



RESEARCH ARTICLE

Effects of Endurance Exercise on Peritoneal Fluid Parameters in Horses: A Comprehensive Study

Abbas A. Hussein^{1*}, Rafid H. Farman², Ahmed K. Munahi³, Amir I. Towfik⁴

^{1,2,3,4} Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq

ARTICLE INFO	ABSTRACT
Received: Apr 29, 2024	<p>This work was designed to investigate the role of endurance exercise effects on some physical and biochemical parameters of peritoneal fluid of Iraqi Arabian horses. Ten local Iraqi Arabian horses were included, under a strict aseptic surgical approach, Peritoneal Fluid (PF) samples (5ml/each) were collected before and after running at different speeds, 48hrs interval between each type of speed. These samples were analyzed to determine the physical (pH, turbidity, viscosity, and color) and biochemical (total nucleated cells count(TNCC), total protein (TP), fibrinogen, creatinine, alkaline phosphatase (ALP), lactate, glucose, lactate dehydrogenase (LDH), sodium, chloride, and potassium) parameters. The physical parameters showed gradual increases in pH, turbidity, and viscosity, and the color was changed to yellow in gallop. The biochemical parameters revealed significant increases in TNCC, TP, fibrinogen, creatinine, ALP, and lactate while there were gradual decreases in glucose, LDH, sodium, chloride, and potassium. A conclusion could be made that there are significant effects of endurance exercise on the physical and biochemical markers of the PF, and checking of sport horses must be done after hard sports. We consider these informational data are very useful for horsemen and veterinarians.</p>
Accepted: June 14, 2024	
<p>Keywords</p> <p>Endurance Exercise Parameters Peritoneal Fluid Arabian horses</p>	
<p>*Corresponding Author:</p> <p>Abbas.Ali@qu.edu.iq</p>	

INTRODUCTION

In Iraq, Arabian horse counts are noticeably growing up during the few last years at the interest of their owners. These horses are used often in sports, running for long distances at different speeds, walking, trotting, cantering, and galloping (Watchrarat et al., 2023). Exercise acts as a stimulator of vital functions as a normal stressor (Gomes et al., 2021; Tashtoush et al., 2022). Histologically, the peritoneum is composed of three layers, the first layer at the base is a loose connective tissue, the second layer is the basal lamina, and the third one is mesothelial cells. These layers are coated with thin film of peritoneal fluid (PF) (Eggleston and Mueller, 2003). PF has many functions, the most important is antifriction for the viscera (Gningue et al., 2022; Jarrah et al., 2022; Reed and Bayly, 2004), prevent adhesions between organs (Smith, 2015; Jam et al., 2011; Wardat et al., 202), and it contains many constituents that act as antibacterial (Walton and Southwood, 2012; Zachary, 2012). PF markers are very important that provide good diagnostic indicators to clinicians (Kilcoyne et al., 2020; Saulez et al., 2018). Previous reports showed the importance of these indicators such as some biochemical parameters in PF; lactate, glucose, and pH, that provide information about the incidence of intestinal ischemia or peritonitis (Apichaimongkonkun et al., 2019; Seabaugh et al., 2014; Ruggles et al., 1993; Van et al., 1999). In the prolonged exercise usually for the muscular activity, the source

of energy which can be produced aerobically is the glucose (TRILK et al., 2010; Teixeira-Neto et al., 2007; Jam et al., 2017).

As exercise intensity is increased, the lactate and H⁺ levels are increased energizing the systems quickly via anaerobic-based glycolysis (McGowan et al., 2008; Tashtoush et al., 2023). Lactate concentration after physical activity has a positive correlation with the competitive performance of the animal (Lindner, 2000). The objective of this work was to study the role of endurance exercise effects on some physical and biochemical parameters of peritoneal fluid of Iraqi Arabian Horses.

