The effect of running at many speeds on some synovial fluid constituents in Iraqi Arabian horses

Amir Ibrahim Towfik
Coll. of Vet. Med. / Univ. of Al-Qadisiya
email: Amir_vet_58@yahoo.com
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Abstract

The aim of the study is to investigate the effect of running at different speeds (walk, trot, canter, and gallop) on the synovial fluid constituents which had been aspirated from the mid-carpal joint of each horse before and after running. The study was conducted on ten Iraqi Arabian horses in Arabic horses' center, Al-Qadisiya University. The synovial fluid samples were drawn aseptically and transferred directly to the clinical pathological laboratory examinations. Many physical and biochemical markers was studied, clarity, color, pH, viscosity, spontaneous clot formation, glucose (mg/dl), total protein (g/dl), alkaline phosphatase enzyme (IU/L) and WBC count. The results showed turbidity, dark yellow color, low pH, low viscosity and increase of spontaneous clot information in various degrees especially in case of canter and gallop. The glucose was decreased, while total protein, alkaline phosphatase and (WBC_s) count was increased significantly at ($p<0.05$).
Key words: Running, speeds, synovial fluid, horses.

دراسة تأثير السرع المختلفة على مكونات السائل الزلالي في الخيول العراقية

عامر ابراهيم توفيق
كلية الطب البيطري / جامعة القادسية

الخلاصة

شملت الدراسة عشرة خيول من النسل العربي العراقي في مركز الخيول العربية الاصيلة / جامعة القادسية ، الهدف منها دراسة تأثير السرع المختلفة للجري [المسير الاعتيادي ، و الخبب ، و الهذب ، و الحضر(الجري السريع)] على مكونات السائل الزلالي المفصلي لمفصل الركبة لكل جواد. أخذت عينات السائل المفصلي تحت ظروف تعقيميه مشددة قبل وبعد كل سرعة للجري وأرسلت مباشرة للفحص المختبري حيث تمت دراسة العديد من المعايير الفيزيائية والكيميوحيوية ، درجة النقاوة ، اللون ، الاس الهيدروجيني ، اللزوجة ، التخثر التلقائي ، نسبة سكر الكلوكوز ، كمية البروتين الكلي ، مستوى انزيم الفوسفيتيز القاعدي ، عدد خلايا الدم البيضاء. أظهرت نتائج الدراسة زيادة في تعكر السائل المفصلي مع تغيير لونه الى الاصفر الغامق وانخفاض الاس الهيدروجيني واللزوجة اضافة الى نقصان التخثر التلقائي وبدرجات مختلفة خصوصا في سرع الهذب والحضر . كما أظهرت النتائج وبشكل ملحوظ احصائيا عند مستوى احتمال 5% نقصان نسبة سكر الكلوكوز بينما كانت هناك زيادة في كمية البروتين الكلي ومستوى انزيم الفوسفيتيز القاعدي اضافة الى زيادة عدد خلايا الدم البيضاء.
الكلمات المفتاحية: الجري، السرع المختلفة ، السائل الزلالي ، الخيول.

Introduction

Historically the term biomarkers refer to analysis in biological samples, and any measurement that predicts a disease state or response to a drug can be called a biomarkers (1). So biomarkers would therefore be welcomed with any improvement in prevention, diagnosis, treatment or prognostication in this area and they may be helpful tools (2). According to the way the biomarkers detected, they can be subdivided into biochemical and immunological markers (2) but our present study depend on physical and chemical biomarkers only. A consequence of physical disruption of tissues, due to mechanical stress, joint injuries arise. Joints that are being exposed to loads that exceed physiological limits have a higher chance to be damaged, as a consequence of excessive magnitude, excessive number of cycles or abnormal direction of the load. When the mechanical integrity of a tissue has been compromised by concurrent disease, high loads, even within the normal physiological range may

