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Hyaluronic Acid Levels and Physical Characteristics of Synovial Fluid in Healthy and Diarrheic Calves with Arthritis

[Sağlıklı ve Artrit Gözlenen İshalli Buzağılarda Sinovial Sıvının Fiziksel Özellikleri ve Hyaluronik Asit Seviyeleri]

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ABSTRACT

Objective: This study aimed to investigate the physical characteristics and levels of hyaluronic acid, protein and glucose in joint fluid and to evaluate findings for a potential clinical application to monitor the diagnosis and prognosis of arthritis developed upon infection in neonatal diarrheic calves.

Methods: The viscosity of joint fluid was evaluated in comparison to the viscosity of water. Mucin coagulation test was employed to determine the quality of synovial fluid. Hyaluronic acid levels were assessed using Enzyme-Linked Immuno Sorbent Assay.

Results: Synovial fluid from healthy calves was bright yellow and displayed high viscosity and low protein levels, whereas the synovial fluid in diarrheic calves showed high protein levels, low viscosity and low glucose levels. Interestingly, the levels of hyaluronic acid (1.72±0.16 mg/ml) were found to be up regulated (i.e.79 % increase) in synovial fluid in the test group in comparison to levels (0.96±0.04 mg/ml) in the control group.

Conclusion: The data demonstrated that joint fluid from newborn diarrheic calves displayed reduced viscosity, decreased glucose levels, and increased hyaluronic acid levels correlating with the severity of the disease. These observations suggest that analysis of viscosity, protein, glucose and hyaluronic acid levels in synovial fluid may potentially be evaluated in the veterinary clinics for prognosis of the disease in neonatal diarrheic calves with arthritis/polyarthritis.

Keywords: synovial fluid, hyaluronic acid, glucose, protein, calf, diarrhea

ÖZET

Amaç: Bu çalışmada, ishalli buzağılarda klinik olarak enfeksiyona bağlı olarak gelişen artritin varlığını ve/veya hastalığın prognozunu değerlendirmede kullanmak amacıyla sinoviyal sıvı hiyaluronik asit, protein ve glukoz düzeyleri ve fiziksel özelliklerinin araştırılması amaçlanmıştır.

Metod: Sinoviyal sıvıda viskozite suyun viskozitesi ile karşılaştırılarak değerlendirildi. Musin koagülasyon testi sinoviyal sıvının kalitesini belirlemek amacıyla kullanıldı. Hiyaluronik asit düzeyleri "Enzyme-Linked Immuno Sorbent Assay" ile ölçüldü.

Bulgular: Sağlıklı buzağılara ait sinoviyal sıvı berrak sarı renkte, yüksek viskozitede ve düşük protein içerirken; ishalli buzağıların sinoviyal sıvısı yüksek protein, düşük viskozite ve düşük glukoz içermektedir. İlginç bir şekilde, test grubuna ait sinoviyal sıvıda hiyaluronik asit düzeyi (1.72±0.16 mg/ml) kontrol grubuna göre (0.96±0.04 mg/ml) önemli düzeyde yüksek bulunmuştur.

Sonuç: Veriler, ishalli buzağılardan alınan sinoviyal sıvının düşük viskozite, yüksek hiyaluronik asit ve düşük glukoz düzeylerinin sahip olmasının hastalığın şiddetiyle paralel olduğunu göstermektedir. Bu gözlemlerden sinoviyal sıvıda viskozite, glukoz, protein ve hiyaluronik asit düzeylerinin analizinin veteriner kliniklere gelen ishalli yenidoğan buzağılarda enfeksiyona bağlı olarak gelişen artrit/poliartrit olgularının varlığının saptanmasında ve/veya hastalığın prognozunda kullanılabileceği kanısına varılmıştır.