MATERIALS and METHODS

Experimental models

The research was designed to include 10 local Iraqi Arabian horses (10 stallions) in Al-Heeraa City, Al-Najaf province, Iraq. They were housed in stalls of the stable with semi-closed system. They were under daily veterinary attention. Their ages were between 4-6.5 years old. Their sub-breeds were Kihalan, Hamadani, and Saqlawi. They were provided with standard quantities of grains and alfalfa hay with ad libitum clean water. Tests were achieved to ensure that horses were healthy which include, blood tests contains complete blood test; the RBCs are 8.6 M/ μ l, hematocrit is 38% and the white blood cells 9.700 cells/ μ l. the urine test discovered that the urine was alkaline with pH value is 7.9, also the stool test stated that the consistency is soft and moist with green glossy color.

Experimental design

Under a strict aseptic surgical approach with local anesthesia by lidocaine 2%, at the end of the sternum in the midline of the linea alba. PF samples (5ml/each) were collected using 19-gauged with 2-inch-long sterile needles with disposable syringes, ultrasound is used to direct the needle to the fluid. Aspiration of fluid was performed before and after running at different speeds, at 48hrs interval between each type of speed; walking (5Km/h), trot(20Km/h), canter(30Km/h), and gallop(50Km/h) on a dry soil track for a distance of 5Km. The speed of running was calculated by dividing the distance on the time which was measured by sports time calculator.

Laboratory biochemical analysis

Peritoneal fluid samples were centrifuged at 1500xg for 10min at 37°C, transferred to plastic tubes, stored at 2°C for 12hrs before analysis, and sent in an icebox to a laboratory for the analyses (Teixeira-Neto et al.,2007).Physical parameters, pH (by pH meter-Sper Scientific.USA), color under the scale of pale amber-amber-yellow-dark yellow, clearance under the scale of clear-turbid, and viscosity under the scale of viscose-watery were measured (Radcliffe et al (2022). The biochemical analyses were done using a spectrophotometer (Chrom Tech, V-1100, USA) with a commercial kit for each parameter; Total Nucleated Cell Count (TNCC), Total Protein (TP), fibrinogen, glucose, creatinine, Lactate dehydrogenase (LDH), Alkaline Phosphatase (ALP), lactate, sodium, chloride, and potassium..

Statistical analysis

The difference-based significance was measured employing SPSS software (version 25) and the data were analyzed by one-sided paired t tests. The percentages were also analyzed using the Chi-square. Data were grouped and summarized as means (\pm) values at $p \leq 0$.

Ethical approval

The care and use ethical approaches were followed according to all important and ethical guidelines 5420/9/7 in University of Al-Qadisiyah College of Veterinary Medicine after agreement of owners for using their horses in this study was declared via consent form.

RESULTS

Physical markers

The pH of the PF for the before and after walking samples were 7.610 ± 0.070 and 7.600 ± 0.050 respectively that was non-significant at $p \leq 0.05$. While it was significant for the before and after trot, 7.640 ± 0.050 and 7.820 ± 0.051 , respectively. It was significant for the before and after canter, 8.010 ± 0.049 and 8.460 ± 0.040 , respectively. It was also significant for the before and after gallop, 8.510 ± 0.043 and 9.120 ± 0.085 , respectively as shown in table (1).

Validity and Reliability of the Questionnaire:

Table 1: The mean \pm SE of the pH values of Peritoneal fluid (PF) according to the speed of exercise.

Speed	Period	pH
Walking	Before	7.610 ± 0.070 A
	After	7.600 ± 0.050 A
Trot	Before	7.640 ± 0.050 B
	After	7.820 ± 0.051 A
Canter	Before	8.010 ± 0.049 B
	After	8.460 ± 0.040 A
Gallop	Before	8.510 ± 0.043 B
	After	9.120 ± 0.085 A

Different letters mean significant difference at $p \leq 0.05$.

The color of the PF was pale amber at a high degree before walking and trot. It was decreased gradually after walking (90*), after trot and canter (70* and 70*) respectively, and before gallop (70*), but it disappeared after canter and gallop. It was yellow at a high degree after canter (100*) and gallop (100*). It was fair after walking (10*) and trot (30*) as shown in table (2).