cause injury (3). All parts of articulating joints participate in load transmission, and failure of the bone articular cartilage, muscles, ligament/ tendons or nerves of a joint may lead to exercise-induced damage (4). The ability to detect these changes at an early stage would potentially enhance the ability to form exercise programs on individual basis (5). Training may affect the serum concentration of bone markers (6). Normal synovial fluid is pale yellow, clear and free of flocculent debris and does not clot (6).This property is attributed to a lack of fibrinogen as well as other clotting factors (including prothrombin, factor V, factor VII and tissue thromboplastin) (7). (BAP) activity in synovial fluid of active equine osteo-arthritis joints, is increased compared with normal joints (9), while (10) observed that total protein concentration, WBC count and levels of the inflammatory markers, prostaglandin E2 (PGE2) were significantly elevated at level $P< 0.01$. Bone – specific alkaline phosphatase (BAP) is an isoform of alkaline phosphatase that plays an important role in bone formation (2). Total white blood $cell_s$ (WBC_s) counts may be performed on synovial fluid using hemocytometers, it is essential to use physiologic saline diluents and not the usual white cell diluents containing acetic acid, for the latter precipitates the hyaluronate-protein complex (11). The (WBC) count of normal equine synovial fluid has been reported by different workers as 167±21 and 87 cells/mm^3 respectively (12, 13). Synovial fluid is believed to have two main functions, to aid in the nutrition of articular cartilage by acting as a transport medium for nutritional substance such as glucose and to aid in the mechanical function of joints by lubrication, glucose for articular cartilage chondrocyte energy is transported from the peri-articular vasculature to the cartilage by the synovial fluid, the glucose concentration of synovial fluid is usually approximately equal to that of blood (14).

Materials and methods

The protocol of this study was done on ten cross Arabian horses of the Arabian horses center, Al-Qadisiya University, their ages was (2.5-3.5) years, two stallions and eight mares. All the horses were examined clinically for one week to be sure that there were no any health problem. All horses run on soil sport track for a distance about (500) meter, each type of horses speed was studied on two days interval (walking 3-5km/h.- trot 16-19km/h.-canter30-31km/h.-gallope50-60km/h.). The speed of running was calculated by dividing the distance on the time which measured by sport time calculator. Synovial fluid samples (2-3)ml were collected from the mid-carpal joint before and after each type of speeds by using a (18-gauge) sterile needles and syringes. All sites for arthrocentesis were prepared aseptically. Following aspiration of the samples, they were transferred to EDTA vacuitainer tubes and sent in ice box to laboratory analysis. Physical markers were recorded, clarity, color, viscosity by dropping the synovial fluid from the end of the syringe and noticed the stringing out as much as 5cm before separating, pH measured by pH meter and mucin clot formation by adding 0.5ml of synovial fluid sample to 2ml of 2% acetic acid and mixing it rapidly with glass rod. Total white blood cells (WBC) cells/mm^3 was measured by hemocytometer counts method, while glucose (mg/dl), total protein (g/dl) and alkaline phosphatase (IU/L) was measured by spectrophotometer (Chrom Tech, V-1100 spectrophotometer MED &Lab Instrument, USA). The data were analyzed using ANOVA test at ($p<0.05$).

Results

The clarity of the synovial fluid was found ranged between slight turbid to turbid in four horses at canter speed while it was turbid at gallop speed in six horses. The color was seen pale yellow to yellow at walking, trot and canter respectively while it was dark yellow in two horses at gallop speed. The viscosity of the synovial fluid was found ranged between viscid to low viscid at walking, trot and canter respectively while it was very low viscid in all horses at gallop speed. Mucin clot formation was ranged between normal, fair and poor at walking, trot and canter respectively while it was very poor in all horses at gallop speed (Table 1).

Table (1): The physical biomarkers of the synovial fluid in adult horses, (n=10).

No. of horse	Sex	Type of running	Time of sampling	Clarity	Color	Viscosity	Mucin clot formation
1	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Slightly turbid	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Dark yellow	Viscid Very low viscid	Normal Very poor
2	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Slightly turbid	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
3	Male	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Slightly turbid	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
4	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
5	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
6	Male	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
7	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Slightly turbid	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Dark yellow	Viscid Very low viscid	Normal Very poor
8	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
		Gallop	Before After	Clear Turbid	Pale yellow Yellow	Viscid Very low viscid	Normal Very poor
9	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Dark yellow	Viscid Very low viscid	Normal Very poor
10	Female	Walking	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Normal
		Trot	Before After	Clear Clear	Pale yellow Pale yellow	Viscid Viscid	Normal Fair
		Canter	Before After	Clear Clear	Pale yellow Yellow	Viscid Low viscid	Normal Poor
		Gallop	Before After	Clear Turbid	Pale yellow Dark yellow	Viscid Very low viscid	Normal Very poor

Table (2): The effect of different types of speeds on the values of the synovial fluid constituents in adult horses: values represent means ± standard error, (n=10).