Anahtar Kelimeler: sinoviyal sıvı, hiyaluronik asit, glukoz, protein, buzağı, ishal

Introduction

Neonatal calf diseases usually exist together with enteritis/diarrhea, pneumonia or pneumoenteritis; however, they occasionally coexist with arthritis/polyarthritis (1-4). Clinically, *E. coli* and similar pathogens are the main causes of enteritis and arthritis in calves (1,5,6). Usually, the calf is infected with more than one agent. Moreover; non-infectious causes, including environmental, management, hygienic and nutritional factors may also be shown as reasons (7). Lack of umbilical hygiene (omphalitis) and inadequate colostrums intake following birth were shown to play an important role in the etiology of diarrheic neonatal calf diseases (8). Inflammatory reactions and cellular changes occur in the joint membrane or joint fluid (9,10). Basoglu et al. (11) indicate that serum TNF concentration is correlated with clinical criteria of sepsis in neonatal calves. The diseased gut may show an increased permeability to potential antigens which could stimulate the production of immune complexes (12). Swollen joints and walking disorders (lameness) are the main clinical symptoms in arthritis. Other symptoms, such as an increase in synovial fluid levels, fever, and pain, are also frequently observed in arthritis (13,14). Biochemical and microbiological analysis, including physical appearance, mucin coagulation test, protein levels, nuclear cellular counts, and cytology tests, are often performed in synovial fluid for differential diagnosis of the various forms of arthritis; these tests are also applied to determine pathological changes during inflammation and the best treatment option for arthritis (14-16). It also causes a change in hyaluronic acid (HA) levels in the synovial fluid (17). The structure and produced amount of HA may alter in arthritis (18). The main function of HA in joint fluid is to retain water and it plays important roles in the hydration, lubrication, and cellular functions of joints (19). Alterations in HA levels in joint fluid associated with arthritis were reported from different laboratories. For example, reduced HA levels were observed in mild and severe arthritis (19) as well as in osteoarthritis (20). Similarly, Dahl et al. (21) showed a reduction in HA levels in synovial fluid with rheumatoid arthritis, osteoarthritis, and other joint diseases. Nonetheless, Praest et al. (22) reported an increase in HA levels in the case of degenerative joint disease and diabetic arthropathies of humans. The three most important disease problems in the young and neonatal calves are septicemia, diarrhea and pneumonia. Early identification and treatment of sick calves are very important.

The purpose of the current study is to describe the physical characteristics of synovial fluid of healthy and diarrheic calves and to evaluate the HA, protein and glucose levels of synovial fluid of calves and to determine if the results vary accordingly to the severity of the disease.

Materials and methods

Animals

A total of 23 calves were used in this study. Animals are divided into two groups: (i) control group (i.e. 7 Holstein calves; 1-28 days) and (ii) test group (i.e. 16 diarrheic calves in different ages "4 young calves ≥30 days, neonatally 7 calves 15-30 days and 5 calves 1-14 days" and different races "10 Holstein, 3 Montafon, 1 Native, 1 Brown and 1 Simmental"). Animals were obtained from Internal Medicine at the Veterinary Clinic and from Institutional Animal Research Farm, Faculty of Veterinary Medicine, Ankara University (Ankara, Turkey). The study protocol was reviewed and approved by an institutional animal care and use committee and it was performed in compliance with institutional guidelines for research on animals. Owner consent was obtained for privately owned animals used in this study.

Synovial joint fluid collection and physical examination

Arthritis was diagnosed according to clinical findings, arthrosynthese, joint radiography, physical and biochemical characteristics of synovial fluid. Samples of joint fluid (1-2 ml) were collected from healthy and diseased calves in sterile glass centrifuge tubes under aseptic conditions. Synovial fluid samples were taken from the right carpal joints of healthy (control) and diarrheic calves under no anesthesia. Physical analysis (amount, color, appearance, viscosity, coagulation) of joint fluid were performed immediately after sample collections. Samples were then centrifuged at 16000xg for 10 min. and supernatants were saved for the determination of HA levels and protein concentration as described (23). The viscosity of joint fluid was measured, and the result was evaluated in comparison to the viscosity of water as described (18). Briefly, one droplet of the synovial fluid was placed on a glass slide, and the sizes of filaments were measured. If the size (length) is more than 2.5 cm, it is considered "weak or low viscosity" or "abnormal" synovial fluid.

Protein, glucose and hyaluronic acid analysis

Protein concentration was determined using the Biuret protein assay (24). An ELISA system was employed to determine the levels of HA in synovial fluid according to the manufacturer's instructions (Echelon Biosciences Inc; product number K-1200; Salt Lake City, Utah, USA). Samples were diluted with PBS (1:1000-1:80000) before they were subjected to the ELISA test.