Table 2: The mean \pm SE of the color of PF according to the speed of exercise.

Speed	Period	PA%	Yellow%	X ²	P value
Walking	Before	100*	0	1.05	0.305*
	After	90*	10		
Trot	Before	100*	0	3.52	0.06*
	After	70*	30		
Canter	Before	70*	30	3.52	0.06*
	After	0	100*		
Gallop	Before	70*	30	3.52	0.06*
	After	0	100*		

Source: * No significant difference at $p < 0.05$.

The clearance ratio of the PF was at a high degree before the walking (100*) and the trot (100*), but it decreased gradually after the trot (70*) and high turbidity after the canter and the gallop (Table 3).

Table 3: The mean \pm SE of the clearance of PF according to the speed of exercise.

Speed	Period	Clear %	Turbid %	X ²	P- value
Walking	Before	100*	0	1.05	0.305*
	After	90*	10		
Trot	Before	100*	0	3.52	0.06*
	After	70*	30		
Canter	Before	70*	30	3.52	0.06*
	After	0	100*		
Gallop	Before	0	100*	0	1*
	After	0	100*		

* No significant difference at $p < 0.05$

The viscosity of the PF was very low at the walking and the trot and was very viscous after the gallop as shown in Table (4).

Table (4): The mean \pm SE of the viscosity of PF according to the speed of exercise.

Speed	Period	Water y%	Viscose %	X2	P value
Walking	Before	100*	0	1.05	0.305*
	After	90*	10		
Trot	Before	100*	0	3.52	0.06*
	After	70*	30		
Canter	Before	30	70*	0	1*
	After	30	70*		
Gallop	Before	100*	0	20	0**
	After	0	100*		

* No significant difference at $P < 0.05$; ** highly significant at $p < 0.01$.

Biochemical markers

For before and after the walking, the effect of the endurance exercise on the TNCC (Cellx109/L) was significant, 1.56 ± 0.094 and 1.63 ± 0.095 respectively. The effect was significant for ALP (IU/L), 391.04 ± 32.497 , 374.65 ± 31.169 respectively. While other biochemical markers were non-significant as showed in Table (5).

Table (5): The mean \pm SE of the biochemical parameters of PF before and after walking.

Biochemical markers	Before	After
TNCC (cellx109/L)	1.56 ± 0.094 B	1.63 ± 0.095 A
TP (g/L)	8.31 ± 0.545 A	8.88 ± 1.274 A
Fibrinogen (mg/dl)	8.31 ± 0.545 A	8.88 ± 1.274 A
Glucose (mg/dl)	154.09 ± 3.741 A	153.70 ± 3.805 A
Creatinine (mg/dl)	1.67 ± 0.052 B	1.8 ± 0.63 A
LDH (IU/L)	1.76 ± 0.024 A	1.73 ± 0.032 A
ALP (IU/L)	391.04 ± 32.497 A	374.65 ± 31.169 B
Lactate (mEq /L)	6.37 ± 0.431 A	6.71 ± 0.395 A
Sodium (mEq /L)	168.66 ± 4.191 A	168.76 ± 4.175 A
Chloride (mEq /L)	124.39 ± 4.042 A	124.59 ± 4.045 A
Potassium (mEq /L)	5.65 ± 0.265 A	5.66 ± 0.225 A

Different letters mean significant variances at $p \leq 0.05$.

For before and after trot, the endurance exercise effect on the TNCC (cellx109/L), TP (g/L), glucose (mg/dl), creatinine(mg/dl), LDH(IU/L), and sodium(mEq/L) were non-significant, table (6). While fibrinogen (mg/dl) was 123.90 ± 6.704 and 125.32 ± 6.561 respectively, ALP (IU/L) was 369.82 ± 31.060 and 360.87 ± 31.324 respectively, lactate (mEq/L) was 6.60 ± 0.367 and 7.07 ± 0.388 respectively, chloride (mEq/L) was $122.77 \pm 3.3.704$ and 118.71 ± 3.699 respectively, and potassium (mEq/L) was 5.39 ± 0.257 and 4.60 ± 0.137 respectively as shown in table (6).

