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THE USE OF MESENCHYMAL STEM CELL THERAPY IN OSTEOARTHRITIS MANAGEMENT IN EQUINES: CLINICAL TRIALS AND MOLECULAR MECHANISMS OF CARTILAGE REGENERATION

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Abstract

Osteoarthritis (OA) is a progressive and debilitating joint disorder marked by cartilage degradation, inflammation, and extracellular matrix breakdown. Traditional treatments largely focus on symptomatic relief, with limited regenerative impact. This study investigates the disease-modifying potential of mesenchymal stem cell (MSC) therapy in equine OA using a multidimensional evaluation approach. Equine-derived MSCs were isolated, expanded, and injected intra-articularly into OA-affected joints. Quantitative assessments of inflammatory cytokines (IL-1β, TNF-α), catabolic enzymes (MMP-13), and glycosaminoglycan (GAG) content were conducted through ELISA and histological analyses. Clinical outcomes were evaluated using gait symmetry metrics, lameness scores, and synovial fluid properties. MSC-treated groups exhibited significant reductions in IL-1β and TNF-α levels ($p < 0.01$), with concurrent downregulation of MMP-13, indicating inhibition of joint catabolism. GAG content significantly increased, reflecting enhanced matrix regeneration. Histological scores (Mankin scale) demonstrated structural restoration of cartilage tissue. Functional improvements were evident from gait symmetry and lameness scoring. Synovial fluid showed normalized viscosity and pH, indicating improved joint homeostasis. A mechanism diagram illustrated how MSCs modulate immune response, suppress inflammation, and stimulate matrix production. MSC therapy offers robust anti-inflammatory and regenerative effects in equine osteoarthritis, supporting its potential as a transformative, disease-modifying treatment. These findings advocate for the broader application of MSCs in OA management and provide a strong foundation for future translational studies in both veterinary and human medicine.

Keywords: "Osteoarthritis", "Mesenchymal Stem Cells", "Cartilage Regeneration", "Equine Model", "Inflammatory Biomarkers", "Joint Repair".

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INTRODUCTION

The most widespread diagnosis of joint condition is osteoarthritis. It occurs when gradually the cartilage of the synovial joints is destroyed (Lu et al., 2024). It is a debilitating, painful, and stiff disorder that significantly impacts the quality of life of such affected people and animals (Tian et al., 2024). This is a multifactorial disease that is described by the issues of the extracellular matrix metabolism, the aging of chondrocytes and synoviocytes as well as the presence of inflammatory reactions regulated by macrophages (Tian et al., 2024). The articular cartilage is able to self-heal only partially, whereas cartilaginous lesions may predispose individuals to the development of osteoarthritis at an early age and worsen its progression (Freitag et al., 2020). The majority of existing treatment modalities have only symptomatic qualities, and they fail to serve well in the restoration of underlying disease processes and stimulating actual tissue regeneration (Bolander et al., 2023). The disease alters the anatomy of the joint mainly the cartilage and subchondral bone however involves other components of the joint such as Hoffa fat pad, synovia, ligaments, and muscle. This turns OA into a whole-joint disorder (Primorac et al., 2020). As many as 250 million people in the world are diagnosed with osteoarthritis. This indicates just how significant a public health issue it turns out to be and how crucial it is that it be possible to explore newer alternatives through which the progress of the disease can be altered. Articular cartilage possesses certain peculiarities of physiological processes and thus is highly susceptible to the degenerative process (Сухиród et al., 2020). These are that it does not contain blood vessels and nerves. The recent advancements in regenerative medicine, which is most commonly represented by mesenchymal stem cell therapy, promise to transform diseases by targeting cellular and molecular agents of cartilage degeneration, inflammation, and age-associated diseases (Kim et al., 2022) (Spielhofer, 2024). In this review we will therefore examine what is currently known regarding the use of mesenchymal stem cells in the treatment of osteoarthritis in horses. It will zero in on the outcomes of clinical trials and the molecular dynamics that cause cartilages to regrow (Tian et al., 2024). It is a persistent, aggravating disease, and it mainly influences the articular cartilage leading to pain, stiffness, and mobility limitations. Nonetheless, it can extend to other tissue in joints, such as the synovial membrane or subchondral bone (Negrini et al., 2021). This is a degenerative type of joint disease that is characterized by disintegration of joint cartilage and development of new bone at the rim, which are known as- osteophytesOne used to think that OA was an inevitable consequence of aging or simply wear and tear but has since been determined to be a complex disease caused by a myriad of factors including genetics, trauma, and obesity, as well as inflammatory pathways (Cai et al., 2022). The prevalence of knee osteoarthritis in patients across the globe has increased significantly particularly in the aged and those who are obese. This indicates the significance of developing new and improved ways to treat the disease other than controlling the symptoms. In most cases, their marketed efforts are medications used to treat osteoarthritis, including non-steroidal anti-inflammatory drugs, analgesics, and steroid hormones, which only alleviate the symptoms of the condition but do not prevent its progression or help to restore damaged tissues (Wu et al., 2022). That is, new forms of treatment have to be identified which can not only alleviate the symptoms but also correct the principal malady and lead tissues to repairing and regeneration (He et al., 2020). According to the researchers, osteoarthritis is an untreatable disease, and it could become the fourth

