for 150 m every day for two weeks before the radiographic evaluation was performed.

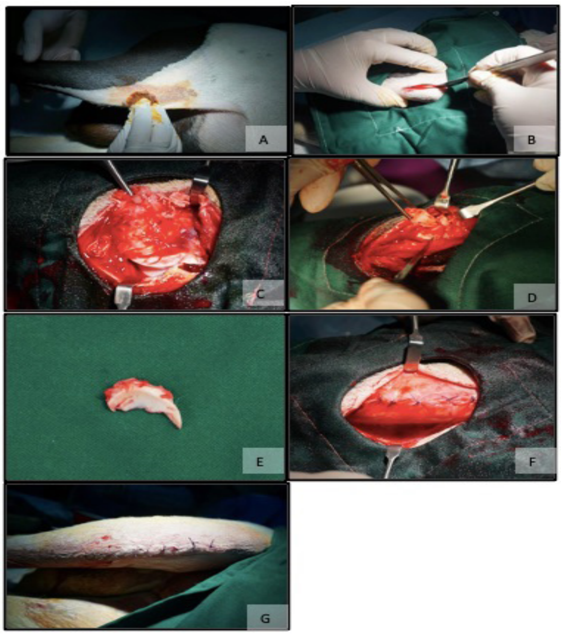

Figure 1. Total lateral meniscectomy. A, Septic and antiseptic procedure; B, skin incision proximal and distal to the stifle joint; C, exposure of the stifle joint and anterior meniscus horn; D, sharp excision of the anterior meniscus horn; E, meniscus lateral excised; F, soft tissue closure; G, skin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

Exosome Preparation

The exosome we used in this study was the one we have successfully isolated in the previous study (14). We successfully isolated the exosome through the ultracentrifugation process. The isolated exosome met Minimal Information for Studies of Extracellular Vesicles (MISEV) exosome criteria. The exosome we isolated had a particle size of 88.7 ± 40 nm, zeta potential ranging from -1.4 to -10.2 mV, conductivity between 14.945 to 14.982 mS/cm, and positive CD63 and CD81 protein markers. Following the exosome isolation, we collected 1 mL of exosome solution to become one dose of exosome injection.

Intra-articular Injection

After all wounds had healed, all ovine were divided into seven groups to receive intraarticular injections, including once 1 ml of NaCl injection as control, 1x exosome injection, 2x exosome injection, 3x exosome injection, 1x hyaluronic acid (HA) injection, 2x HA injection, and 3x HA injection. The exosome AD-MSC was prepared according to our previous protocol. Meanwhile, the HA used was 1 ml of high viscosity HA, Durolane®, which has been reported to contain the highest molecular weight HA (10^{15}kDa) with a safety confirmation of repeated injection.

Histology Examination

After sacrifice, the knee joint specimens were fixed in 10% formaldehyde and decalcified in an electrolytic decalcifying solution. After the decalcification process had finished, the most destroyed area of 5x5x5 mm was extracted sharply for each region including, lateral femoral condyle, lateral tibial plateau, trochlear groove, and patellar region and further embedded in paraffin and sectioned with 5 µm thickness. Histological staining was done using 0,04% toluidine blue, hematoxylin, and eosin staining (15, 16). Observations from several low-power fields were stitched to display the whole cartilage surface using Fiji's ImageJ plugin (17). Observations were done on cartilage surface, cell density, cloning, and matrix staining (18).

RESULT

Fourteen ovines weighing 28-38 kg with early OA and a mean pre-intervention total radiographic OA score of 5.21± 0.57 out of 11 points can be categorized as mild OA. There was no significant difference in the mean progression of radiographic OA score between pre-and post-intervention (5.21±0.57 vs. 5.78±0.83); p = 0.842. Table I describes the mean scores of each OA parameter between groups before and after intervention.

Table I shows that the highest increase in radiologic score was consecutively in the NaCl group, 1x HA, 2x HA, 3x HA, 1x exosome, 2x exosome, and 3x exosome injection groups. This data means that the 2x and 3x injections of exosome AD-MSC produced the lowest OA progression, indicating a chondroprotective behavior compared to other groups (Figure 2).