Table (6): The mean \pm SE of the biochemical parameters of PF before and after trot.

Biochemical markers	Before	After
TNCC (cellx109/L)	1.71 \pm 0.088 B	2.45 \pm 0.550 A
TP (g/L)	9.01 \pm 1.183 A	10.16 \pm 1.416 A
Fibrinogen (mg/dl)	123.90 \pm 6.704 A	125.32 \pm 6.561 A
Glucose (mg/dl)	154.57 \pm 3.627 B	250.82 \pm 96.474 A
Creatinine (mg/dl)	1.68 \pm 0.044 A	1.70 \pm 0.045 A
LDH (IU/L)	88.42 \pm 7.838 A	85.66 \pm 7.865 A
ALP (IU/L)	369.82 \pm 31.060 A	360.87 \pm 31.3249B
Lactate (mEq/L)	6.60 \pm 0.367 B	7.07 \pm 0.388 A
Sodium (mEq/L)	168.03 \pm 3.971 A	162.99 \pm 4.187 A
Chloride (mEq/L)	122.77 \pm 3.704 A	118.71 \pm 3.699 B
Potassium (mEq/L)	5.39 \pm 0.257 A	4.60 \pm 0.137 B

Different letters mean significant variances at $p \leq 0.05$.

For before and after canter, only lactate (mEq/L) was non-significant, 6.71 \pm 0.380 and 6.75 \pm 0.484 respectively. All the other biochemical parameters were significant, Table (7).

Table (7): The mean \pm SE of the biochemical parameters of PF before and after canter

Biochemical markers	Before	After
TNCC (cellx109/L)	1.63 \pm 0.087 B	16.25 \pm 1.638 A
TP (g/L)	8.88 \pm 1.167 B	10.56 \pm 1.344 A
Fibrinogen (mg/dl)	122.74 \pm 6.474 B	157.52 \pm 7.208 A
Glucose (mg/dl)	153.70 \pm 3.970 A	121.84 \pm 2.599 B
Creatinine (mg/dl)	1.66 \pm 0.053 B	1.80 \pm 0.034 A
LDH (IU/L)	87.59 \pm 7.930 A	74.76 \pm 6.456 B
ALP (IU/L)	374.65 \pm 29.067 A	324.87 \pm 29.364 B
Lactate (mEq/L)	6.71 \pm 0.380 A	6.75 \pm 0.484 A
Sodium (mEq/L)	168.66 \pm 3.984 A	150.41 \pm 3.557 B
Chloride (mEq/L)	124.39 \pm 3.864 A	100.61 \pm 9.849 B
Potassium (mEq/L)	5.65 \pm 0.190 A	4.12 \pm 0.144 B

For before and after gallop, all the biochemical parameters were significant. TNCC (Cellx109/L) was 1.89 \pm 0.126 and 30.75 \pm 3.256 respectively, TP (g/L) was 8.41 \pm 0.555 and 17.76 \pm 1.323 respectively, fibrinogen (mg/dl) was 123.84 \pm 7.338 and 218.22 \pm 7.419 respectively, glucose (md/dl) was 160.80 \pm 4.284 and 77.45 \pm 3.793 respectively, creatinine (mg/dl) was 1.55 \pm 0.051 and 1.90 \pm 0.047 respectively, LDH (IU/L) was 79.52 \pm 8.161 and 58.15 \pm 5.786 respectively, ALP (IU/L) was 366.78 \pm 31.624 and 271.07 \pm 29.452 respectively, lactate (mEq/L) was 6.64 \pm 0.373 and 7.77 \pm 0.454 respectively, sodium (mEq/L) was 168.46 \pm 5.903 and 114.41 \pm 1.719 respectively, chloride (mEq/L) was 108.39 \pm 2.398 and 87.11 \pm 3.915 respectively, and potassium (mEq/L) was 4.46 \pm 0.170 and 3.14 \pm 0.151 respectively as shown in table(8).