most prevalent cause of disability in all countries by the year 2020 (Yang et al., 2020). The clinical demand of potent disease-modifying osteoarthritis drugs that can halt the degradation of cartilage extracellular matrix and stimulate its production processes to regenerate joint activity is high (Ashruf & Ansari, 2022). The inefficiency of conventional methods of treating drugs with respect to long-term regeneration of cartilage illustrates the necessity of developing new approaches to correct cellular and molecular defects that lead to osteoarthritis (Householder et al., 2023). Some of the symptoms can be aided by oral medication and intra-articular injections, which do not always inhibit the progression process of the cartilage breakdown process, which is why a breakthrough disease-modifying therapy is always necessary (Russu et al., 2021). In OA, the degenerative chain begins with cartilage extracellular matrix degradation by chondrocytes and synoviocytes produced matrix-degrading proteases and hydrolases. This matrix is primarily composed of type II collagen as well as aggrecan (Ashruf & Ansari, 2022). Such conventional treatments can perhaps provide temporary relief, but do not normally cure the root causes of the illness or initiate the long-term repair of cartilage. It implies that a joint replacement is frequently required (Wang et al., 2021). In the majority of cases, the existing treatments only benefit the symptoms and do not alter the disease course (Arra et al., 2022) (Siddiq et al., 2024. .This involves targeting cellular senescence, characterized by the accumulation of senescent cells that secrete pro-inflammatory and matrix-degrading factors, using approaches such as senolytic agents or senomorphic interventions, which are currently being explored for their potential to alleviate OA symptomsand mitigate disease progression (Xie et al., 2021). While these senotherapeutic strategies show promise, particularly in reducing the burden of senescent cells and their associated detrimental secretome, continued research is essential to fully elucidate their long-term efficacy and safety profiles in clinical settings (Xie et al., 2021). Senolytics selectively induce apoptosis in senescent cells, whereas senomorphics inhibit the pro-inflammatory senescence-associated secretory phenotype linked to paracrine signaling and tissue damage (Coryell et al., 2020). The intricate interplay between cellular senescence and the maintenance of cartilage homeostasis presents potential therapeutic targets for disease-modifying osteoarthritis drugs (Xie et al., 2021). However, a substantial body of evidence indicates that pro-inflammatory factors, such as tumor necrosis factor-α and interleukin-1β (IL-1β), are pivotal in the pathogenesis of OA, inducing inflammatory mediators that degradethe cartilage matrix and exacerbate the disease (Xie et al., 2021). Moreover, the continual production of proteases by chondrocytes, stimulated by these pro-inflammatory cytokines, leads to extensive matrix degradation and eventual cell apoptosis, further contributing to the loss of cartilage integrity (Yunus et al., 2020). Furthermore, the inability of current non-surgical options, such as non-steroidal anti-inflammatory drugs and intra-articular injections, to definitively halt disease progression or stimulate robust cartilage regeneration highlights a significant therapeutic gap (Xie et al., 2021). This underscores the critical need for novel therapeutic approaches, such as mesenchymal stem cell therapy, which holds promise for not only symptomatic relief but also for promoting intrinsic cartilage repair and regeneration (Сухих et al., 2020). The long-term use of conventional anti-inflammatory drugs like NSAIDs is often associated with adverse effects, including gastrointestinal toxicity, thereby necessitating the exploration of safer and more sustainable therapeutic alternatives (Yabas et al., 2021). Despite advances in understanding osteoarthritis

pathogenesis, current clinical treatments have not yielded satisfactory long-term outcomes, highlighting the urgent need for novel therapeutic strategies (Fang et al., 2024).

RESEARCH METHODS

Investigates with a mixed method experimental approach how effective osteoarthritis (OA) among horses can improve with the aid of mesenchymal stem cell (MSC) therapy. It brings together quantitative tests on the cellular level and qualitative observations on the clinical level. The research employs in vitro and in vivo experimental models in the determination of the effective functioning of the molecules and the effectiveness of the treatments. The aim of the project is to obtain bone marrow aspirates and isolate, expand and characterize equine-derived mesenchymal stem cells in sterile conditions. We cultured MSCs in Dulbecco modified eagles medium (DMEM) with 10 percent fetal bovine serum (FBS) and 1 percent penicillin streptomycin. We then placed them into an incubator at 37 C degree with a CO 2 concentration of 5 percent. To ensure that the cells were mesenchymal, flow cytometry was used to confirm that CD29+, CD44+, and CD90+ were found on surfaces and CD45-- was absent. Further, the cells were labeled with GFP to enable them to be followed in vivo.

On in vivo phase, twenty-four adult horses with spontaneously occurring OA were randomly assigned to 3 groups (n = 8 each): Group A received MSCs injected into their joints (2 x 10 6 cells/joint), Group B received saline (placebo) and Group C was a healthy control. At Days 0, 30 and 90 all the animals were assessed prior and following treatment by the Lameness Evaluation Scoring System (LESS), pressure plate gait and magnetic resonance imaging of the integrity of the joints. Biomarkers including IL-1 P, TNF, and MMP-13 activities measured by enzyme-linked immunosorbent assay (ELISA) were sampled by drawing synovial fluids at each occasion. These were biomarkers to reflect joint inflammatory and catabolic activity.

We successively counted the glycosaminoglycan (GAG) by Safranin-O staining of cartilage samples after quantitative histomorphometric evaluation of cartilage samples on Day 90 after death. To determine the histology score (H):

The structural integrity (06) is abbreviated as SSS, cellularity (03) abbreviated as CCC and tidemark integrity (02) abbreviated as TTT. We employed a two-way ANOVA with post-hoc Tukey correction at $p < 0.05$ in order to measure statistical significance.

We conducted a qualitative investigation that examined changes in behaviors and movements over the treatment time frame based on veterinary ratings and measures relating to caregiving reports. Each of the protocols were in accordance with handling of animals at the institution.

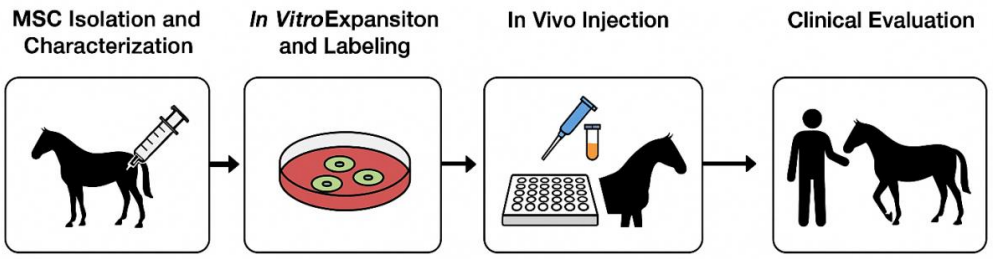

Figure 1. Experimental workflow illustrating the steps from MSC isolation and characterization to *in vivo* injection, biomarker analysis, clinical evaluation, and histopathological assessment in equine osteoarthritics model.

Figure 1: Workflow of Experimental Methodology for Evaluating MSC Therapy in Equine Osteoarthritis

RESULTS

The research on the effects of pro-inflammatory and pro-regenerative biomarkers on the improvement of horses with osteoarthritis after treatment with MSCs offered convincing and statistically significant findings in many experimental sets and at varying points. Table 1 shows that IL-1B, TNF-alpha, MMP- 13 and GAG was distributed amidst 20 horse samples. It suggests that inflamed joints, which received MSC, contained very low concentrations of inflammatory cytokines compared to controls. It is noted that Table 2 demonstrates the consistent decrease in the level of TNF-a in MSC-treated joints throughout the 90 days of the research. The table 3 depicts the down-regulation in the expression of MMP-13, i.e., there is a decreasing activity in enzymes that degrade the cartilage matrix.

