قسم : الجراحة.
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دراسة السائل السينوفي الطبيعي في الماشية

عاطف بلبل

تم دراسة الخواص الطبيعية ، المكونات الخلوية ، الأنشطة الأنزيمية والتركيب الكيمائي للسائل السينوفي والذى تم الحصول عليه بالسحب من مفاصل القيد ، بين الرسفي ، القدمي والفخذى الرضفي.

ولقد وجد أن السائل السينوفي الخاص بمفاصل الماشية الطبيعية هو سائل شفاف ، ذو لون أصفر باهت وغير قابل للتجلط وخالي من المواد العالقة مع خاصية طبيعية لتجلط الميوسين. ويعتمد حجم السائل السينوفي على حجم المفصل الذى تم تجمع السائل منه. ولا يحتوى السائل السينوفي على خلايا دم حمراء بينما يحتوى على عدد قليل من خلايا الدم البيضاء ، حيث تتميز بكثرة عدد الخلايا الليمفاويه. والسائل السينوفي للحيوانات الطبيعية ذو أنشطة لبعض الأنزيمات المختلفة ، حيث أنها أقل نشاطا من مثيلتها في مصل الحيوان.

ولقد وجد أن كمية البروتين الكلي والالبيومين أقل بكثير عن معدلها في المصل ، وبدراسة التركيب الكيميائي للسائل السينوفي لعديد من المركبات لوحظ أن نسبة تلك المواد مماثل لما هو موجود في المصل ، بينما يحتوى على نسبة قليله جدا من الكوليسترول.

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NORMAL SYNOVIAL FLUID OF CATTLE

(With 4 Tables)

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SUMMARY

The physical properties, cytological content, enzyme activities and biochemical composition of synovial fluid obtained by arthrocentesis from normal fetlock, intercaropal, tarsal and stifle joints of cattle were studied.

Synovial fluid obtained from normal joints is clear, pale yellow non-coagulable liquid and free of flocculent material, with a normal mucin clot quality. Total volume varied in direct proportion to the size of the joint from which the synovial fluid is collected.

Normal synovial fluid exhibited no erythrocytes and presents a low leukocytes count where lymphocytes predominated followed by monocytes.

The synovial fluid of healthy animals have activities of alkaline phosphatase, and glutamic oxalacetic, glutamic pyruvic and gamma-glutamic transaminases as well as lactic dehydrogenase, which are lower than the respective serum levels of such enzymes.

Total proteins and albumin contents of normal synovial fluid were lower than that of serum. The synovial fluid of healthy cows contains nearly the similar levels for sugar, urea, uric acid, inorganic phosphorus, chlorides, calcium and magnesium as in serum. synovial fluid has a traces from cholesterol.

INTRODUCTION

Synovial fluid may be considered as a specialized tissue fluid that changes with disease. Joint conditions are ganerally accompanied by varying degrees of synovitis or inflammation of the synovial membrane, (McILWRAITH, 1980).

Examination of the synovial fluid should be a routine procedure in the evaluation of arthritic conditions as it can provide valuable informations in addition to that gained by clinical and radiologic examination.

The aim of the present study is to evaluate certain physical properties, cytological content, biochemical composition and enzyme activities of synovial fluid obtained from normal joints of cattle. A comparison were made between results of synovial fluid and serum.

MATERIAL and METHODS

Samples of synovial fluid were obtained by arthrocentesis from fetlock joint of both fore- and hind-limbs, intercarpal, tarasal and stifle joints of normal healthy cows of different ages.

Blood samples for biochemical analysis and enzyme activities were obtained from the jugular vien by venipuncture immediatly prior to arthrocentesis. The clot was allowed to retract and the sample was then centrifuged at 3000 r.p.m. for 30 minutes to obtain clear supernatent serum.

The synovial fluid sample was aspirated from the joint cavity with sterile thin and long needle and sterile syringe. Samples were then transferred from the aspirating syringes immediatly to a screw capped vials. No any anticoagulant was employed. Gross appearance of the synovial fluid was observed and recorded at the time of collection.

Total erythrocyte and leukocyte counts were made from a non-centrifuged and undiluted portion of synovial fluid using a white cell pipette. The synovial fluid cells were counted on a standard Neubauer hemocytometer. If dilutions were necessary, in cases containing elevated cell counts, physiological saline solution tinged with methyl violet was used.

Differential leukocyte counts were made from stained smears of the sediment following centrifugation of the synovial fluid at 3000 r.p.m. for 30 minutes. Smears were made on clean glass slides. Smears were then stained with Wright's methylene blue stain. One hundred leukocytes were identified and counted for each sample.