Table(8): The mean \pm SE of the biochemical parameters of PF before and after gallop

Biochemical markers	Before	After
TNCC (cellx109/L)	1.89 \pm 0.126 B	30.75 \pm 3.256 A
TP (g/L)	8.41 \pm 0.555 B	17.76 \pm 1.323 A
Fibrinogen (mg/dl)	123.84 \pm 7.338 B	218.22 \pm 7.419 A

Glucose (mg/dl)	160.80±4.284 A	77.45±3.793 B
Creatinine (mg/dl)	1.55±0.051 B	1.90±0.047 A
LDH (IU/L)	79.52±8.161 A	58.15±5.786 B
ALP (IU/L)	366.78±31.624 A	271.07±29.452 B
Lactate (mEq/L)	6.64±0.373 B	7.77±0.454 A
Sodium (mEq/L)	168.46±5.903 A	114.41±1.719 B
Chloride (mEq/L)	108.39±2.398 A	87.11±3.915 B
Potassium (mEq/L)	4.46±0.170 A	3.14±0.151 B

Different letters mean significant variances at $p \leq 0.05$.

DISCUSSION

PF analysis is very useful for a lot of equine health problems like septic peritonitis, colic, intestinal pathologic lesions Etc (Huo et al.,2021). According to our knowledge, there are no previous studies of the effect of endurance exercise at different speeds (walking, trot, canter, and gallop) on the constituents of PF. This fact may be difficult to compare the results. The PF analysis may give important information about the performance of the sport horses(Walton, 2021). The speed may change many properties which reflect the pathophysiological state of the parietal and visceral mesothelial surfaces.

Abdominocentesis is a very simple, fast, and useful technique to collect PF in the field(Lindner,2000). Gradual increases of pH according to the hardness of the endurance exercise in which for walking was 7.60 ± 0.050 and in gallop was 9.12 ± 0.085 . This result might have been due to the rise of total proteins which move the pH shaft to the (Hooijberg and Lyle, 2020). Normal adult horses have little volume and clear or pale amber color of PF(Reed and Bayly,2004). While the results showed gradual changes in the clearance according to the speed of running and transformed to turbid fluid because of the increase in the TNCC and proteins (Smith,2015). Interestingly, there was an increase in the viscosity of PF, and that might have been due to increases in the hyaluronic acid, an essential anti-inflammatory constant, that might have happened.

The increase in the TNCC to $30.75 \pm 3.256 \times 10^9/L$ after galloping may indicate clearly that this high speed caused this effect. The normal value of TNCC may not exceed $5 \times 10^9/L$ in adult horses. This result indicates that the endurance exercise is a stress factor and this agreed with (Birckhead et al., 2023) who found that stress cause increase in the TCNN in horses. The TP level was increased significantly, reached to the tip especially after galloping ($17.76 \pm 1.323g/L$), because of the extravasations of proteins during the high speed which reached 80km/hr. Due to the inhibition of fibrinolytic activity and damage of mesothelial cells of peritoneum by the exposure of tissue thromboplastin and plasminogen activator level decreasing which promotes the precipitation of fibrin on the viscera, there was a visible significant increase of fibrinogen level at galloping, $218.22 \pm 7.419mg/dl$ and this compatible with(Ткаченко et al.,2020).

The endurance-exercise-based response of insulin is well-understood and recorded by McKeever KH(McKeever,2002). Insulin will prevent glucose from leaving the cells through their membranes, so the significant decrease of glucose in all speeds especially after galloping, $77.45 \pm 3.793mg/dl$, is rationally explained. The results of this work show a significant increase in lactate, $7.77 \pm 0.454 mEq/L$, after the endurance exercise, and this result agrees with (Gomide et al.,2006) who documented that there was an important increase of lactate in blood after the end of a demanding-based physical activity. After galloping, creatinine level, $1.90 \pm 0.047mg/dl$, was significantly increased due to the high physical activity of the muscular system anaerobically.

