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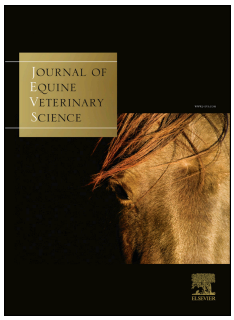

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EFFECT OF REPEATED ARTHROCENTESIS ON CYTOLOGY OF SYNOVIAL FLUID

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Abstract

Repeated arthrocentesis is necessary to diagnose and monitor the evolution of joint diseases, but the procedure may worsen any inflammation and lead to an alteration in synovial fluid. The aim of this study was to determine the effect of repeated arthrocentesis on synovial fluid cytology in healthy horses with normal joints. The experimental study was approved by Ethics Committee (University of Pisa, Italy).

Four horses were enrolled in this study on the basis of inclusion criteria and underwent repeated arthrocentesis of the inter-carpal joint of both left and right forelimbs. The synovial fluid samples were processed for total protein concentration, total nucleated cell count and differential leukocyte count. Data distribution was performed with the Komolgorov-Smirnov test and a Friedman test for repeated measures and Dunn's test as *post hoc* were performed in order to verify differences related to sampling times comparing each time point. Significance was set at $p<0.05$.

All horses remained free of lameness throughout the study period. Statistical differences were found for macrophage and lymphocyte related to sampling time.

Our results support the finding that repeated arthrocentesis does not induce detectable synovial fluid alterations. Although mild statistically significant changes in macrophage and lymphocyte populations were found, the values were always within normal ranges, suggesting that these changes were not clinically significant. Moreover, the cytological alterations rapidly solved. In conclusion, repeated arthrocentesis does not cause long term and clinically relevant alterations in synovial fluid cytology in healthy horses with normal joints.

Keywords

Horse; repeated arthrocentesis; synovial fluid; cytology.

Introduction

Joint disease is a significant cause of lameness in horses and arthrocentesis is common equine practice in order to obtain synovial fluid for analysis, instill diagnostic anesthetics, and administer therapeutic medications [1]. Nonetheless, repeated arthrocentesis is an invasive procedure that may lead to the introduction of bacteria into joints and the development of septic arthritis, which is a potentially devastating complication [2]. Repeated arthrocentesis may be necessary for repeated blocks or for treatment of joints shortly after blocking or to monitor the development of joint diseases, as reported in other species [3]. Given the importance of arthrocentesis in horses, the aim of this study was to determine the effect of repeated samples on synovial fluid cytology in healthy horses. Only one paper has reported on the effect of repeated arthrocentesis on synovial fluid cytology in dogs [3] and one on calves [4]. To the best of our knowledge, no papers have been published regarding the effect of repeated arthrocentesis in horses.

Materials and methods

Approval to conduct this study was obtained from the Ethics Committee on Animal Experimentation of the University of Pisa, Italy, No. 14875/2012.

Animals

Four horses owned by the Department of Veterinary Sciences, University of Pisa, were enrolled in this study on the basis of the following inclusion criteria: (1) no lameness or signs of joint pain on any of their four legs; (2) no local or systemic drug administration during the three months preceding the study. The four horses enrolled were female and included three were Trotters and one Warmblood. They were aged between 3-10 years and weighed 430-560 kg, with a median Body Condition Score of 4/5 [5].

Procedure

All horses underwent a complete orthopedic exam before starting the experimental protocol and then the presence of lameness were assessed before each arthrocentesis. The horse was evaluated while walking straight on a loose lead and then made to circle in left and right directions. Then, the horse was examined for swelling, heat, and pain and trotted on a loose lead in a straight line and a circle. A passive flexion test that involved the carpal region was performed, for 60 seconds, after which the horse was immediately trotted off. The horses were housed in single 4X4 meters boxes for all the study period and were not submitted to exercise.