We employed human HA-ELISA test system to measure HA levels in joint fluid. As indicated by the manufacturer (Echelon Biosciences Inc.), this test can be successfully used to measure HA levels in biologic fluids from different sources, such as blood, serum, urine, and synovial fluid obtained from humans, animals, or cell cultures.

A mucin coagulation test was conducted to determine the quality of joint fluid as described (19,25). Briefly, a droplet of joint fluid was mixed with 20 ml of 7 N glacial acetic acid, followed by shaking. The quality of synovial fluid was assessed based on the characteristics of mucin coagulation. If the test results in a clear and sharp-edged coagulation, it is considered as "normal" synovial fluid; however, if the test shows no clear coagulation or display a cloudy or small coagulation or suspension, it is marked as "weak" synovial fluid. Levels of glucose were measured using commercial test kits by an autoanalyser (Alcyon 300/300i Analyzer, Abbot). Microbiological analysis of faeces samples taken from five calves with diarrhea were performed in the department of microbiology.

Statistical analysis

The results are expressed as mean ± standard deviation and as median (range) values. For statistical analysis SPSS 15.0 software, Kruskal Wallis and Mann-Whitney U-test were used.

Results

Calves in the test group were clinically evaluated, and results showed that among 16 calves, 15 of them showed symptoms of monoarthritis, and only one of them displayed characteristics of polyarthritis. Faeces samples of five diarrheic calves (from one to 30 days old) were screened microbiologically. Enteropathogenic *Escherichia coli* was the most frequently isolated organism in the faeces samples of five diarrheic calves as well as some other minor polymicrobial infections were observed as *BVDV*, *coronavirus* and *cryptosporidium*.

Clinical signs

Diarrhea causes varying degrees of fluid and electrolyte loss to the calf resulting in; dehydration, electrolyte imbalance, acidosis, depression and loss of appetite.

Clinical and physical examination of joints

Observation (swelling, position-angulation); palpation (nature of the swelling, heat, pain) and manipulation (flex-bend) and extend (straighten) the joint, checking for pain and altered range of motion. Severity of the disease in diarrheic arthritic calves were evaluated clinically as severe (n=7), mild (n=4) and moderate (n=5). Synovial HA levels were compared statistically with the severity of the disease.

Physical examination indicated that synovial joint fluid from healthy calves displayed a light yellow color with clear content, whereas the synovial fluid obtained from arthritic calves appeared cloudy with no clear content (Table 1). Blood and fibrin in the synovial fluid associated with arthritis were also observed. The synovial fluid from control calves was characterized as "normal" because it showed a high viscosity and resulted in good mucin coagulation and no blood and fibrin; however, joint fluid in arthritis was characterized as "abnormal" due to weak mucin coagulation and low viscosity. We further evaluated the synovial fluid based on protein levels: those less than 2 mg/ml were marked as "normal," but more than 2 mg/ml were considered as "abnormal" joint fluid (Table 1).

Glucose levels in synovial fluid from diarrheic calves with arthritis were found to be lower (55 %) significantly in comparison to control grup (Table 1). Mean glucose level proportion of the serum level in healthy calves were 45.28±1.55 (average 40-52) and in arthritic-diarrheic calves were 21.63±4.06 (average 7–38).

The results presented in Table 2 and Figure 1 showed that a 79 % increase in HA levels (1.72±0.16 mg/ml) was observed in joint fluid from diarrheic-arthritic calves in comparison to the levels (0.96±0.04 mg/ml) in normal synovial fluid from healthy calves, and such differences in HA levels between the two groups were statistically significant ($p<0.05$). In addition, the results from clinical evaluations and laboratory investigations suggest that there is a strong correlation between HA levels and the severity of diarrheic-arthritis (Figure 2 and Table 3), especially HA levels above 2 mg/ml most likely indicating severe arthritis in darrheic calves.

We have demonstrated the altered HA, glucose and protein levels and physical properties of joint fluid in diarrheic arthritic calves, and such alterations might correlate with the severity of arthritis (Figure 2 and Table 3).