This work shows very interesting results in which the LDH and ALP activities were decreased significantly especially after galloping, $58.15 \pm 5.786 \text{ IU/L}$ and $271.07 \pm 29.452 \text{ IU/L}$ respectively, because there might be no hepatic exposure to endotoxins in contrast to the cases of intestinal problems like colic, inflammatory-based mediators or bacteria of enteric origin, and bile-duct-based stretching due to ileus (Zohier et al., 2023).

The results also showed significant decreases in sodium ($114.41 \pm 1.719 \text{ mEq/L}$), chloride ($87.11 \pm 3.915 \text{ mEq/L}$), and potassium ($3.14 \pm 0.151 \text{ mEq/L}$) after galloping, and that might have been due to reverse permeability of these elements through peritoneum to excrete outside the body with sweat or urine (Gomes et al., 2023).

CONCLUSION

A conclusion could be made that there were significant effects of endurance exercise on the physical and biochemical markers of the PF, and checking this fluid is crucial in sport horses because it is considered as a diagnostic tool and monitoring the disease progression as well as reflecting pathophysiology status. We consider these informational data are very useful for horsemen and veterinarians.

Disclosure of potential conflicts of interest

No conflicts of interest are present.

ACKNOWLEDGMENT

The authors would like to thank the University of Al-Qadisiyah Research Council and Iraqi National Science Foundation of the Ministry of Higher Education and Scientific Research for their support.

REFERENCES

- Apichaimongkonkun, T., Limratchapong, S., & Phetudomsinsuk, K., (2019). A retrospective analysis of the data of blood lactate concentrations and composite pain scores in Thai native crossbred ponies and full-sized horses underwent colic surgery. *Veterinary Integrative Sciences*, 17(3), 221–231. Retrieved from <https://he02.tci-thaijo.org/index.php/vis/article/view/177446>
- Birckhead, E. M., Das, S., Tidd, N., Raidal, S. L., & Raidal, S. R., (2023). Visualizing neutrophil extracellular traps in septic equine synovial and peritoneal fluid samples using immunofluorescence microscopy. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 35(6), 751–760. <https://doi.org/10.1177/10406387231196552>
- Eggleston RB, Mueller POE., 2003. Prevention and treatment of gastrointestinal adhesions. *Vet Clin North Am Equine Pract* [Internet]. Dec [cited (2018) Oct 2];19(3):741–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14740767>
- Gningue, S. M., Peach, R., Jarrah, A. M., & Wardat, Y. (2022). The relationship between teacher leadership and school climate: Findings from a teacher-leadership project. *Education Sciences*, 12(11), 749. <https://doi.org/10.3390/educsci12110749>
- Gomes, C. L., Alves, A. M., Ribeiro Filho, J. D., Moraes Júnior, F. J., Barreto Júnior, R. A., Fucuta, R. S., & Miranda, L. M., (2021). Physiological and biochemical responses and hydration status in equines after two barrel racing courses. *Pesquisa Veterinária Brasileira*, 40, 992-1001.).
Booth FW, Thomason DB. Molecular and cellular adaptation of muscle in response to