All horses were submitted to repeated arthrocentesis of the intercarpal (IC) joint of the left and right forelimbs. In particular, the right IC joint was sampled at Time 0 (T0), at 2 (T2), 7 (T7) days and then every week for three times (T14, T21, T28, respectively). After T0 the left IC joint was sampled twice every 10 days (T10 and T20, respectively). An arthrocentesis was also performed on both, left and right, IC joints 60 days (T60) after T0. The IC joint was chosen for ease of use and sample consistency. The times for arthrocentesis were chosen because most intra articular treatments for joint disease are usually done weekly [6], while T10, T20 and T60 were chosen to assess the synovial fluid changes in a longer period of time.

Arthrocentesis was performed by the same operator (FBR). The hair over the joints was clipped and a 10-minute scrub in a circular motion using gauze sponges soaked in povidone–iodine 10% was performed. Excess povidone–iodine was removed from the arthrocentesis site by a single 70% isopropyl alcohol wipe with a gauze sponge. Each arthrocentesis was performed by introducing a non-lubricated 20 Gauge 1.5'' needle [7] into the IC joint between the tendons of the *M. extensor carpi radialis* and the *M. extensor digitorum communis*. The correct placement of the needle was confirmed by the presence of synovial fluid in the hub of the needle. A 2.5 ml syringe was then attached to the hub, and 2 ml of synovial fluid was collected in sterile EDTA tubes for each sampling time. The presence of blood in the sample or the re-placement of the needle was not recorded.

The synovial fluid samples were processed within one hour to evaluate: 1) total protein (mg/dl) (TP) concentration by a rephractometer [8]; 2) total nucleated cell count (cell/μl) (TNCC) by an automatic hematology analyzer (Lasercyte®, Idexx, USA) with hyaluronidase pre-treatment [9,10]; to reduce the viscosity; 3) differential leukocyte count (expressed as absolute values and percentages of macrophages, lymphocytes, neutrophils, eosinophils) after cytospin preparation (1500 gpm, 5') (Cytofuge 2, StatSpin, USA) to improve smear quality [9]. Smears were colored with a modified Romanowsky staining (Diff Quick®, Dade Spa, Milano, Italia), coded with random numbers, and stored in the dark at room temperature. Smears were then evaluated by microscope at 40X and 100 X by a single, experienced clinical pathologist who was blinded to sample identities and time points (V.M.). The activation of macrophages or the presence of red blood cells (RBCs) in the smear was not evaluated.

Statistical analysis

Data distribution was performed with the Komolgorov-Smirnov test to check normal distribution of data. Data did not show a Gaussian distribution, thus a Friedman test for repeated measures and Dunn's test as *post hoc* were performed in order to verify differences in TP concentration, TNCC, in absolute values and percentages of macrophages, lymphocytes, neutrophils and eosinophils related to sampling times comparing each time point. Significance was set at $p<0.05$.

Results

All horses remained free of lameness throughout the study period. Results concerning TP concentration, TNCC, absolute values and percentages of macrophages, lymphocytes, neutrophils and eosinophils were expressed as mean±standard deviation. Results for both right and left IC joints for each horse enrolled were reported in Tables 1 and 2, respectively. Statistical differences related to time were found in the right IC joint for percentage of macrophages, percentage and absolute

values of lymphocytes. Differences were also found for percentage and absolute values of lymphocyte in the left IC joint.

Regarding the right IC joint, the percentage of macrophages increased at T14, showed a plateau between T14 and T21, and then decreased at T28 returning to basal values at T60. The percentages and absolute values of lymphocytes decreased at T14, showed a plateau between T14 and T21, and then increased at T28 returning to basal values at T.

In the left IC joint, the percentage and absolute value of lymphocytes decreased at T10, and then increased at T30 returning to basal values at T60.

Discussion

The aim of this study was to evaluate the effect of repeated arthrocentesis on synovial fluid in horses. Our results support the finding that repeated arthrocentesis does not induce synovial fluid alterations with clinical importance.

Similar studies have already been conducted in dogs [3] and cattle [4]. Berg et al. (2009) [3] demonstrated that serial arthrocentesis at 3-week intervals can rarely be associated with mild mononuclear joint inflammation, however it does not appear to induce neutrophilic inflammation in the joints of healthy dogs. The increase of mononuclear cells was related to exercise. In our study macrophage percentages showed an increase at two weeks after the first arthrocentesis, then values returned to the baseline at 60 days. Our results are similar to those reported in healthy dogs regarding the increase of macrophages.

