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EVALUATION OF OXIDATIVE STRESS PARAMETERS OF HORSES HOUSED WITH GOATS IN INDIVIDUAL BOXES

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ABSTRACT: Horses are animals that are affected very quickly by the warnings coming from the environment. In this study, it was aimed to evaluate oxidative stress parameters of horses obtained from saliva analysis related to animal welfare as a result of keeping horses together with goats. While the research was being prepared, three groups were developed based on the time spent sheltering goats and horses. The horses were housed alone in the first and last 15-day groups, and along with the goats in the second 15-day group. In these stages, the levels of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and ischemia-modified albumin (IMA) level in saliva were examined. Results showed that MDA and IMA levels decreased, but CAT, GPx, and SOD activities increased. It was concluded that goats had a positive effect on horses according to the oxidative stress parameters examined in terms of animal welfare. However, there is still a need for research that will house horses with various animals in acceptable animal welfare circumstances, analyse their stress metrics, and maintain a high level of welfare.

Keywords: Animal welfare, Equus caballus, Oxidative stress, Single stall housing, horse, goat.

INTRODUCTION

Behavior is described as a change in the condition of an animal's body in all or part of it, or in other words, the animal's reaction to its environment while it is in direct contact with it (Özbeyaz and Akçapınar, 2006). Most animals use behavior to adapt to and regulate their surroundings (Kappeler, 2010). Unsuitable care and feeding circumstances in animal husbandry might result in aberrant behavior in animals (Mellor, 2015; Arndt et al., 2022). Horse abnormal behaviors are characterized by a succession of unwanted, ineffective, and recurrent negative habits (McAfee et al., 2002; Gill et al., 2005).

Most research believes that abnormal behaviors are induced by the suppression of friendship, i.e., loneliness in individual compartments. As a result, offering friendship to other animals or establishing conditions that are not harmful to horses will benefit their welfare.

As with most animals, horses need someone to live their daily lives and social interactions and care. Isolating horses completely from their surroundings or from their social environment might result in the development of undesired disorders (Landsberg and Denenberg, 2019). Therefore, the primary goal of this research was to see how the enhanced environment created by the breeders for the goats influenced the horses, and the horses were allowed to interact with the goats.

It is known that unstimulated saliva plays an important role in oral immunity, enamel integrity and keeping the oral mucosa wet. Insufficient saliva flow causes dental caries, mucosal deterioration, and dry mouth (Ono et al., 2006). In the last 10 years, the use of saliva as a diagnostic fluid has become increasingly important. The use of saliva as a diagnostic material in the monitoring of drug metabolisms in autoimmune disorders, pharmacology, endocrinology, nephrology, infectious diseases, cardiovascular diseases, psychiatry, oncology, and many other fields is becoming widespread (Streckfus and Bigler, 2002). There are many defenses in the body against oxidative stress caused by free radicals, and the first line of these defenses is saliva. It has been stated that the attacks of free radicals on the oral mucosa can cause different results from infection to cancer. Saliva is the first defense step against free radicals with the antioxidant molecules (a rich source of antioxidants) and enzymes it contains (the salivary peroxidase system) (Maciejczyk et al., 2021).

Various antioxidant defense systems have been developed in biological systems to limit the formation of free radicals and the damage they cause. Glutathione peroxidase (GPx) is an antioxidant enzyme that contains the amino acid selenocysteine in its active site and is effective on H_2O_2, steroid and lipid hydroperoxides. The primary scavenging enzyme in scavenging oxygen free radicals (ROS) is superoxide dismutase, which converts O_2- to H_2O_2. GPx, on the other hand, is

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the key enzyme responsible for the detoxification of cellular H_2O_2. It transforms H_2O_2 into molecular oxygen and water (McCord, 2000; Wei et al., 2004). Saliva is the first biological agent to encounter foreign substances that enter our body through food, drink or inhalation and is the first defense step against free radicals (Streckfus and Bigler, 2002).