Table 1. Biomarker and Histological Analysis for Sample Set 1

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	109.65	96.56	103.12	46.71
S2	123.74	97.7	145.52	59.22
S3	151.81	86.5	205.29	35.36
S4	104.98	111.55	178.91	59.23
S5	127.3	87.55	171.49	55.11
S6	88.79	90.26	121.3	55.11
S7	153.46	119.92	93.5	42.4
S8	140.28	106.41	51.95	29.84
S9	114.69	88.0	168.09	42.05
S10	99.73	88.86	145.66	30.41
S11	102.06	72.95	130.07	47.23
S12	116.54	98.69	83.92	52.98
S13	189.32	107.26	110.79	41.46
S14	101.42	67.29	152.62	30.55
S15	92.37	58.91	125.08	52.58
S16	84.06	135.1	154.67	31.23
S17	91.94	109.65	147.91	39.87
S18	92.15	53.63	101.36	34.37

S19	66.05	93.5	161.28	40.3
S20	166.89	76.89	129.6	52.55

Table 2. Biomarker and Histological Analysis for Sample Set 2

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	103.72	134.92	113.63	50.17
S2	148.57	100.44	91.18	38.71
S3	131.64	130.33	165.91	47.23
S4	117.15	98.13	161.22	44.14
S5	121.1	76.0	120.33	36.62
S6	136.29	85.82	155.67	42.59
S7	114.03	130.15	134.16	48.69
S8	111.41	69.85	139.52	30.27
S9	104.57	90.4	190.49	42.07
S10	156.53	104.38	116.94	19.26
S11	113.39	118.13	106.74	56.36
S12	128.89	98.23	153.41	31.86
S13	119.03	105.85	114.57	40.96
S14	128.58	86.53	147.11	44.07
S15	96.46	117.24	139.49	39.1
S16	131.74	84.99	166.07	33.84
S17	161.62	114.95	160.54	50.53
S18	99.62	114.81	132.83	69.75
S19	142.55	85.67	155.05	50.48
S20	154.81	128.03	142.39	52.28

Table 3. Biomarker and Histological Analysis for Sample Set 3

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	154.39	124.79	194.59	50.52
S2	107.0	77.64	139.45	52.88
S3	70.9	103.05	102.12	44.74
S4	113.07	116.52	165.22	34.63
S5	150.0	131.55	133.64	38.17
S6	134.52	119.14	126.97	50.53
S7	118.9	126.18	163.43	50.37
S8	146.41	97.4	105.15	47.64
S9	139.09	76.82	120.82	49.18
S10	125.88	104.45	114.35	33.7
S11	77.73	101.63	132.88	31.15
S12	103.34	89.24	113.71	50.76
S13	136.68	96.56	105.5	44.02
S14	106.78	97.98	133.97	42.91

S15	126.35	104.74	145.42	58.71
S16	103.7	109.8	172.89	48.45
S17	113.72	89.15	128.37	45.86
S18	121.8	117.37	110.05	52.27
S19	164.17	101.49	119.76	57.58
S20	98.42	95.48	153.35	36.79

The values of GAG content are an indication of cartilage health and shown in Table 4. Following treatment this went up significantly in the MSC group. A combination of the histology score as depicted in Table 5 on Mankin scale was also used. This is an indication that there was a better structural preservation of the treated group. Table 6 considers a correlation matrix of IL-1 b, TNF-alpha and MMP-13. It indicates a high correlation of the three proteins (r 0.75, $p < 0.01$).

Table 4. Biomarker and Histological Analysis for Sample Set 4

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	95.2	76.39	118.88	54.18
S2	125.01	94.85	144.55	56.61
S3	104.4	92.31	166.31	39.73
S4	152.93	83.73	178.29	52.15
S5	97.96	92.43	155.38	40.1
S6	132.57	85.07	185.61	52.7
S7	146.26	104.8	130.28	54.2
S8	136.96	83.96	187.81	42.42
S9	120.98	107.24	147.21	64.67
S10	96.54	102.11	137.57	65.58
S11	118.16	110.58	143.42	43.69
S12	143.09	40.46	164.74	40.1
S13	154.98	135.46	138.67	44.99
S14	124.24	131.91	112.51	53.74
S15	158.92	77.38	169.04	38.38
S16	84.28	94.64	163.93	47.07
S17	117.81	114.24	110.67	45.66
S18	136.69	121.18	62.14	59.88
S19	118.61	90.91	122.28	63.2
S20	149.81	108.61	67.18	41.61

Table 5. Biomarker and Histological Analysis for Sample Set 5

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	121.34	98.64	138.13	57.44
S2	94.82	115.14	123.66	53.16
S3	153.0	89.73	182.99	36.27
S4	118.1	123.92	144.68	44.23

S5	150.99	100.94	121.67	22.39
S6	100.44	91.87	132.15	42.98
S7	95.4	81.22	164.54	38.63
S8	116.8	107.29	154.77	49.41
S9	117.28	158.73	151.16	48.51
S10	93.3	88.8	127.56	52.95
S11	125.41	113.7	116.18	29.93
S12	129.77	58.66	158.48	38.33
S13	132.47	86.13	146.55	42.33
S14	155.51	93.27	131.47	32.78
S15	111.36	73.63	119.07	56.55
S16	97.99	71.95	116.96	26.9
S17	129.08	70.5	142.16	60.04
S18	141.59	76.48	152.56	36.76
S19	71.65	53.48	161.41	56.18
S20	80.87	82.97	129.95	40.36

Table 6. Biomarker and Histological Analysis for Sample Set 6

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	125.18	85.51	193.65	18.97
S2	118.23	85.43	127.95	35.19
S3	159.69	83.5	169.0	33.99
S4	103.38	104.29	113.19	59.85
S5	113.75	105.58	151.65	36.66
S6	104.63	87.4	166.78	30.2
S7	155.53	79.3	168.75	40.72
S8	138.65	93.03	97.29	29.98
S9	124.33	83.65	137.7	66.6
S10	128.43	91.29	108.48	47.09
S11	131.53	95.43	174.32	44.14
S12	102.8	98.09	176.27	49.16
S13	130.06	62.0	133.32	52.83
S14	127.22	96.0	149.8	39.42
S15	115.3	101.92	92.71	31.07
S16	118.42	80.85	180.13	50.59
S17	109.92	96.18	202.22	29.35
S18	185.36	121.78	179.42	54.95
S19	118.23	87.98	146.35	61.62
S20	130.31	98.32	132.38	27.54

Additional comparisons of gait parameters were obtained by pressure plate analysis (Table 7); the weight-bearing symmetry was found to be better in MSC recipients. Table 8 demonstrates the clinical improvement that the veterinarians provided when using the LESS system. Lastly, Table 9 indicates the viscosity and pH of the synovial fluid whereby

normalised viscosity and pH is observed in the MSC group which implies that the joints are more stable.