Table 1. Physical and biochemical characteristics of synovial joint fluid obtained from healthy or diarrheic arthritic calves

Sample	Healthy calves (n=7)	Arthritic calves (n=16)
Quantity	Low	High
Color	Colorless or light yellow	Yellow
Appearance	Clear	Cloudy/purulent
Viscosity	Strong	Weak
Spontaneous coagulation	No	Yes
Mucin coagulation test	Strong mucin	Weak or very weak mucin
Blood and Fibrin	No	Yes
Protein (g/dl)	1.66±0.27	3.74±0.45
Glucose (serum level %)	45.28±1.55 (40-52)	21,63±4.06 (7–38)
Remarks	Normal	Abnormal

Table 2. Statistical significance and HA levels of synovial joint fluid obtained from healthy (control) or arthritic calves

Parameter	Control Group			Diarrheic Arthritic Group			P
	n	x	Sx	n	x	Sx	
HA (mg/ml)	7	0,96 (0.88–1.20)	0,04	16	1,72 (0.80–3.20)	0,16	**P ≤ 0.05**

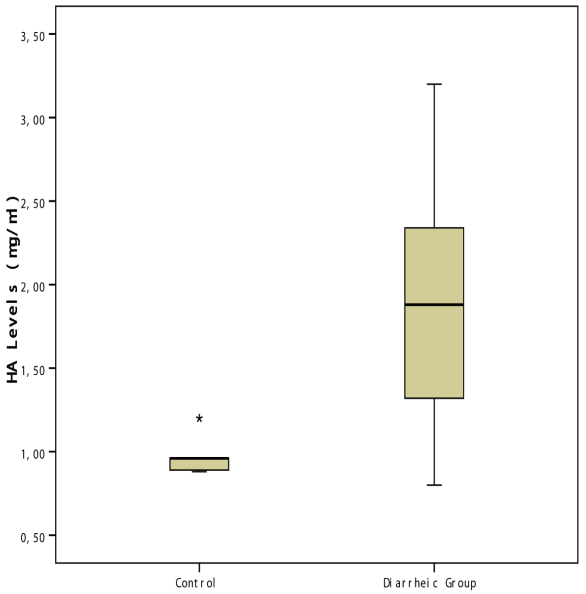

Figure 1. Hyaluronic acid (HA) levels in the synovial fluid from healthy (control group; n=7) and calves with diarrheic arthritis (test group; n=16).

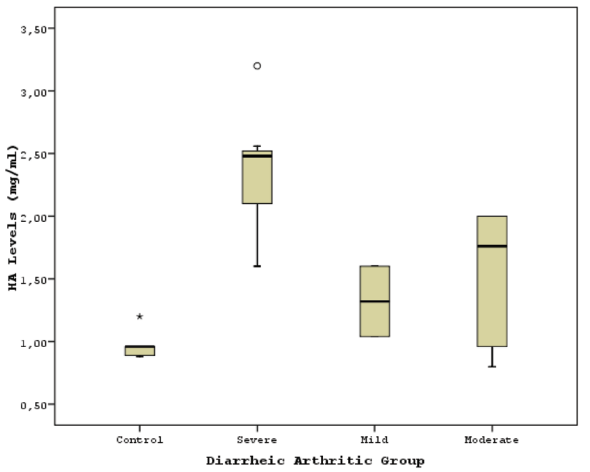

Figure 2. Correlation between hyaluronic acid (HA) levels in synovial fluid and the severity of diarrheic arthritis. HA levels were determined in synovial fluid from severe (n=7), mild (n=4) and moderate (n=5) arthritis cases. Data are representative of individual experiments. P-value: 0.01; HA: Hyaluronic acid.

Table 3. Relationship between the severity of the disease and HA levels of synovial joint fluid obtained from diarrheic arthritic calves

Parametre	Severe				Mild				Moderate				P
	n	Min.	Max.	Median	n	Min.	Max.	Median	n	Min.	Max.	Median	
HA (mg/ml)	7	1.20	3.20	2.48	4	1.04	1.60	1.32	5	0.8	2.0	1.76	P ≤ 0.01

HA levels were increased significantly especially in 1-14 days old diarrheic calves with arthritis (Figure 3 and Table 4).