There is no scientific research in the literature that shows the change in some oxidative stress values that are considered important for animal welfare when goats and horses are housed together. For this reason, this research aims to show the changes in some oxidative stress parameters MDA, IMA, SOD, GPx and CAT activities that occur when keeping goats and horses in a paddock. In accordance with all of the observations, literature, and reviews, the hypothesis of this study is that goats will contribute positively to some oxidative stress parameters of horses that are regarded as important in terms of animal welfare.

MATERIALS AND METHODS

Research plan

While forming the study groups of this research, three groups were formed considering the residence times of goats and horses together and separately. During the first and last 15 days of these groups, horses were housed alone, and in the second 15-day group, horses were housed together with goats. At the end of each 15-day stage, saliva samples were taken from horses, and the materials for the research were created.

Ethical approval

Experimental procedures adopted in this study were complied with the ARRIVE guidelines (Percie du sert et al., 2020).

The test area, animals and management

Figures 1 and 2 show the research areas in the barn where the horses stay alone or with the goats. In these areas, horses and goats were housed together (19:00 to 08:00 h) while the horses were in the stables.

The horse barn where the research was conducted had a length of 19.5 meters, a width of 9.5 meters, and a middle roof height of 6 meters. The horses in the stable used in the research were sheltered in six individual boxes surrounded by iron fences over the walls. The individual compartment of each horse, where the study was carried out, had an area of 3.5 x 3 m2. The service road in the middle of the barn is 2.8 meters wide.

The breeds of six horses used in the study were 3 Thoroughbred (1 gelding, and 2 mare) and 3 Haflinger (1 stallion, 1 gelding, and 1 mare), and their ages ranged from 9 to 20. Horses and goats were given both concentrate and roughage twice a day at 08:00 and 19:00 while they were in the barn. The nutritional needs of the animals used in the study were given daily by the breeders. The needs of these nutrients were calculated by the enterprise and presented as roughage and concentrate. Goats used in the research—breed-hair goats—were used to be 6 months old and female.

To meet the daily water needs of horses and goats, they always had clean water in front of them. The cleaning and maintenance of their litter material was done daily at 08:00. The breeders and observers at the shelter constantly checked the general condition of the horses and goats. Everything needed by horses and goats in the animal shelter was met as much as possible. In addition, horses and goats were grazed and irrigated in an outdoor paddock area during daylight hours convenient for observers.

Figure 1 - The horse's box stalls.

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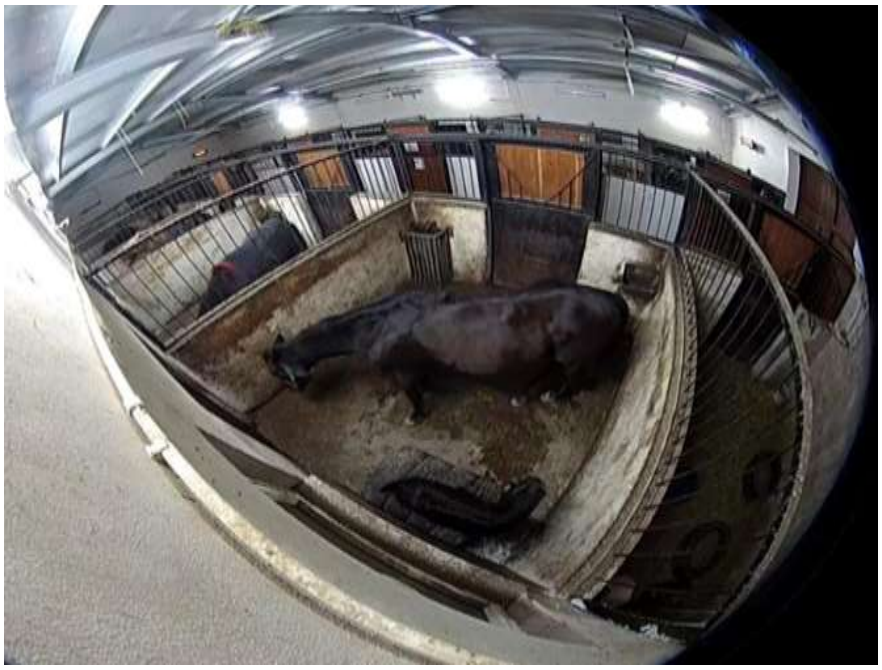

Figure 2 - Housing horse and goat in the box stall.