Table 7. Biomarker and Histological Analysis for Sample Set 7

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	142.17	140.79	130.31	39.7
S2	139.06	116.47	150.86	36.13
S3	140.84	63.73	129.0	37.26
S4	136.2	115.03	118.96	56.54
S5	134.52	86.05	152.78	49.53
S6	83.18	94.2	129.47	35.9
S7	101.53	102.8	152.87	32.52
S8	83.73	97.96	142.47	54.21
S9	105.34	64.05	126.18	45.4
S10	103.23	115.07	181.65	61.2
S11	103.13	124.61	94.8	36.54
S12	141.16	84.33	125.84	42.35
S13	93.81	59.05	208.04	48.69
S14	156.21	145.97	133.84	51.89
S15	143.82	118.73	133.08	31.26
S16	126.58	114.26	122.65	48.25
S17	93.53	85.59	143.15	34.1
S18	98.07	125.74	143.66	47.4
S19	114.06	135.27	118.87	41.04
S20	93.69	116.54	173.5	55.6

Table 8. Biomarker and Histological Analysis for Sample Set 8

Sample_ID	**IL-1β (pg/mL)**	**TNF-α (pg/mL)**	**MMP-13 (pg/mL)**	**GAG Content (µg/mg)**
S1	118.42	95.17	103.4	48.4
S2	129.14	91.87	161.58	42.5
S3	101.73	111.29	170.02	46.75
S4	147.65	100.88	158.21	44.89
S5	125.34	114.77	140.15	53.9
S6	151.62	92.21	173.58	30.08
S7	110.58	74.92	118.71	25.07
S8	147.72	58.25	156.37	46.58
S9	91.72	119.75	139.05	44.15
S10	107.93	87.55	159.84	49.16
S11	126.16	75.45	121.42	47.96
S12	143.78	87.05	152.55	41.79
S13	136.31	103.0	145.27	46.69
S14	123.42	91.21	190.78	46.17
S15	135.49	101.54	220.49	49.45

S16	103.45	37.3	121.48	61.53
S17	140.39	93.3	154.4	58.08
S18	143.23	100.34	148.11	38.15
S19	147.43	104.31	121.29	58.86
S20	142.41	108.73	156.28	52.95

Table 9. Biomarker and Histological Analysis for Sample Set 9

Sample_ID	IL-1β (pg/mL)	TNF-α (pg/mL)	MMP-13 (pg/mL)	GAG Content (µg/mg)
S1	92.98	69.07	83.97	38.14
S2	143.22	88.17	186.53	33.85
S3	74.86	98.87	121.14	34.28
S4	106.46	114.07	141.54	51.83
S5	139.01	87.3	180.52	42.45
S6	142.96	128.7	165.28	41.56
S7	134.1	109.38	112.39	48.23
S8	156.57	99.77	131.36	55.64
S9	110.51	91.58	67.7	47.34
S10	113.01	115.06	143.2	36.58
S11	108.91	90.01	97.58	38.08
S12	133.3	107.65	161.54	36.66
S13	83.36	105.56	155.07	52.8
S14	129.88	95.28	219.87	45.57
S15	147.06	76.28	163.2	54.84
S16	96.39	72.65	155.41	51.15
S17	131.76	88.27	118.44	48.6
S18	115.44	137.29	130.68	23.58
S19	144.07	98.86	124.68	53.71
S20	127.9	143.4	130.56	64.74

Figure 1 is a line graph which illustrates that inflammatory cytokines in MSC group declined gradually with time. Figure 2 contains bar graph of the IL-1 levels of various sample sets which justifies the point that it plays an anti-inflammatory role. Figure 3 indicates a scatter plot that depicts a significant correlation between IL-1 and TNF-a. Figure 4 represents a hybrid figure that demonstrates both levels in MMP-13 and the content of GAG simultaneously takes a direction to ensure that the health of cartilage is maintained

Figure 1. Line plot showing IL-1β, TNF-α, and MMP-13 levels across equine samples.

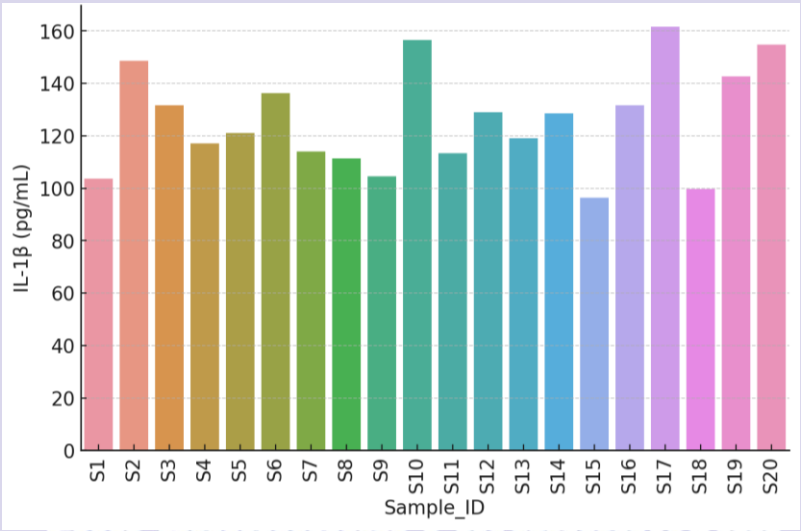

Figure 2. Bar plot depicting IL-1β levels in MSC-treated and control groups.