Francoz et al. (2007) [4] reported a moderate inflammatory response in the joints of healthy cows after repeated arthrocentesis characterized by an increase in mononuclear cells, lymphocytes, and neutrophils 24 h after the first arthrocentesis and then began to return to normal 24 h later. In our study, we found an increase in the percentage of macrophages (right IC joint) and a decrease of

lymphocytes (percentages and absolute values in both IC joints) 10-14 day after the first centesis, while neutrophils remained constant during time. The inflammatory cells returned to the baseline after 60 days, suggesting that the time passed has enabled the joint to recover from the insult.

Gottschalk et al. (1998) [11] find a mild inflammatory response characterized by an increased nucleated cell count and neutrophils after repeated aseptic arthrocentesis (at 4h, 8h, 24h and 72h after 0h) of the left intercarpal joint in clinically normal horses. In this study, we did not found an increase of both nucleated cell count and neutrophils.

These differences could be due to different sampling times. Moreover, in our study only arthrocentesis for the collection of synovial fluid was performed, while in previous studies, a joint lavage with Ringers lactate at T24 [4] and the introduction of sterile Ringer lactate solution into the joint at T0 [11] were performed.

In a study performed in horses [12] submitted to repeated arthrocentesis of the middle carpal joint, the authors reported an increase in total cell count that peaked 24h after the first procedure and then returned to normal values 24h later. However, the authors injected anesthetic agents (lidocaine HCL and mepivacaine HCL) before sampling synovial fluid, while only arthrocentesis for the collection of synovial fluid was performed in our study. Local anesthetics are irritating to the synovial fluid and may lead to an increase in synovial fluid cellularity more rapidly than using arthrocentesis alone.

In the study by Stover et al. (1985) [13], horses were euthanized 1 to 10 days (one horse per day) after the first arthrocentesis to evaluate the effect of arthrocentesis alone on the total and differential leukocyte counts. The leukocyte counts increased, but with a predominance of mononuclear cells instead of neutrophils, in line with our findings.

We found that neuthophils (percentages and absolute values) did not change over time, in line with the study by Sanchez Teran and collegues (2012) [14] who performed arthocentesis for 5 consecutive times in their control group.

Also eosinophils remained unchanged over time, however this data cannot be related to the literature because to the best of our knowledge there are no papers on this issue.

In our study, macrophage and lymphocyte showed similar values to T0 two months after the first arthrocentesis. These results support the hypothesis that joints adjusted to repeated centesis and tolerance progressively developing, as already suggested by others in healthy calves [4] and horses [12].

The total protein concentration remained constant over time, both for right and left IC joints, in line with some authors [12], but not with others who found an increase over time [4,14]. The difference could be related to different analytical methods, different sampling times [4,14], and previous treatment of the sampled joint [11,15].

The values that we found concerning total and differential cell counts were always within reference intervals [8] in line with other studies [3,14], while total protein concentrations were slightly higher [8].

Our study has some limitations. One is that we did not evaluate macrophages activation. Berg et al. (2006) [3] found the concurrent presence of mononuclear reactivity, defined as macrophages with increased cytoplasmic volume with or without increased cytoplasmic vacuolization and foaminess, and a slight increase in large mononuclear cells. Since we did not evaluate macrophages activation, we cannot say whether the increase in macrophages is related to a real activation of these cells secondary to the mild inflammatory stimulus or whether an activation of small mononuclear cells could be interpreted as “macrophages” by the cell counter, as already reported by others [16].

The evaluation of the presence of RBC in the smear has not been done, however, a previous paper reported that hemorrhage is an unlikely cause of mild inflammation [3].

Our results support the finding that repeated arthrocentesis do not induce detectable synovial fluid alterations. Although mild changes in macrophages and lymphocytes were found, values were always within normal ranges and the TNCC is low. These findings suggest that these changes were

not clinically significant and cytological alterations rapidly solved, probably due to an adjustment of the joints to mechanical stimulation, as suggested by others [4].