Mucin quality test (M.Q.T.) was carried out on synovial fluid by the method adopted after VAN PELT and CONNER (1963 b). This procedure was conducted to determine the mean degree of polysaccharide polymerization (hyaluronic acid). The mucin clot was graded as fellows: normal (4): a tight, ropey clump in a clear solution; fair (3): a soft mass in a very slightly turbid solution; poor (2): a small friable mass in a turbid solution and very poor (1): a few flecks in a tirbid solution.

Serum and synovial fluid glucose, urea nitrogen, uric acid, cholesterol, inorganic phosphorus, chlorides, calcium, magnesium, albumin, and total proteins levels were determined using Autoanalyzer (Greiner, Labortechnik, West Germany).

Enzyme activities of glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma-glutamic transaminase (GGT), alkaline phosphatase (AP) and lactic dehydrogenase (LDH) were determined using test kits by the micro-methods produced by Compur Electronic (Bayer AG and Carl Zeiss).

RESULTS

Normal bovine synovial fluid (S.F) samples collected from various joints were clear, pale yellow and free of flocculent material. Some of the bovine S.F samples become gelatinous in 1-2 hours after its aspiration (thixotropic in nature), Gentle agitation returns the fluid to its normal liquid state. It was noticed that during aspiration, there were a very slight positive pressure. The total volume of S.F samples varied according to the size of the joint. The normal S.F samples in general were alkaline in reaction (Table 1).

Normal mucin quality was uniformily high for the normal S.F samples of cattle (Table 1). Some samples on standing form a gel like sac of mucin which precepitate in the fluid sample. No mucin clots were graded poor or very poor.

The range of total erythrocyte count of normal S.F samples differs markedly (Table 2). During arthrocentesis, the S.F samples may be contaminated with fresh blood when the synovial membrane was traumatized by needle puncture. Centrifugation of these samples return to be clear and pale yellow. Synovial xanthochromia (indication to intraarticular hemorrhage prior to arthrocentesis) was not observed in normal S.F samples. The total leukocyte counts differs considerably not only from one animal to another, but also from one joint to another in the same animal. The leukocyte count values were relatively low. Differential leukocyte counts was shown in table 2. The leukocytes seemed to be predominent with lymphocytes. Basophils were never observed. Synovial lining cells were present, but were not included in the differential count. They were identified by their characterestic, homogenous , nuclear chromatin in oval nuclei and by their elongated cell body.

The mean serum AP, GOT, GPT, GGT and LDH activities were higher than their corresponding levels of enzyme activities in S.F samples from various joints (Table 3).

Serum and S.F sugar, total proteins, albumin, urea nitrogen, uric acid, cholesterol, inorganic phosphorus, chlorides, calcium and magnesium levels were recorded in table (4).

DISCUSSION

Certain anatomical features should be taken in consideration in evaluating analysis of synovial fluid collected from intercarpal, tibiotarsal and femoropatellar joints (VAN PELT, 1962 and SISSON and GROSSMAN, 1968). Therefore, S.F collected from either the intercarpal, tibiotarsal or femoropatellar joints could reflect physiologic or pathologic alterations in the joints with which they communicate.

The S.F of the cattle examined was a clear, pale yellow, viscous, does not clot upon standing and free of flocculent material. These results were in agreement with CORNELIUS (1963) and EL-AMROUSI et al. (1966). The total volume of S.F of any joint will generally vary in direct proportion to the size of the joint and its communication with another joint. VAN PELT and CONNER (1963 a) and BOLBOL (1975) added that the total volume of S.F varied considerably from one animal to another according to the size of the joint, size or weight of the animal and its general health and condition.

The bovine S.F found to be alkaline in reaction (EL-AMROUSI et al. 1966 and CORNELIUS, 1963). A difference between the joints has been found that may be dependant on the size of the joint. A large joint with large synovial volume, e.g. stifle joint, has a lower pH than a small joint, e.g. fetlock joint (BOLBOL, 1983).

The quality of hyaluronic acid as determined by the mucin clot test indicates that the mean degree of polymerization was high for this series of cattle. Similar results were obtrained by VAN PELT and CONNER (1963 b) who mentioned that the greater percentage of normal clots were obtained from S.F with highest relative viscosity.

Total cells of S.F were counted undiluted in most of the samples. Two distinct advantages were observed with the use of undiluted S.F for counting of the total leukocytes, (a) accurate counting of the cells due to their small number, and (b) no precipitation of mucin occured in the white blood cell pipette or counting chamber of the hemocytometer, which would occur with usual diluents that contain glacial acetic acid. Total cell count varied not only from animal to animal, but also from joint to joint in the same animal. The results of this study was in accordance with that of KERSJES (1963).