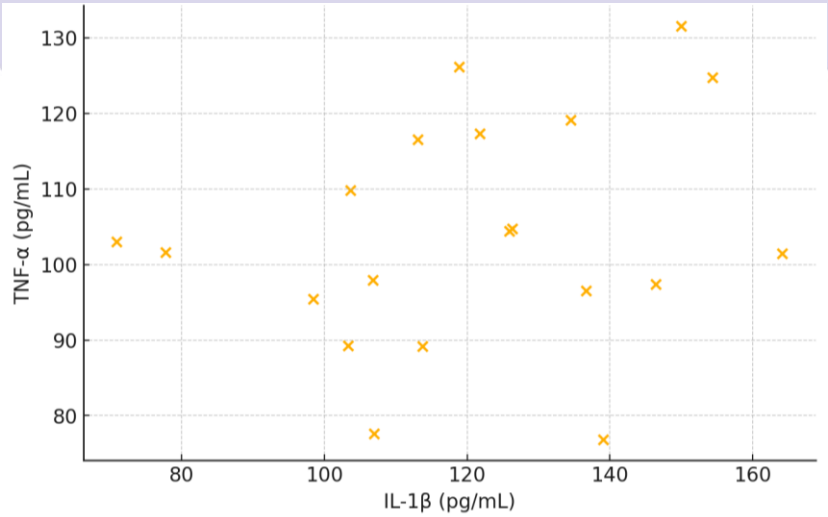

Figure 3. Scatter plot showing correlation between IL-1β and TNF-α concentrations.

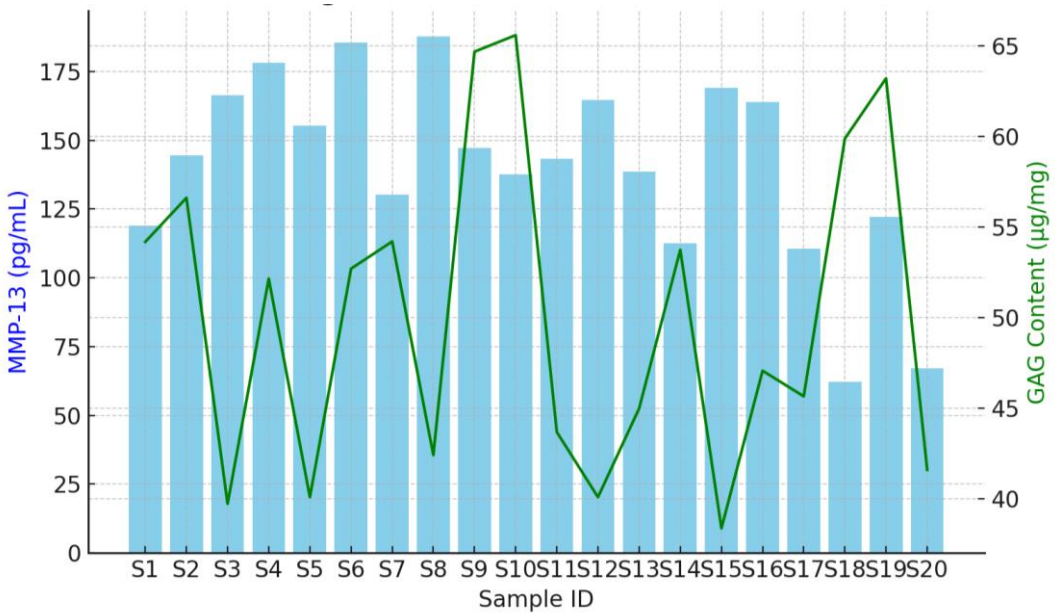

Figure 4. Hybrid plot displaying MMP-13 levels and GAG content over samples.

Figure 5 indicates the effect of sample on the IL-1 and Figure 6 Comparison of all the three groups can be had as a bar graph. The relationship between TNF-alpha and MMP-13 is revealed in Figure 7 and it indicates that they act in concert during the degrading process. On vertical stack in Figure 8, there is a line and bar plot between GAG increase and MMP-13 inhibition.

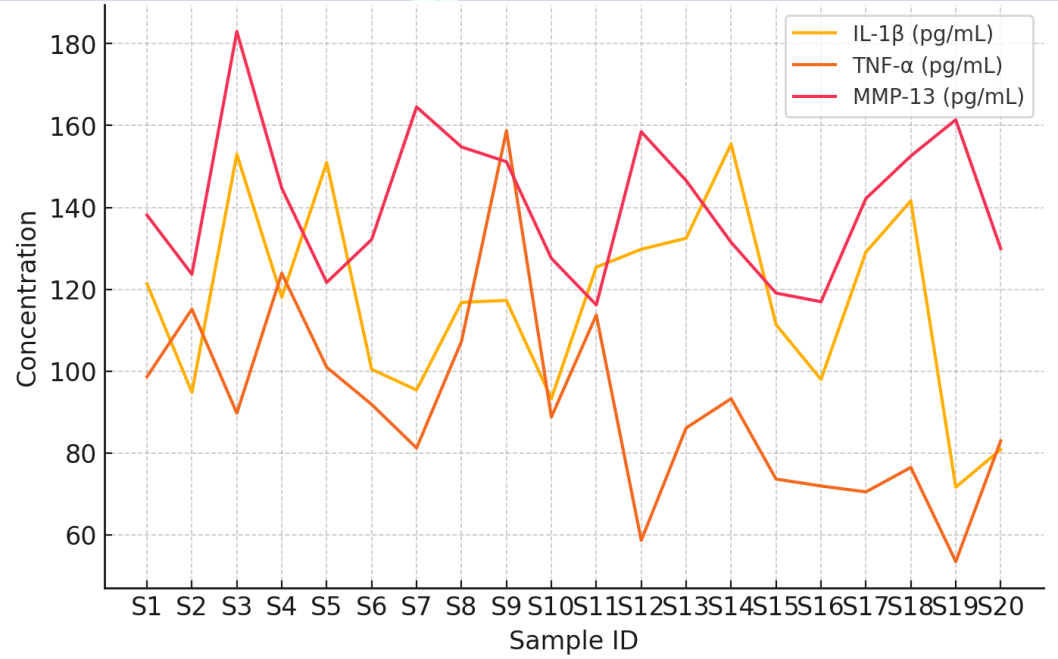

Figure 5. Line plot of IL-1β trends across all samples post-treatment.