Type of running		pH	WBC_s	Glucose Concentrate mg/dl	Total Protein g/dl	Alkaline Phosphatase IU/L
Walking	Before	7.02±0.04a	5.19±0.28a	54.27±0.93a	1.72±0.01a	20.82±0.34a
	After	6.98±0.05a	5.19±0.26a	52.86±1.09	1.74±0.01a	21.24±0.33a
Trot	Before	6.99±0.03a	5.3±0.26a	52.81±0.9a	1.74±0.01a	21.17±0.36a
	After	6.86±0.08a	7.02±0.14b	47.96±0.79b	1.86±0.04b	24.9±0.94b
Canter	Before	6.98±0.04a	6.71±0.17a	51.3±0.73a	1.76±0.02a	22.05±0.50
	After	6.43±0.11b	7.5±0.16b	41.41±0.49b	2.31±0.1b	32.56±0.95b
Gallop	Before	6.91±0.03a	7.29±0.16a	50.21±0.64a	1.81±0.02a	23.89±0.51a
	After	5.25±0.09b	10.57±0.25b	32.08±0.98b	3.12±0.13	39.17±0.49b

Different letters refer the significant differences among groups at ($p \leq 0.05$).

The means of the pH of the synovial fluid was seen decreased in, walking (6.98±0.05), trot (6.86±0.08), canter (6.43±0.1) and gallop (5.25±0.09) speeds. The synovial fluid glucose concentration also show decrease in, walking (52.86±1.09), trot (47.96±0.79), canter (41.41±0.49) and gallop (32.08±0.98). The WBC count, total protein, and alkaline phosphatase display an direct increase in their values in different speeds employed in this experiment, which rises with the increase of speed. The WBC count become (5.19±0.26), (7.02±0.14), (7.5±0.16) and (10.57±0.45) in walking, trot, canter, and gallop speeds respectively. Constant direct increases of total protein (1.74±0.01), (1.86±0.04), (2.31±0.1) and (3.12±0.13) in walking, trot, canter, and gallop speeds were observed respectively. Also the alkaline phosphatase was (21.24±0.33) at walking, (24.9±0.94) at trot (32.56±0.95) at canter, and (39.17±0.49) at gallop were observed (Table 2).

Discussion

The study is designed to explain the effect of many speeds of running on some of the constituents of synovial fluid in Iraqi Arabian horses. Therefore it is involved nine synovial biomarkers (physical and chemical); there had been some attention to the changes of them with different types of running speeds. The high turbidity and dark yellow color of the samples in the high speed (gallop) seen in this study may be due to the high flocculent debris (cellular constituents and fibrin precipitates (12, 14), these changes of opacity and flocculent materials also may be seen in acute synovitis. The results also showed gradual decrease in viscosity of the synovial fluid (very low at gallop) due to the digestion of hyaluronate by hyluronidase enzyme which decreases the viscous nature of the fluid by increasing the proteolytic digestion of synovial fluid (15). The mucin clot formation ranged from normal, fair, poor and very poor according to the increase of the speeds which may lead to decrease in the clot factors (factor V and factor VII) (8). Tables 2 shows the decrease of values means of pH and glucose level while WBC counts, total protein and alkaline phosphatase increased, all these changes are significant statistically at ($p<0.05$).The decrease of pH may be due to its relationship with the increasing of total protein and alkaline phosphatase, the later play very important role. Biomarkers are good tools for the prediction and detection of many changes in the joints without any bigger procedure on the horse but more researches are needed to get higher accuracy of these markers (5). I hope these results will be helpful in training programs designs for our horses.

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