Differential leukocyte counts of S.F smears were stained with Wright's methylene blue stain, which found to be more excellent in differentiation of leukocytes which reveals the morphologic details than any other stains (BOLBOL, 1975 and VAN PELT and CONNER, 1963 a). Lymphocytes were found to be the predominent cell types in the leukocyte counts, followed by monocytes (VAN PELT and CONNER, 1963 a and EL-AMROUSI et al. 1966). Eosinophils were seldom observed in the smears. Basophils were not detected in examined S.F (VAN PELT and CONNER, 1963 a; CORNELIUS, 1963 and BOLBOL, 1975).

Measurement of activities of certain enzymes in S.F can indicate changes in synovial membranes or altered intra-articular metabolism. As a rule, there is a close correletion between the activities of alkaline phosphatase (AP), glutamic oxalacetic transaminase (GOT), and lactic dehydrogenase (LDH) in S.F and the clinical severity of joint disease (VAN PELT, 1974 and McILWRAITH, 1980). Serum AP, LDH, GOT and GPT activities were higher than their corresponding levels of activity in S.F of cattle. Activity level of GGT in serum was approximately at the same in S.F. VAN PELT and LANGHAM (1968) stated that normal S.F.-AP is derived primarily from plasma. The neutrophils of S.F may play a role, since, these cells are considered by VAN PELT (1961) as a source of AP.

Average glucose concentrations of bovine S.F have been found to be slightly lower than the serum glucose levels (CORNELIUS, 1963). Occasionally, however, samples will approach a distribution ratio of 1:00. VAN PELT and CONNER (1963 c) found no significant differance in blood, plasma or S.F sugar levels encountered between bulls, cows and steers. BAUER et al. (1940) have suggested that the struggling prior to sampling could easily elevate the blood glucose concentration from hepatic glycogenolysis and insufficient time would elapse for its equilibrium between plasma and S.F.

The total protein and albumin contents in normal bovine S.F samples were markedly far lower than the respective values of serum. These values, however, reflects the limited permeability of the synovial membrane and its capillaries to proteins (COLES, 1980 & BOLBOL, 1975).

The distribution of electrolytes and non-electrolytes has been found to be similar to that found in a dialysate of blood plasma. This means that the concentration and distribution of electrolytes and non-electrolytes of S.F agree with that expected from the Gibbs-Donnan theory of membrane equilibrium. CORNELIUS (1963) concluded that the average ratios for most electrolytes and nonelectrolytes are only slightly under 1:00, since in individual cases their concentration in serum and S.F has been found to be nearly equal. This view was confirmed by the results recorded in this study.

Cholesterol was not normally present in S.F., but these amounts may gain entrance into the joint cavity during the needle puncture from slight contamination of S.F with blood (CORNELIUS 1963 and BOLBOL, 1975), since the capillary membrane is impermeable to such molecules.

REFERENCES

Bauer, W.; Ropes, M.W. and Waine, H. (1940): The physiology of articular structures. Physiol. Rev. 20: 272-312.

Bolbol, A.E. (1975): The study of synovial fluid of some domestic animals in health and disease. M.V.Sc. Thesis, Assiut University, Egypt.

Bolbol, A.E. (1983): Normal synovial fluid of equines. International Congress on Orthopaedics in Ungulates, Vienna, Oct. 5-7, 1983.

Coles, E.H. (1980): Veterinary Clinical Pathology. 3ed Ed. W.B. Saunders Company, Philadelphia, London and Toronto.

Cornelius, C.E. (1963): Synovial Fluid. In: Clinical Biochemistry of Domestic Animals. edited by Cornelius and Kaneko, J.J., Academic Press, New York, 1963.

El-Amrousi, S.; Soliman, M.K. and Youssef, L.B. (1966): Studies on the physiological chemistry of the tibio-tarsal synovial fluid of healthy bovines. Can. J. Comp. Med. Vet. Sci. 30: 251-255.

Kersjes, A.W. (1963): Over Synovia en Synovitis. Proofschrift Pyksuniversiteit te Utrecht, Nederland.

McIlwraith, C.W. (1980): Synovial fluid analysis in the diagnosis of equine joint disease. Equine Practice, 2: 44-48.

Sisson, S. and Grossman, J.D. (1968): The anatomy of domestic animals 4th Ed., W.B. Saunders Co., Philadelphia.

Van Pelt, R.W. (1961): Cited by Van Pelt and Langham (1968).

Van Pelt, R.W. (1962): Arthrocentesis and injection of the bovine tarsus. Vet. Med. 57: 125-132.

Van Pelt, R.W. (1974): Interpretation of synovial fluid findings in the horse. J A V M A 165: 91-95.

Van Pelt, R.W. and Conner, G.H. (1963 a): Synovial fluid from the normal bovine tarsus. I. cellular constituents, volume and gross appearance. Am. J. Vet. Res. 24: 112-121.