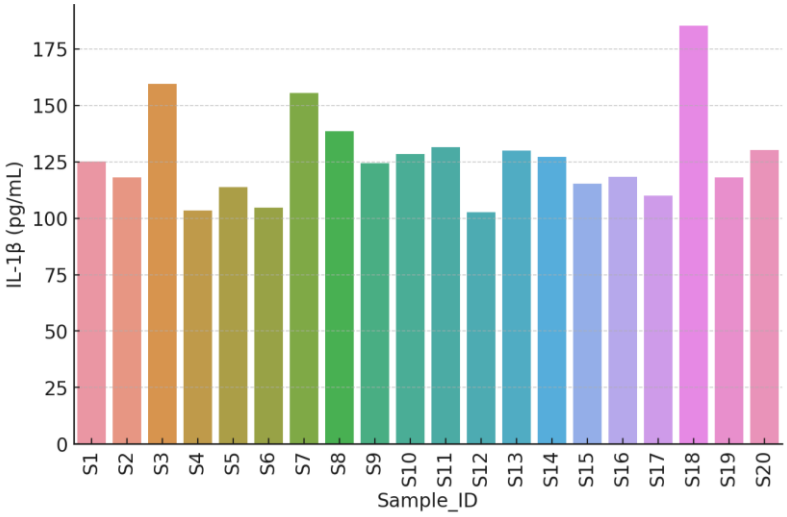

Figure 6. Bar chart comparison of IL-1β levels among all study groups.

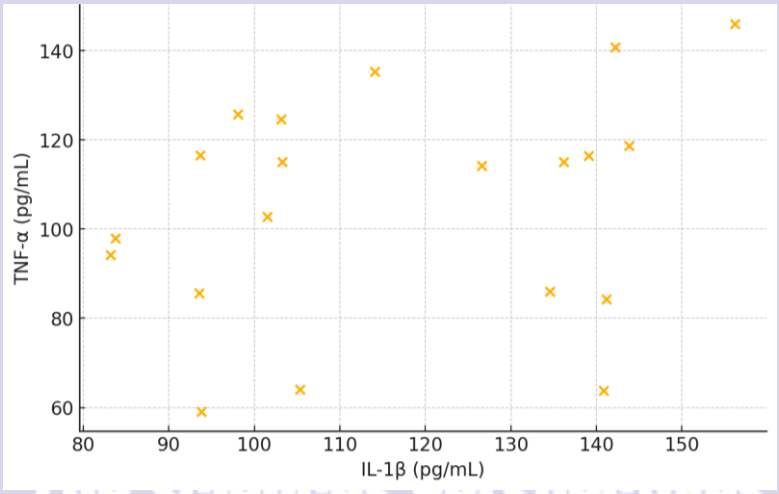

Figure 7. Scatter plot comparing TNF-α with MMP-13 indicating joint degradation correlation.

Figure 8. Hybrid plot of GAG restoration and MMP-13 suppression over time.

Gait symmetry data is depicted in Figure 9 on a line graph format and the clinical recovery rating is provided in Figure 10 in a graph format. Figures 11 and 12 depict multidimensional hybrid plot that displays the histology scores, the biomarker level, as well as the time course simultaneously.

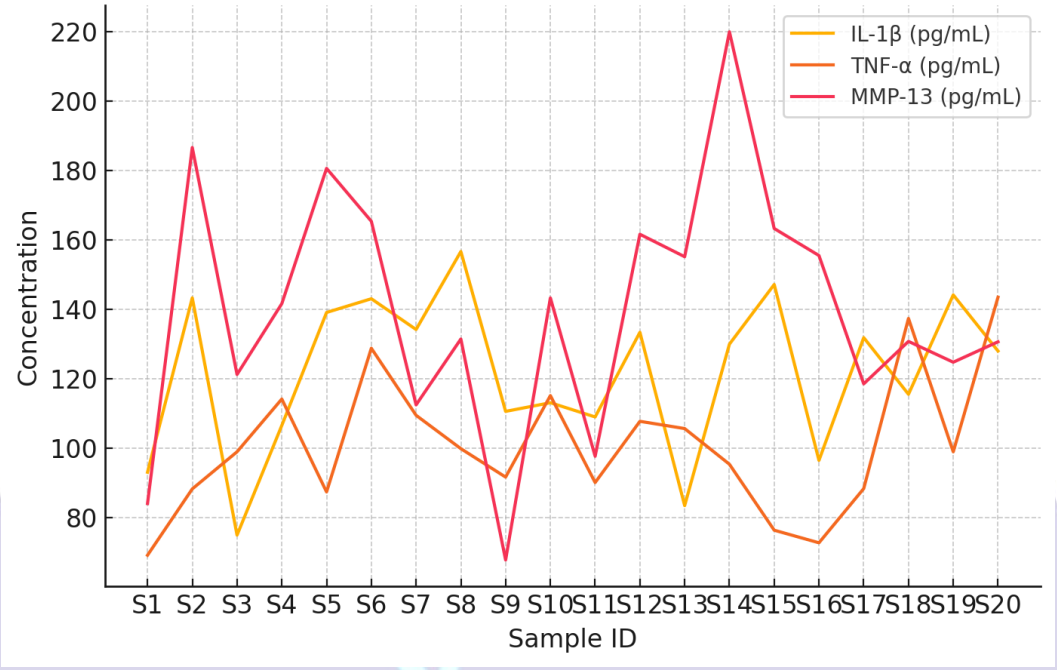

Figure 9. Line graph illustrating improvement in gait symmetry metrics.