Table I. Mean Radiographic Score Pre and Post Intervention Each Group. Data presented in mean

	NaCl	Exo-some -1	Exo-some -2	Exo-some -3	HA-1	HA-2	HA-3
Global pre	2	2	2	2	2	2	2
Global post	3	3	2	2	3	2.5	2.5
Effusion pre	2	2	2	2	2	2	2
Effusion post	1	1	1.5	1	1	1	1
Osteo-phyte pre	1	1	1	1.5	1	1.5	1
Osteo-phyte post	2	2	2	2	2	2	2
Mineral pre	0	0.5	0	0	0	0	0
Mineral post	1	0.5	0	0	0	0	0
Sclerosis pre	0	0	0	0	0	0	0
Sclerosis post	0	0	0.5	0.5	0.5	0	1
Total pre	5	5.5	5	5.5	5	5.5	5
Total post	7	6.5	5.5	6	6.5	6.5	6
Delta	2	1	0.5	0.5	1.5	1	1

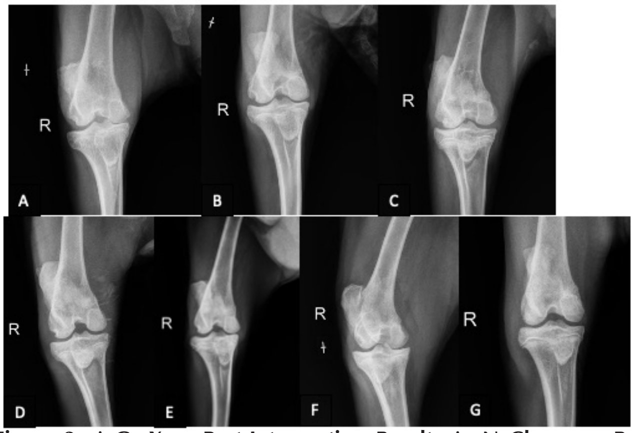

Figure 2, A-G. Xray Post-Intervention Result. A, NaCl group; B, HA-1 group; C, HA-2 group; D, HA-3 group; E, Exosome-1 group; F, Exosome-2 group; G, Exosome-3 group

The post-intervention X-ray showed prominent osteophyte formation in all groups compared to the pre-intervention condition. There was also sclerosis formation in groups 1x HA, 3x HA, 2x exosome, and 3x exosome injection groups, and a general increase in the global radiographic severity in the NaCl (Figure 2A), 1x HA (Figure 2B), 2x HA (Figure 2C), 3x HA (Figure 2D), and 1x exosome (Figure 2E) injection groups. In contrast, the other 2x exosome (Figure 2F) and 3x exosome (Figure 2G) injection groups seemed more benign.

Histologic Evaluation

Cartilage lesions were found on all subjects. Sections with the worst lesions were histologically analyzed. In the control group, cartilage had eroded, and subchondral bone was exposed. The remaining surrounding cartilage had increased cellularity, with cells in doublet or triplets. No staining was observed on the matrix (Figure 3A).

In the group treated with 1x HA injection, lesions were found to reach calcified cartilage. Cellularity was increased with cells in doublets, triplets, or clusters. Matrix staining was decreased to the middle zone or calcified cartilage (Figure 3B). In the group treated with 2x HA injections, lesions reached calcified cartilage. Slight fibrillation was observed on the surface of the remaining surrounding cartilage. Cellularity was increased with cells in clusters. Matrix staining was decreased to the middle zone (Figure 3C). In the group treated with 3x HA injections, the lesion reached the subchondral bone. Cellularity was normal, and cells were found in doublets or triplets. Matrix staining decreased to the middle zone (Figure 3D).

In the group treated with 1x exosome injection, the lesions were found to reach calcified cartilage or subchondral bone. The number of cells observed tended to increase with cells in doublets or triplets found in the remaining surrounding cartilage. Matrix staining was decreased to the middle zone (Figure 3E). Similar results were observed in the group treated with 2x exosome injections. Cells were found primarily on doublets or clusters. Matrix staining was decreased to the middle or deep zone (Figure 3F). In the group treated with 3x exosome injections, lesions reached the subchondral bone. Normal cellularity was observed, but cells in doublets or triplets were found. However, matrix staining was observed to be normal (Figure 3G).