In conclusion the effect of repeated arthrocentesis does not cause long term and clinically cytological alterations in synovial fluid samples collected from healthy horses.

Right IC joint	T0	T2	T7	T14	T21	T28	T60	
TNCC (cells/μL)	265±98.8	272.5±77.6	327.5±100,5	167.5±64.0	332.5±123.7	305.0±134.8	262.5±160.9	NS
TP (gr/dl)	6.2±1.6	5.7±0.6	4.6±0.2	4.2±0.6	5.1±0.5	5.1±1.1	6.2±0.9	NS
M (cells/μL)	192.8±85.9	79.5±7.8	282.7±105.4	154.8±62.1	307.1.0±142.7	266.5±107.4	189.0±126.0	NS
M (%)	72.2±8.8^{a}	79.5±7.8^{a}	85.2±10.2^{ab}	92.0±2.2^{b}	92.0±1.4^{b}	87.5±3.1^{ab}	70.7±6.0^{a}	P<0.05
L (cells/μL)	67.2±28.4^{a}	53.8±29.7^{a}	32.4±27.7^{a}	10.0±2.4^{b}	21.5±5.3^{ab}	26.9±18.8^{ab}	64.7±32.7^{a}	P<0.05
L (%)	25.7±8.4^{a}	19.0±8.3^{a}	11.2±9.6^{a}	6.2±1.3^{b}	6.7±1.5^{b}	8.7±2.9^{ab}	25.7±6.2^{a}	P<0.05
N (cells/μL)	17,2±16.0	2.7±0.8	12.3±8.6	2.6±0.9	4.3±2.7	11.0±5.4	8.8±4.4	NS
N (%)	1.7±1.0	1.2±0.5	3.5±1.9	1.7±1.0	1.2±0.5	3.7±1.7	3.5±0.6	NS
E (cells/μL)	0.5±1.1	0.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	NS
E (%)	0.25±0.5	0.2±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	NS

Table 1 – Results obtained for right intercarpal (IC) joint at different sampling times. Data are expressed as mean±standard deviation.

Legend

TNCC: total nucleated cell count; M: macrophages; L: lymphocytes; N: neutrophils; E: eosinophils; TP: total protein; NS: no significant difference. Within row, different superscripts denote a significant difference (a≠ab≠b).

Left IC joint	T0	T10	T20	T60	
TNCC (cell/μL)	257.5±83.4	192.5±47.9	322.5±62.9	250.0±118.0	NS
TP (mg/dl)	6.5±0.7	4.9±0.4	5.1±0.6	6.4±0.6	NS
M (cells/μL)	190.2±49.2	177.9±48.4	287.7±48.8	188.4±74.9	NS
M (%)	75.8±9.1	92.0±2.2	88.3±5.0	77.0±6.3	NS
L (cells/μL)	21.6±11.0^{a}	9.7±0.9^{b}	32.2±17.6^{ab}	56.2±45.2^{a}	P<0.05
L (%)	22.0±11.0^{a}	5.3±1.3^{b}	9.8±3.9^{ab}	20.5±7.0^{a}	P<0.05
N (cells/μL)	5.6±2.9	5.0±2.0	6.6±4.4	5.4±1.3	NS
N (%)	2.7±2.4	2.8±1.3	2.0±1.2	2.5±1.0	NS
E (cells/μL)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	NS
E (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	NS

Table 2 – Results obtained for left intercarpal (IC) joint at different sampling times. Data are expressed as mean±standard deviation.

Legend

TNCC: total nucleated cell count; M: macrophages; L: lymphocytes; N: neutrophils; E: eosinophils; TP: total protein; NS: no significant difference. Within row, different superscripts denote a significant difference (a≠ab≠b).

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Highlights

1) Repeated arthrocentesis is necessary to monitor the development of joint diseases.
2) The procedure may worsen inflammation and alter the synovial fluid.
3) Repeated arthrocentesis were done on left and right intercarpal joints in 4 horses.
4) The procedure does not cause relevant alterations in synovial fluid cytology.