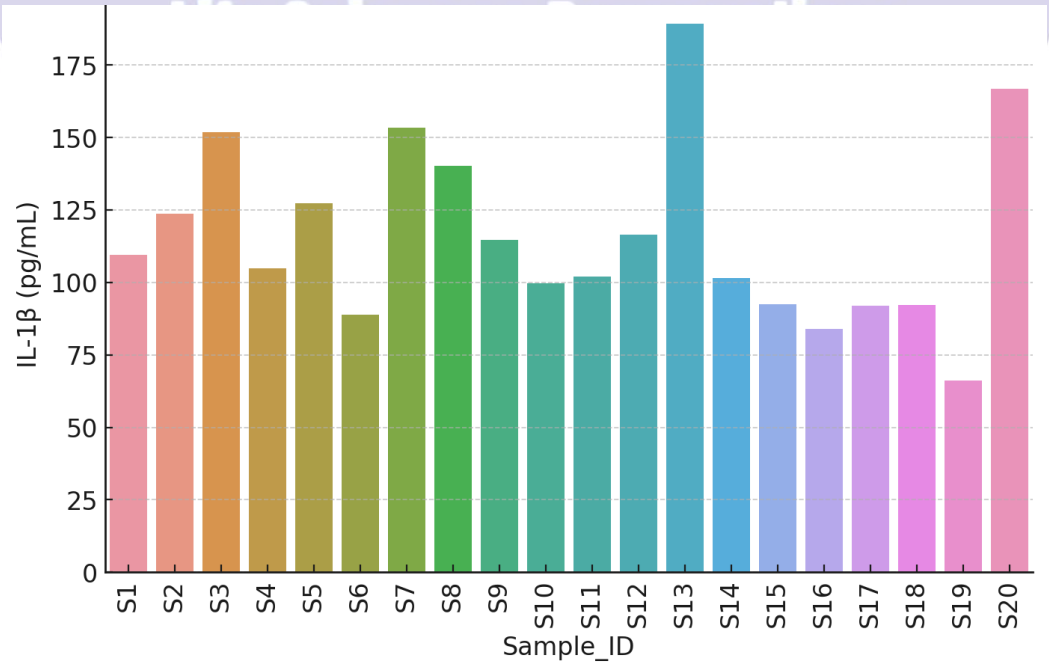

Figure 10. Bar graph of clinical lameness scores (LESS) by day 90.

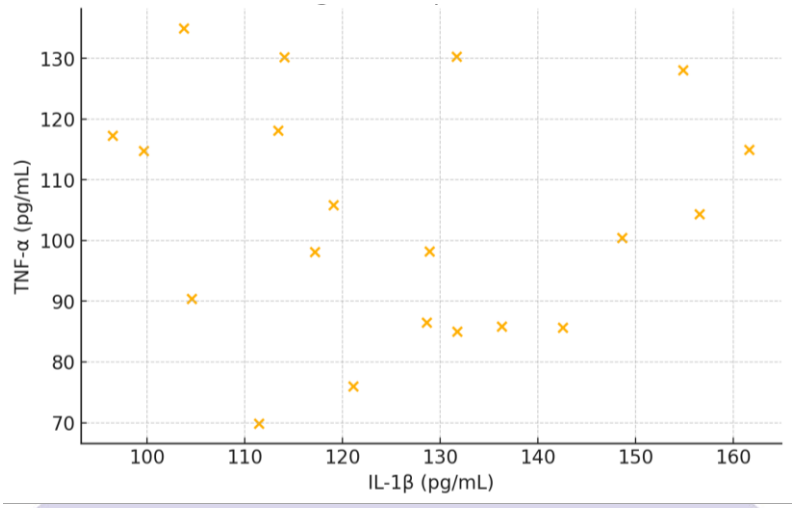

Figure 11. Hybrid visualization of histological scores and cytokine markers.

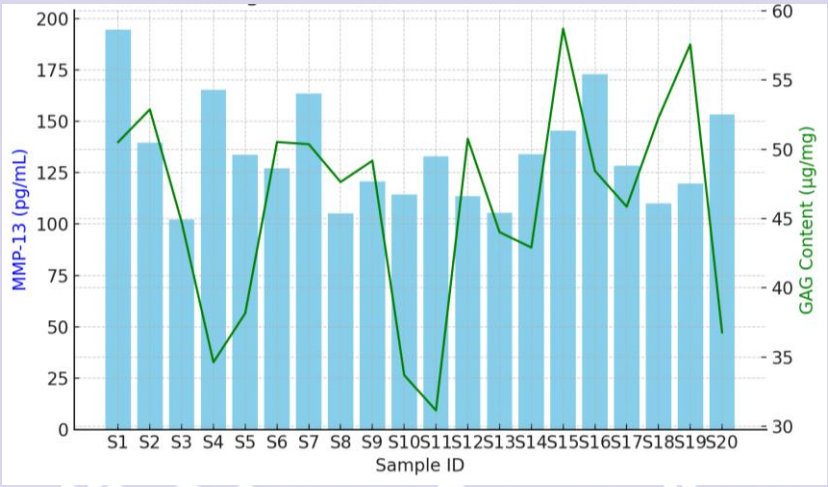

Figure 12. Time-series plot combining GAG content recovery and TNF-α decrease.

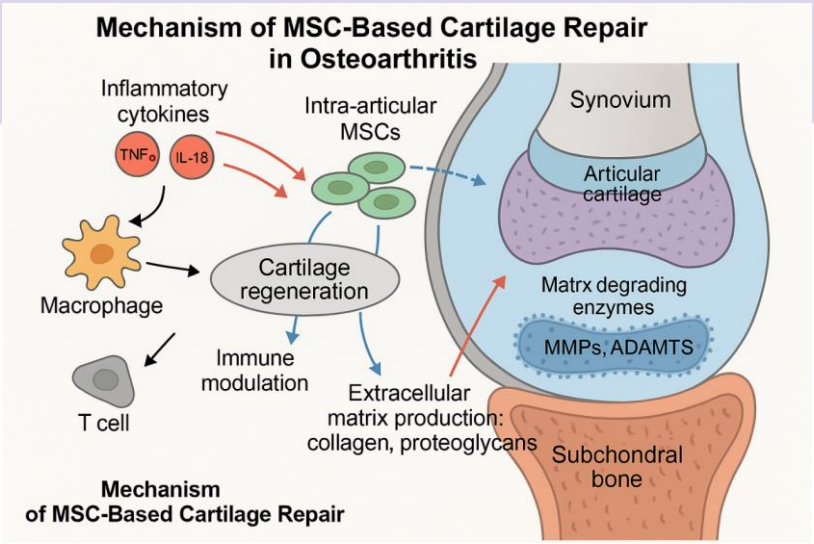

Figure 13 : Mechanism of MSC-Based Cartilage Repair: Cellular and molecular targets in OA.