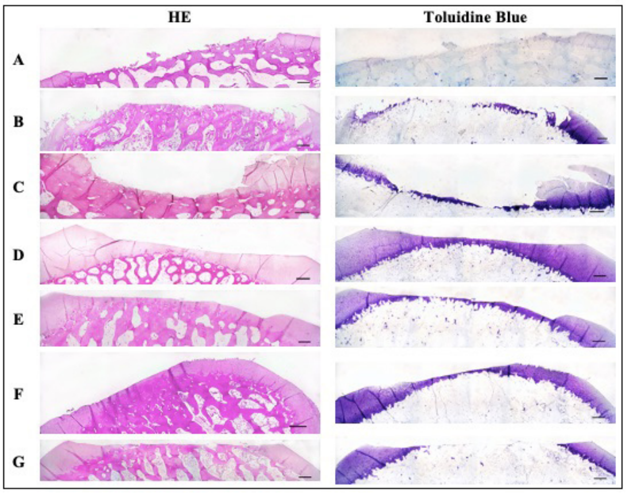

Figure 3 A-G. Representative histologic staining (HE and Toluidine Blue. 40Ч. 500 µm scale bar). A, NaCl group; B, HA-1 group; C, HA-2 group; D, HA-3 group; E, Exosome-1 group; F, Exosome-2 group; G, Exosome-3 group

DISCUSSION

This is the first worldwide study that evaluated the effect of Ad-MSC-exosome in the treatment of OA in large animal. Sheep stifle joint has the topography similar to human knee and the size of 1/3 human knee which made it as a better animal model since we can evaluate the parameters better. For conventional radiology evaluation, we evaluated the global score for the overall disease severity, joint effusion, osteophytosis, intra-articular mineralization, and tibial subchondral sclerosis (10). Radiographic OA score showed decreased effusion from all groups after the intervention compared to post-meniscectomy. The reduction in effusion component was due to the reduced inflammation phase commonly happened in post-operative period. The osteophyte formation from all groups slightly increased before and after meniscectomy and was relatively stable after the intervention. The most prominent difference was found in the development of sclerosis and global radiographic score, which is remained minimal in the 3x exosome and 2x exosome group while the other group showed more advanced progression.

The best radiographic score after intervention showing the slower progression of low-grade OA was observed in 3x exosome, 2x exosome, 1x exosome, 3x HA, 2x HA, 1x HA, and NaCl injection groups respectively. According to previous literature, exosome treatment increases chondrogenic markers such as β-cathenin and type II collagen (19, 20). An increase in chondrogenic markers indicates the occurrence of chondrogenesis, thus delaying the development of OA. The administration of HA helps to counteract the effect of damaged HA in the joints with OA (21). However, HA does not generate a chondrogenic effect, unlike exosomes. Nonetheless, no significant difference was found in the comparison between groups in this study which could be due to the smaller number of samples in each group and difficulties in obtaining weight-bearing images and image interposition (10, 22). This finding was also similar in the clinical setting where conventional radiographic evaluation is often if not almost always, performed in daily outpatient clinic to evaluate OA and mostly showed statistical insignificancies but correlates well with a more detailed MRI result (23).

Histological analysis was done on the worst lesions found in each subject. The worst lesion and cartilage quality were observed and compared between groups. The most notable difference was seen in matrix staining across observed groups. Toluidine blue did not stain the cartilage matrix of the control group, but staining was observed in groups treated with HA and exosome (Figure 3). Matrix staining in the exosome-treated groups was variable, but the group treated with three times exosome injections showed normal and even staining (Figure 3E-3G).

Positive toluidine blue staining represents the amount of proteoglycans present in the matrix (18). Wang et al. previously reported that exosomes derived from embryonic stem cell-induced MSCs (ESC-MSCs) were able to alleviate matrix degradation in mice OA models by supporting collagen type II synthesis and decreasing expression of ADAMTS-5 (24). However, Li et al. reported that AD-MSCs EVs could support matrix synthesis and were superior to EVs derived from BMSC or SMSC (25). Therefore, as described in our study, the 3x exosome injection group (Figure 3G) produced better outcomes than all treatment groups by maintaining the proteoglycan content in the cartilage matrix. In the HA-treated group, matrix staining decreased in the middle zone. Although it is not as potent as the 3x exosome injection group, compared to the NaCl-treated group, HA-treated groups can retain proteoglycan content to some degree (Figure 3B-3D).