Discussion

Calf diarrhea remains the major cause of mortality in dairy calves. In this study, we showed that the levels of HA and the physical properties of synovial fluid from diarrheic calves with arthritis were significantly altered. We classified the synovial fluid based on protein and glucose concentration, viscosity and mucin coagulation test "normal" or "abnormal" (Table 1). Similarly, an increase in HA levels was observed in synovial fluid in diarrheic calves with arthritis in comparison to controls (Figure 1). Our results also showed that the viscosity of synovial fluid was reduced in diarrheic calves with arthritis. This reduction was most likely caused by the depolymerization and hydration of HA due to inflammation (17,26). Hyaluronic acid is also known as mucin. Normally, synovial joint fluid was capable of producing strong mucin coagulations. However, we have observed the induction of "weak mucin" coagulation in synovial fluid from diarrheic calves with arthritis (Table 1). These attributes of synovial fluid may be caused by the structural change of HA by bacterial enzymes during infection in joints, and this interpretation is consistent with published studies (26-28). Interestingly, we observed statistically significant ($p<0.05$) elevated HA levels (1.72±0.16 mg/ml) in joint fluid from the test group (n=16, diarrheic calves with arthritis), compared to HA levels (0.96±0.04 mg/ml) in the control group (n=7; healthy calves) (Figure 1). Our observation is consistent with the literature, in which an increase in HA levels (2.00±0.57 mg/ml) was reported in joint fluid in arthritis (29).

This finding suggests that an increase in HA levels may be an important indicator for the prognosis of arthritis in diarrheic calves (30). Moreover, there may be a corre-

Table 4. Distribution of hyaluronic acid levels according to the age (day) groups of calves with diarrheic arthritis.

Age (day)	1-14	15-30	> 31 day	Control (1-28) day	
n	5	7	4	7	
HA (mg/ml)	1.60	0.80	0.96	0.88	
	3.20	1.04	1.20	0.96	
	2.20	2.56	0.96	1.20	
	2.48	0.96	1.76	0.96	
	1.60	2.48		0.90	
		2.00		0.96	
		1.04		0.88	
Mean	2.216±0.299[a]	1.554±0.289[b]	1.220±0.188[b]	0.96±0.04[b]	p<0.05

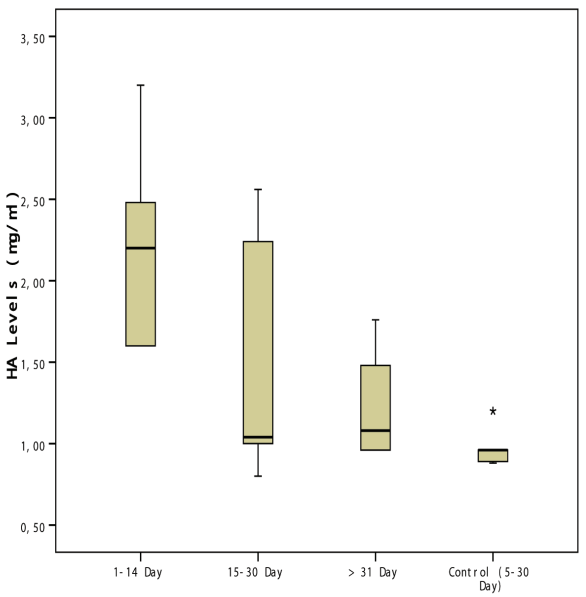

Figure 3. Distribution of hyaluronic acid (HA) levels according to the age groups of diseased calves with diarrheic arthritis.

lation between the severity of arthritis and levels of HA; however, the observation in the current study (Figure 2) and studies from others (18, 25) suggest that HA levels may not always correlate with the clinical symptoms associated with mild and weak arthritis in diarrheic calves.

The mechanism of increased HA levels in diarrheic calves with arthritis is unknown. Kruskal Wallis Test results suggest that increased HA levels may only be important for the ages between one and fourteen days (Figure 3 and Table 4). Older calves can normally have different HA values when compared to younger calves. The articulation is very active in young animals. The cartilage is remodelling according to the forces applied on it. For calves neonatal period is 1-28 days.