- exercise: perspectives of various models. *Physiol Rev* [Internet]. 1991 Apr [cited 2018 Oct 2];71(2):541–85. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2006222>
- Gomes, C. L., Alves, A. M., Ribeiro Filho, J. D., Moraes Júnior, F. J., Barreto Júnior, R. A., Fucuta, R. S., Miranda, L. M., (2021). Physiological and biochemical responses and hydration status in equines after two barrel racing courses. *Pesquisa Veterinária Brasileira*, 40, 992-1001.
- Gomide, L. M. W., Martins, C. B., Orozco, C. A. G., Sampaio, R. de C. de L., Belli, T., Baldissera, V., & Lacerda Neto, J. C. de., (2006). Blood lactate concentrations in horses during the distance test of the complete horse riding competition. *Rural Science*, 36(2), 509–513. <https://doi.org/10.1590/S0103-84782006000200022> (in Portuguese).
- Halyna Tkachenko, Natalia Kurhaluk, Irina Tkachova., (2020). EXERCISE-INDUCED CHANGES IN THE LEVEL OF TOTAL PROTEIN AND ITS FRACTIONS IN THE BLOOD OF HORSES INVOLVED IN RECREATIONAL HORSEBACK RIDING The Scientific and Technical Bulletin of the Institute of Animal Science NAAS of Ukraine, № 124, p. 35-46
- Hooijberg, E., & Lyle, C., 2020. Abdominal and Thoracic Fluid Analysis in Horses. *Veterinary Cytology*, 713-726.)
- Huo, X. ., Wongkwanklom, M. ., Phonraksa, T. ., & Na-Lampang, P., (2021). Effects of playing classical music on behavior of stabled horses : <https://doi.org/10.12982/VIS.2021.023>. *Veterinary Integrative Sciences*, 19(2), 259–267. Retrieved from <https://he02.tci-thaijo.org/index.php/vis/article/view/250739>
- Jam, F. A., Sheikh, R. A., Iqbal, H., Zaidi, B. H., Anis, Y., & Muzaffar, M. (2011). Combined effects of perception of politics and political skill on employee job outcomes. *African Journal of Business Management*, 5(23), 9896-9904.
- Jam, F., Donia, M., Raja, U., & Ling, C. (2017). A time-lagged study on the moderating role of overall satisfaction in perceived politics: Job outcomes relationships. *Journal of Management & Organization*, 23(3), 321-336. doi:10.1017/jmo.2016.13
- Jarrah, A. M., Almassri, H., Johnson, J. D., & Wardat, Y. (2022a). Assessing the impact of digital games-based learning on students' performance in learning fractions using (ABACUS) software application. *Eurasia Journal of Mathematics, Science and Technology Education*, 18(10), em2159. <https://doi.org/10.29333/ejmste/12421>
- Kilcoyne I, Nieto JE, (2020). Dechant JE. Diagnostic value of plasma and peritoneal fluid procalcitonin concentrations in horses with strangulating intestinal lesions. *J Am Vet Med Assoc*. 15;256(8):927-933. doi: 10.2460/javma.256.8.927.
- Lindner A., (2000). Use of blood biochemistry for positive performance diagnosis of sport horses in practice MOTS-CLÉS : performance-diagnostic-biochimie-sang-lactate-cheval [Internet]. Vol. 151, *Revue Méd. Vét.* [cited 2018 Oct 2]. Available from: https://www.revmedvet.com/2000/RMV151_611_618.pdf
- McGowan C., (2008). Clinical pathology in the racing horse: the role of clinical pathology in assessing fitness and performance in the racehorse. *Vet Clin North Am Equine Pract* [Internet]. [cited 2018 Oct 2];24(2):405–21, vii. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0749073908000199>
- McKeever K. H., (2002). The endocrine system and the challenge of exercise. *The Veterinary clinics of North America. Equine practice*, 18(2), 321–vii. [https://doi.org/10.1016/s0749-0739\(02\)00005-6](https://doi.org/10.1016/s0749-0739(02)00005-6)
- Radcliffe, R. M., Liu, S. Y., Cook, V. L., Hurcombe, S. D. A., & Divers, T. J. (2022). Interpreting abdominal fluid in colic horses: Understanding and applying peritoneal fluid evidence. *Journal of veterinary emergency and critical care (San Antonio, Tex. : 2001)*, 32(S1), 81–96. <https://doi.org/10.1111/vec.13117>
- Raquel M. Walton, Rick L. Cowell, Amy C. Valenciano, (2020). Pleural, Peritoneal, and Synovial Fluid Analysis. *Equine Hematology, Cytology, and Clinical Chemistry*, 275-292.) DOI:10.1002/9781119500186.