Furthermore, histological analysis in this study showed normal cellularity in the group treated with 3x exosome injections. Despite that, cells were found in doublets and triplets. Proliferating cells were also found in other treatment groups. Even though the overall morphology of chondrocytes is normal, chondrocytes have already started proliferating, indicating that the development of OA is progressing (11). This finding is linear with the previous study that AD-MSC exosome could protect cartilage and bone degradation in OA by increasing the expression of type II collagen and reducing metabolic markers such as MMP-13 and ADAMTS5 hence protecting chondrocytes from apoptosis and blocking macrophage activation along with increasing chondrocyte migration and proliferation (26). MicroRNA (miRNA) was one of the most important bioactive molecule ingredient in exosome that could alter the cellular communication process. Several miRNA have been identified in previous studies that could modulate the progression of OA in animal model, such as miR-92a-3p that could promote chondrocyte proliferation, miR-136-5p promote chondrocyte migration and reduce degradation of ECM and miR-338-3p that stimulate cell proliferation and inhibit cell apoptosis (27).

The most crucial finding in this study is that the injection of 3x intra-articular AD-MSC exosome showed the slowest progression of low-grade OA through radiological and histological evaluation. In histological evaluation, the 3x exosome injection group demonstrated better proteoglycan maintenance than other groups. However, cells were proliferating, suggesting that OA's development had already occurred. Thus, further evaluation through other means is needed to confirm AD-MSC exosome injections' effect on the ovine OA cartilage model and biomolecular analysis to elucidate its mechanism of action.

The strength of this study is that it is the first study that has demonstrated the potency of exosome AD-MSC injection in treating low-grade OA through conventional radiology and histologic evaluation. Furthermore, we identify the minimum optimum dose for exosome AD-MSC injections to elicit their beneficial effects. The limitation of this study is the limited samples distributed to each group due to financial limitations. However, despite financial limitations, we have successfully compared the exosome group with the control group (NaCl-treated group) and the current treatment group (HA-treated groups). Future studies should evaluate the long-term effect of exosome AD-MSC treatment for low-grade OA through various components of evaluations with larger samples.

CONCLUSION

This study evaluated the effects of various treatments on low-grade osteoarthritis (OA) using conventional radiology and histological analysis. The best radiographic outcomes were seen in groups treated with 3x exosome injections. Histological analysis revealed better proteoglycan maintenance in the 3x exosome injections group, compared to HA and control groups. Despite positive results, further evaluation with larger samples is needed to confirm the long-term efficacy of AD-MSC exosome injections in treating OA.

ACKNOWLEDGMENTS

This study was supported by RIIM 2022-2023 grant.