Diarrhea is the most common cause of death in young calves. Bacteria, viruses and/or parasites cause diarrhea in calves. Usually, the calf is infected with more than one agent. The highest risk period for diarrhea is from birth until about 1 month of age.

We found significantly elevated HA levels in the synovial fluid of young diarrheic calves with arthritis. It should be indicated that this is the first study reports the increased levels of HA in the synovial fluid of young diarrheic calves with arthritis. Interestingly, some other reports suggested the reduced levels of HA in synovial fluid in horses with arthritis (31,32) and in humans with arthritis, osteoarthritis, and rheumatoid arthritis (19-22). During diarrhea, pathogens may enter into blood circulation and result in arthritis or polyarthritis in calves. Polyarthritis in calves may be immune-mediated as reported for canines (12). These observations suggest the possibility that presence of both symptoms (diarrhea and arthritis) in calves may result in an unexpected increase in HA levels. This may be an explanation for the differences between our findings and published results (20-22). Nevertheless, the potential effects of diarrhea that can cause an increase in HA levels in synovial fluid deserve further investigation.

Finally, although it is very rare, elevations of protein concentration (3 mg/ml) and HA levels (2 mg/ml) in humans have been implicated as reflecting a severe inflammation in the joints (18,29). Consistently, we also observed an increase in HA levels (>2 mg/ml) and protein concentration (>4 mg/ml) in the synovial fluid with arthritis in five calves. Therefore, these observations suggest that HA levels may increase as a result of inflammation, depending on the conditions of arthritis.

In conclusion, this report shows a significant increase in synovial HA and protein levels and decrease in glucose levels in diarrheic calves with arthritis. Alterations of the biochemical and physical attributes of synovial fluid strongly indicate the significant changes occurring at the joints of young calves. Our data also suggest the pos-

sibility that alterations in HA levels may be correlated with the degree of arthritis in neonatal diarrheic calves. Thus, the determination of HA levels may potentially be used as a prognostic or, in some cases, as a differential diagnostic marker for arthritis in diarrheic calves in the veterinary clinics.

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References

[1] Brenner J, Elad D, Markovics A, Grinberg A and Trainin A. (1993) Epidemiological study of neonatal calf diarrhoea in Israel-one year survey of fecal samples. Isr J Vet Med. 48: 113-116.

[2] Kahrs RF. (2001) Adenovirus. Viral diseases of cattle. Iowa State University Pres, Ames.

[3] Hewicker-Trautwein M, Feldmann M, Kehler W, Schmidt R, Thiede S, Seeliger F, Wohlsein P, Ball HJ, Buchenau I, Spergser J, Rosengarten R, (2002) Outbreak of pneumonia and arthritis in beef calves associated with *Mycoplasma bovis* and *Mycoplasma californicum*. Vet Rec. 151: 699-703.

[4] Svensson C, Jensen MB. (2007) Identification of diseased calves by use of data from automatic milk feeders. J Dairy Sci. 90: 994-997.

[5] Caple IW. (1989) Neonatal viral diarrhoeas. Aust Vet J. 66 (12): 407–408.

[6] Trotz-Williams LA, Wayne Martin S, Leslie KE, Duffield T, Nydam DV, Peregrine AS. (2007) Calf-level risk factors for neonatal diarrhea and shedding of Cryptosporidium parvum in Ontario dairy calves. Prev Vet Med. 82 (1-2): 12-28.

[7] Aldridge BM, Garry FB, Adams RA. (1993) Neonatal septicemia in calves: 25 cases (1985-1990). JAVMA. 203: 1324-1329.

[8] Samsar E, Akin F. (2000) Özel Cerrahi. 3. baski. Eklemlerin Sirurjikal hastaliklari. Medipress, Malatya, Turkey, pp. 403-430.

[9] Morris CJ, Farr M, Hollywell CA, Hawkins CF, Scott DL, Walton KW. (1983) Ultrastructure of the synovial membrane in seronegative inflammatory arthropathies. J Roy Soc Med. 76: 27–31.

[10] Ley C, Ekman S, Elmén A, Nilson G, Elorante ML. (2007) Interleukin-6 and tumour necrosis factor in synovial fluid from horses with carpal joint pathology. J Vet Med A. 54: 346-351.