- Reed S. M., Bayly W. M. SDC.,(2004). Equine Internal Medicine. 2nd ed. Elsevier, editor. St Louis;, 941-949 p.
- Ruggles AJ, Freeman DE, Acland HM, FitzSimmons M.,(1993) . Changes in fluid composition on the serosal surface of jejunum and small colon subjected to venous strangulation obstruction in ponies. Am J Vet Res [Internet]. [cited 2018 Oct 2];54(2):333–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8430944>
- Saulez MN, Cebra CK, Tornquist SJ.,(2018) . The diagnostic and prognostic value of alkaline phosphatase activity in serum and peritoneal fluid from horses with acute colic. J Vet Intern Med [Internet]. [cited 2];18(4):564–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15320599>
- Seabaugh KA, Goodrich LR, Bohn AA, Morley PS, Hendrickson DA., (2014).A comparison of peritoneal fluid values in mares following bilateral laparoscopic ovariectomy using a vessel sealing and dividing device versus placement of two ligating loops. Vet J. 2014 Nov;202(2):297-302. doi: 10.1016/j.tvjl.2014.08.025. Epub.
- Smith B.P., (2015). Large Animal Internal Medicine. 5th ed. Mosby, editor. Mosby. 723-728 p.
- Tashtoush, M. A, Wardat, Y., Aloufi, F., & Taani, O. (2022). The effect of a training program based on TIMSS to developing the levels of habits of mind and mathematical reasoning skills among pre-service mathematics teachers. Eurasia Journal of Mathematics, Science and Technology Education, 18(11), em2182. <https://doi.org/10.29333/ejmste/12557>
- Teixeira-Neto AR, Ferraz G de C, D'Angelis FH de F, Lacerda-Neto JC de, Queiroz-Neto A de., (2007) .Exercise intensity, but not electrolyte reposition, alters plasmatic cortisol and glucose levels of horses submitted to 30 and 60km distance endurance rides. Ciência Rural [Internet]. [cited 2018 Oct 2];37(3):740–3. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782007000300021&lng=en&tlng=en
- TRILK JL, LINDNER AJ, GREENE HM, ALBERGHINA D, WICKLER SJ., (2010) . A lactate-guided conditioning programme to improve endurance performance. Equine Vet J [Internet]. [cited 2018 Oct 2];34(S34):122–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12405671>
- Van Hoogmoed L, Rodger LD, Spier SJ, Gardner IA, Yarbrough TB, Snyder JR., (1999). Evaluation of peritoneal fluid pH, glucose concentration, and lactate dehydrogenase activity for detection of septic peritonitis in horses. J Am Vet Med Assoc [Internet]. [cited 2018 Oct 2];214(7):1032–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10200799>
- Walton, R.M. and Southwood, L.L.,(2012). Abdominocentesis and Peritoneal Fluid Analysis. In Practical Guide to Equine Colic, L.L. Southwood (Ed.). <https://doi.org/10.1002/9781118704783.ch10>
- Wardat, Y., Jarrah, A. M., & Stoica, G. (2021). Understanding the meaning of the equal sign: A case study of middle school students in the United Arab Emirates. European Journal of Educational Research, 10(3), 1505-1514. <https://doi.org/10.12973/eu-jer.10.3.1505>
- Watchrarat, K., Kidtiwong, A., Isawirodom, A., Phetkarl, T., Sthaporn, T., Na Lampang, K., Warrit, K., & Rungsri, P., (2023). Clinical study of palmar foot pain in 30 polo ponies with forelimb lameness in Thailand: <https://doi.org/10.12982/VIS.2024.047>. Veterinary Integrative Sciences, 22(3), 693–711.
- Zachary J.F., (2012).DMM. Pathologic Basis of Veterinary Disease. 5th ed. Mosby, editor. St. Louis: Mosby; 401-402 p.
- Zohier, A. S., Baraka, T. A., Abdelgalil, A. I., Aboelmaaty, A. M., & Yehia, S. G.,(2023). Oxidative stress biomarkers status in selected equine sports. Comparative Clinical Pathology, 32(3), 461-466.