REFERENCES

1. Primorac D, Molnar V, Rod E. Jelec Z. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes. 2020;11:854. doi: 10.3390/genes11080854
2. Ronn K, Reischl N, Gautier E, Jacobi M. Current surgical treatment of knee osteoarthritis. Arthritis. 2011;454873. doi: 10.1155/2011/454873
3. Voskamp C, Koevoet JLM, Somoza RA, Caplan AI, Lefebvre V, van Osch GJVM, et al. Enhanced Chondrogenic Capcaity of Mesenchymal Stem Cells After TNFa Pre-treatment. Front in Bioeng and Biotech. 2020;9:658. doi: 10.3389/fbioe.2020.00658
4. Somoza RA, Welter JF, Correa D, Caplan AI. Chondrogenic differentiation of mesenchymal stem cells: challenges and unfulfilled expectations. Tissue Eng Part B, Rev. 2014;20(6):596-608. doi: 10.1089/ten.teb.2013.0771
5. Linero I, Chaparro O, Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. Plos One. 2014;9(9), e107001. doi: 10.1371/journal.pone.0107001
6. Chang C, Yan J, Yao Z, Zhang C, Li X, Mao HQ. Effects of mesenchymal stem cell-derived paracrine signals and their delivery strategy. Adv health mat. 2011;10(7): e2001689. doi: 10.1002/adhm.202001689
7. Yaya T, Yan Z, Hong-Jun L. Advances in mesenchymal stem cell exosomes: a review. Stem Cell Res & Therapy. 2021;71:12. doi: https://doi.org/10.1186/s13287-021-02138-7
8. Sepideh N, Jafar R, N MZ, Reza J. Mesenchymal stem cell derived-exosomes: a modern approach in translational medicine. J of Transl Med. 2020; 18:449. doi: 10.1186/s12967-020-02622-3
9. Song H, Zhao J, Cheng J, Feng Z, Wang J, Momtazi-Borojeni AA, et al. Extracellular Vesicles in chondrogenesis and cartilage regeneration. J of Cell and Moll Med. 2021; 25, 11. doi: 10.1111/jcmm.16290
10. Innes JF, Costello M, Barr FJ, Rudolf H, Barr ARS. Radiographic progression of osteoarthritis of the canine stifle joint: a prospective study. Vet Radiol & Ultrasound. 2004;45(2):143-8. doi: 10.1111/j.1740-8261.2004.04024.x.
11. Pritzker KPH, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoar and cart. 2006;14:13-29. doi: 10.1016/j.joca.2005.07.014.
12. Potes JC, da Costa Reis J, Silva FC, Relvas C, Cabrita AS, Simoes JA. The Sheep as an animal model in orthopaedic research. Exp path and health sci. 2008;2(1): 29-32.
13. Fiolin J, Priosoeryanto BP, Tri Isyani RDA, Marcel Prasetyo ARH, Pakasi TA, Dilogo IH, Pontoh LA. Early low-grade knee osteoarthritis in sheep (ovis aries) after 6-weeks of total unilateral meniscectomy: a radiographic evaluation. Int J Vet Sci. 2024;13(1):94-9. doi: https://doi.org/10.47278/journal.ijvs/2023.065
14. Fiolin J, Dilogo IH, Antarianto RD, Pontoh LA. Isolation and characterization of adipose-derived mesenchymal stem cell exosomes: an in-vitro study. Study J Prof Medika. 2022;16(2). doi: https://doi.org/10.33533/jpm.v16i2.4867
15. Jusuf AA. Histoteknik Dasar. Fak Kedokter Univ Indo. 2008; 32.
16. Schmitz N, Laverty S, Kraus VB, Aigner T. Basic methods in histopathology of joint tissues. Osteoar and cart. 2010;18: S113-S116. doi: 10.1016/j.joca.2010.05.026
17. Preisbich S, Saalfeld S, Tomancak P. Globally optimal stitching of tiled 3D microscopic image acquisitions. Bioinformatics. 2009; 25(11):1463-65. doi: 10.1093/bioinformatics/btp184
18. Bergholt NJ, Lysdahl M, Lind M, Foldager CB. A standardized method of applying toluidine blue metachromatic staining for assessment of chondrogenesis. Cartilage. 2019; 10(3): 370-4. doi: 10.1177/1947603518764262.
19. Yang Z, Zou Y, Guo XM, et al.: Temporal activation of β-catenin signaling in the chondrogenic process of mesenchymal stem cells affects the phenotype of the cartilage generated. Stem Cells Dev. 2012; 21: 1966–1976. doi: 10.1089/scd.2011.0376.
20. Li Q, Yu H, Sun M, et al.: The tissue origin effect of extracellular vesicles on cartilage and bone regeneration. Acta Biomater. 2021; 125: 253–266. doi: 10.1016/j.actbio.2021.02.039.
21. Chavda S, Rabbani SA, Wadhwa T: Role and effectiveness of intra-articular injection of hyaluronic acid in the treatment of knee osteoarthritis: a systematic review. Cureus. 2022; 4: 26. doi: 10.7759/cureus.24503.
22. Ol6h T, Reinhard J, Gao L, Haberkamp S, Goebel LKH, Cucchiarini M, Madry H. Topographic modeling of early human osteoarthritis in sheep. Sci Transl Med. 2019;11(508):eaax6775. doi: 10.1126/scitranslmed.aax6775.
23. Bensaoud N, Rostom S, Tazi L, et al. Correlation between MRI and conventional radiography findings in knee osteoarthritis. Ann of Rheum Dis. 2015;74:925.
24. Wang Y, Yu D, Liu Z, Zhou F, Dai J, Wu B, et al. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing syntesis and degradation of cartilage extracellular matrix. Stem Cell Res & Ther. 2017; 8:189. doi: 10.1186/s13287-017-0632-0.
25. Li Q, Yu H, Sun M, Yang P, Hu X, Ao Y, et al. The tissue origin effect of extracellular vesicles on cartilage and bone regeneration. Acta Biomater. 2021;125:253-266. doi: 10.1016/j.actbio.2021.02.039.
26. Mianehsaz, E., Mirzaei, H.R., Mahjoubin-Tehran, M. et al. Mesenchymal stem cell-derived exosomes: a new therapeutic approach to osteoarthritis?. Stem Cell Res Ther. 2019; 10: 340. doi: 10.1186/s13287-019-1445-0
27. Luo D, Zhu H, Li S, Wang Z and Xiao J.Mesenchymal stem cell-derived exosomes as a promising cell-free therapy for knee osteoarthritis. Front Bioeng Biotechnol. 2024; 12:1309946. doi: 10.3389/fbioe.2024.1309946