[11] Basoglu A, Sen I, Sevinc M, Simsek A. (2004) Serum concentrations of tumor necrosis factor-alpha in neonatal calves with presumed septicemia. J Vet Intern Med. 18 (2): 238-241.

[12] Kohn B. (2003) Canine immune-mediated polyarthritis. World Small Animal VeterinaryAssociation World Congress Proceedings,. 24-27 October, 2003. Bangkok, Thailand.

[13] Johnstone AC, King CM. (2003) Bovine and ovine mycoplasmal arthritis and synovitis. New Zeal Vet J. 51 (1): 46.

[14] Bani Ismail Z, Al-Rukibat R, Al-Tarazi Y, Al-Zghoul MB. (2007) Synovial fluid analysis and bacterial findings in arthritic joints of juvenile male camel (Camelus dromedarius) calves. J Vet Med A. 54 (2): 66-69.

[15] Van Pelt RW. (1970) Synovial effusion changes in idiopathic septic arthritis in calves. JAVMA. 156: 84-92.

[16] Yehia SR, Duncan H. (1975) Synovial fluid analysis. Clin Orthop Relat Res. 107: 11-24.

[17] Karagül H, Altintas A, Fidanci UR, Sel T. (2000) Klinik Biyokimya. Medisan, Ankara.

[18] McIlwraith CW, Billinghurst RC, Frisbie DD. (2001) Current and future Diagnostic Means to Better Characterize Osteoarthritis in the Horse-Routine Synovial Fluid Analysis and Synovial Fluid and Serum Markers. Proceedings of the Annual Convention of the AAEP. 47: 171-179.

[19] Kofoed JA, Barcelo AC. (1967) The synovial fluid hyaluronic acid in rheumatoid Arthritis. Arthritis Rheum. 10: 357-376.

[20] McCarty MF. (1998) Enhanced synovial production of hyaluronic acid may explain rapid clinical response to high-dose glucosamine in osteoarthritis. Med Hypotheses. 50: 507-510.

[21] Dahl LB, Dahl IM, Engström-Laurent A, Granath K. (1985) Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoidarthritis and other arthropathies. Ann Rheum Dis. 44: 817-822.

[22] Praest BM, Greiling H, Kock R. (1997) Assay of synovial fluid parameters: hyaluronan concentration as a potential marker for joint diseases. Clin Chim Acta. 266: 117-128.

[23] Rowley G, Antonas KN, Hilbert BJ. (1982) Quantitation of Hyaluronic acid in equine synovia. AJVR. 43: 1096-1099.

[24] Weichselbaum TE. (1946) An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol. 16: 40-49.

[25] Kolasinski SL. (2000) Synovial fluid analysis and synovial Biopsy. In: Burkholder JF (Editor): Rheumatology 4: 1-12.

[26] Gronefeld S, Moore HA, Latimer KS. (2006) Canine rheumatoid arthritis. Available at: http://www.vet.uga.edu/vpp/clerk/gronfeld. Accessed Jun 2, 2007.

[27] Ponnuraj K, Jedrzejas MJ. (2000) Mechanism of 275 hyaluronan binding and degradation: structure of Streptococcus pneumoniae hyaluronate lyase in complex with hyaluronicacid disaccharide at 1.7 A resolution. J Mol Biol. 299: 885-895.

[28] Kelly SJ, Taylor KB, Li S. Jedrzejas M.J. (2001) Kinetic properties of *Streptococcus pneumoniae* hyaluronate lyase. Glycobiology. 11: 297-304.

[29] Levine MG and Kling DH. (1956) Rheologic studies on synovial fluid. J Clin Invest 35: 1419-1427.

[30] Laurent TC, Laurent BG, Fraser JR. (1996) The structure and function of hyaluronan. Immunol Cell Biol 74: A1–A7.

[31] Hilbert BJ, Rowley G, Antonas KN. (1984) Hyaluronic acid concentration in synovial fluid from normal and arthritic joints of horses. Aust Vet J 61 (1): 22-24.

[32] Little CB, Hilbert BJ, Wickstrom S, Hedlund BE. (1990) Quantitative microanalysis of equine synovial fluid glycosaminoglycan concentration. AJVR 51:1534-1539.