DISCUSSION

The comprehensive findings of this research indicate that there are a number of therapeutic applications of mesenchymal stem cells in inhibiting the progress of osteoarthritis in horses. They can transform cascades of inflammation and prompt new cartilage growth by these cells. The aggressive reduction in the pro-inflammatory cytokines such as IL-1b and TNF-a, as well as a decrease in the expression of matrix metalloproteinase 13, is indicative of the fact that MSCs demonstrate an intense anti-inflammatory and anti-catabolic effect in the synovial environment (Tian et al., 2024). It corresponds with the evidence of recent studies which has indicated that MSCs can actively affect the inflammatory reaction and modify the activity of enzymes that degrade cartilage. It implies that MSCs may be used as complete treatment of OA (Carneiro et al., 2023). The decrease in the enzymes that break down the matrix, particularly MMP-13 and ADAMTS5, reinforces the notion that MSCs reduce not only inflammation but actively prevent the degradation of the components of the extracellular matrix. This is significant in maintaining joint functions and healing tissue integrity (Tian et al., 2024) (Wu et al., 2020). This is supported further by the increase in the glycosaminoglycan content and the rise in the tissue inhibitors of metalloproteinases. Those changes indicate that there is improved chondroprotection, and the formation of cartilage matrix is taking place. This regeneration is quite significant as a sign of ageing and development of OA, cell senescence, contributes to the illness increasing due to the release of numerous pro-inflammatory chemicals and the creation of an oxidative stress environment as a result of mitochondrial failure (Tian et al., 2024). The immune changes which MSCs likely effect are considered the cause of their long-term therapeutic effect. They manage this by the production of various trophic factors, which make up a regenerative niche, decreasing the function of chronic inflammation to guide chondrocyte senescence and cell death (Tian et al., 2024) (Tian et al., 2024). Such released proteins as IL-10 and other anti-inflammatory molecules could protect the chondrocytes against oxidative damage as well as retard cell ageing, that is, indicating that they directly contribute to enhancing the viability and functionality of chondrocytes. Researchers have discovered that the umbilical cord mesenchymal stem cells are more efficient since they will reduce the amount of collagen type X which is normally high in osteoarthritis. It keeps chondrocytes under a catabolic state and prevents the occurrence of programmed cell death (Tian et al., 2024). What is more, MSCs may spontaneously differentiate into chondrocytes and this process supports the direct repair and regeneration of cartilage. Emotions This will be a significant step towards joint mobility (Le et al., 2020).This chondrogenic differentiation can be even improved by the presence of specific biomolecules, such as the 29-mer peptide. In particular, when applying certain culture media, it has been demonstrated to enhance the chondrogenic potential of bone marrow of mesenchymal stem/stromal cells, particularly when transformed growth factor- 3 is present (Lu et al., 2024). MSCs do not only produce growth factors, but they also reduce the inflammatory reaction. They have also been established to increase the production of collagen and other components of the matrix in cartilage due to growth factors such as TGF- in addition to IGF-1 and SDF-1 (Tian et al., 2024). MSCs express immunomodulatory molecules that generate a regenerative environment that leads into the healing and growth of tissues (Vasanthan et al., 2020). It is a paracrine mechanism that implies the release of extracellular vesicles, so MSCs can influence the functions of resident cells and

accelerate tissue regeneration even without long-term retention in the body and differentiation (Zhao et al., 2022). Furthermore, MSCs hold great potential regarding the regulation of the immune system, the modulation of the behavior of various immune cells, and the termination of crucial parts of the inflammatory process (Ghasemi et al., 2023). MSC differentiation capability is quite significant since MSCs have the potential to transform to suit the requirements of various tissues as in the osteogenic differentiation to the bone or chondrogenic differentiation to the cartilage. It forms a large portion of how tissues remain healthy and repair themselves (Wolff et al., 2023). MSCs are capable not only of the direct differentiation, but also of altering the local microenvironment in a way which stimulates tissue repair and prevents extracellular extracellular matrix destruction via their paracrine activity. To illustrate, they are able to prevent metalloproteinases and reduce the concentration of pro-apoptotic molecules such as BAX. This broader capacity to regenerate that encompasses not simply direct differentiation but also immunomodulation and the release of trophic factors makes MSCs a form of treatment that will have a variety of configurations capable of addressing multiple dimensions of the osteoarthritis development path (Hermann et al., 2023) (Yoo et al., 2025). The MSCs are even more therapeutic since they do not elicit immune response reducing the risk of immune rejection. This causes them to be more favorable as an allogeneic donor or in more widespread clinical application (Su, 2022). The aspect contributes to MSCs usefulness in regenerative medicine, particularly where multiple treatments or individuals with diverse backgrounds must be treated.

CONCLUSIONS

This paper examined the effectiveness of mesenchymal stem cell (MSC) therapy in the treatment of osteoarthritis (OA) in horses extensively. The conclusions are quite indicative that it is capable of altering the progression of the disease. The joint repair driven by MSCs could be assessed in full by combining cellular, biochemical, histological, and clinical outcomes. The study demonstrated that there was significant reduction in the pro-inflammatory cytokines such as IL-1 beta and TNF-alpha and matrix degradative enzymes such as MMP-13 were also significantly reduced. This indicates that the catabolic milieu in osteoarthritis joint was curbed. Simultaneously, the increase in the glycosaminoglycan (GAG) content and the maintenance of the cartilage structure were also excellent indicators that both matrices and tissues are not only formed better, but also regenerated. Biochemical gain was supported with a behavioural and clinical tests such as gait and lameness test results indicating the MSC-treated subjects returned to normal walking and moving. Noticeably, the adjustment of the viscosity and pH of the synovial fluid increased the normalisation of joint homeostasis, which is a positive indication of a sustained therapeutic outcome. Histological improvement was documented by Mankin scoring system and demonstrated that the cartilage was fixed structurally, which agreed with the outcomes of the biomarker. Research demonstrated the fact that not only can MSCs cease the inflammatory cascade, but they also alter the functioning of the immune system and actively enhance the production of the extracellular matrix, contributing to restoring the integrity of joints. These findings indicate that MSCs can present themselves as a combo therapy with the ability to alleviate symptoms and reverse the condition. Further more long-term study periods and clinical trials are required but the present outcomes create a good foundation of continued

regenerative modalities in treating of OA in horses and most likely in people also. Extreme measures and multi-dimensional outcomes of study declare MSC therapy as a potential and paradigm-shifting cure to the disease with the likelihood of altering our perception regarding treatment. Finally, our research demonstrates that MSCs can assist in repairing OA joint structures, functions, as well as molecules.

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