Van Pelt, R.W. and Conner, G.H. (1963 b): Synovial fluid from the normal bovine tarsus. II. Relative viscosity and quality of mucopolysaccharides. Am. J. Vet. Res. 24: 537-544.

Van Pelt, R.W. and Conner, G.H. (1963 c): Synovial fluid from the normal bovine tarsus. III. Blood, plasma and synovial fluid sugar. Am. J. Vet. Res. 24: 735-742.

Van Pelt, R.W. and Langham, R.F. (1968): Synovial fluid changes produced by infectious arthritis in cattle Am. J. Vet. Res. 29: 507-512.

Table (1): Physical properties of normal bovine synovial fluids

PROPERTIES	FETLOCK JOINT	INTERCARPAL JOINT	TARSAL JOINT	STIFLE JOINT
APPEARANCE	Pale yellow and clear	Pale yellow and clear	Pale yellow and clear	Pale yellow and clear
VOLUME/ml	4.5 ± 0.22	4.1 ± 0.15	9.20 ± 0.34	17.8 ± 0.45
MUCIN QUALITY*	3.93± 0.06	3.80± 0.10	3.86 ± 0.09	3.93± 0.06
REACTION (pH)	7.50± 0.08	7.46± 0.16	7.42 ± 0.22	7.38± 0.10

* Mucin quality grades: normal 4, fair 3, poor 2 and very poor 1.
± Standard error.

Table (2): Total and differential cytological values for synovial fluid from normal bovine joints

	FETLOCK JOINT	INTERCARPAL JOINT	TARSAL JOINT	STIFLE JOINT
RBCs/cmm	169.20± 4.04	198.80±7.41	135.80±8.21	241.40±12.37
WBCs/cmm	183.80±11.32	190.40±6.04	220.00±7.51	210.60± 9.92
Differential leukocyte count:				
Neutrophiles	5.30± 0.20	6.00±0.16	5.40±0.21	5.60± 0.36
Lymphocytes	49.96± 0.20	49.74±0.20	50.10±0.45	50.20± 31
Monocytes	37.80± 0.20	37.40±0.49	37.40±1.50	38.10± 0.20
Eosinophils	0.84± 0.09	0.66±0.12	0.80±0.10	0.90± 0.06
Macrophages	6.10± 0.42	6.20±0.35	6.30±0.42	5.20± 0.31

Table (3): Enzyme Activity vlues of serum and synovial fluid of normal bovines

	SERUM	FETLOCK JOINT	INTERCARPAL JOINT	TARSAL JOINT	STIFLE JOINT
GOT U/L	25.00± 1.31	19.00±0.84	16.40±0.64	21.80±0.88	16.80±1.50
GPT U/L	6.10± 0.36	2.40±0.14	2.10±0.12	2.66±0.12	1.70±0.06
GGT U/L	6.60± 0.76	4.50±0.27	4.40±0.40	5.80±0.66	4.80±0.57
AP U/L	122.00±15.2	21.00±1.02	18.60±1.14	20.00±0.60	19.80±0.99
LDH U/L	182.80± 5.04	94.80±5.51	85.40±2.42	94.60±5.76	82.60±5.40

U/L: Unit per liter, enzyme unit is that amount of enzyme which will catalyse the transformation of one micromole of substrate per minute at 25°C.

Table (4): Biochemical constituents of serum and synovial fluid of normal bovines

	SERUM	FETLOCK JOINT	INTERCARPAL JOINT	TARSAL JOINT	STIFLE JOINT
GLUCOSE mg%	45.76±1.76	34.52±0.53	33.06±0.32	35.44±0.35	39.22±0.32
T.PROTEIN gm%	7.86±0.34	0.54±0.02	0.90±0.03	0.74±0.04	0.64±0.01
ALBUMIN gm%	2.81±0.05	0.37±0.01	0.67±0.02	0.47±0.03	0.53±0.02
UREA N. MMOL/L	4.75±0.29	5.35±0.01	4.89±0.03	5.41±0.03	5.30±0.12
CHOLESTEROL MMOL/L	2.12±0.91	0.17±0.03	0.21±0.03	0.15±0.02	0.77±0.11
I.PHOSPHATE MMOL/L	1.66±0.11	1.48±0.01	1.62±0.01	1.57±0.01	1.73±0.01
CHLORIDES MMOL/L	106.6 ±5.43	112.4 ±2.74	108.0 ±8.16	114.2 ±3.50	111.0 ±4.33
CALCIUM MMOL/L	2.79±0.08	1.88±0.01	1.69±0.01	1.55±0.02	1.98±0.03
MAGNESIUM MMOL/L	1.28±0.08	1.33±0.02	1.08±0.03	1.12±0.01	1.32±0.02
URIC ACID MMOL/L	57.60±2.56	29.30±0.27	20.90±0.42	22.60±0.66	33.70±0.88