Accustoming goats to horses

Initially, for a certain period of time, horses and goats eventually become acquainted with one another. The goats were permitted to stay with the horses for 15 days (16-30 days) after becoming acclimated to them during the second groups days of the research. One horse stayed with a goat during the research while the goats and horses were together. The same goat was placed next to the same horse each time.

Saliva analysis

Saliva samples were taken from the mouths of all Horses in the morning on an empty stomach by means of swaps. A total of 18 (6x3) saliva samples were collected on the 15th, 30th and 45th days of the study. In saliva samples taken; MDA level (Yoshioka et al., 1979), CAT (Goth, 1991), GPx (Matkovics et al., 1988), SOD activities (Sun et al., 1988) and IMA (Bar-Or et al., 2000) levels were measured with Biotek ELISA Reader (Bio Tek µQuant MQX200 Elisa reader/USA).

Statistical analysis

To examine the outcomes of salivary data from horses, a statistical program was used. (SPSS, Version 25, IBM Corp., Armonk, NY, USA). With this program, oxidative stress parameter variables (MDA, SOD, CAT, GPx, and IMA) obtained from horse saliva analysis were analyzed using the GLM procedure. Tukey multiple range tests were performed on unique groups according to the results. If the level of difference was $P<0.05$ in the whole data analysis, it was considered statistically significant.

RESULTS AND DISCUSSION

The results obtained between the study groups formed at 15-day intervals are shown in Table 1. As a consequence of the research groups, the investigated parameters of horses, MDA level ($P<0.05$), SOD ($P<0.01$), CAT ($P<0.01$), GPx activities ($P<0.01$) and IMA level ($P<0.001$) were significantly affected by goats. According to the evaluations made between the observation groups (1-3), it was determined that MDA and IMA levels decreased, but CAT, GPx, and SOD activities increased after the goats were placed next to the horses.

While Figure 3 shows that a goat even rides a horse, and the horse did not showed any attitude towards it, the results of the saliva analysis were also an indication that the goats have a beneficial impact on the horses in the same direction. Therefore, it can be concluded that keeping the goat with the horse does not prevent the welfare of the horse but is also positive for the health of the animal and reduces stress. However, as with every animal, it should not be forgotten that horses can unintentionally harm goats due to their large body size, and precautions should be taken to prevent this.

The current study is the first to report on the effect of goats on animal welfare-related oxidative stress parameters (MDA, CAT, GPx, SOD and IMA) in box stall horses. Individual stable boxes are normal practice for many racehorse breeders when it comes to performing horses like racehorses (Munoz et al., 2014; Munoz et al., 2018). Similarly to prior research, the six box stalls used for this study kept their horses in separate stable boxes (Normando et al., 2002; Hockenhull and Creighton, 2010; Leme et al., 2014; Hanis et al., 2020).

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Horses are still mostly confined in single stalls, despite mounting scientific evidence that single-stall living is detrimental to horse wellbeing. As a result, social relationships are critical, and they are a requirement for ensuring excellent horse welfare (Lesimple et al., 2020). In this research, we used goat, a live material, for horse socialization, and we discovered positive responses in certain oxidative stress measures related to animal wellbeing.

As with many creatures, horses are creatures that need social contact or communication with other living things during the day. Isolating horses from social life and communication may cause undesirable problems for them. The communication between goats and horses was ensured in this study, and their idle time was reduced. Also, they were observed less as a consequence of these regulations of undesired blood results related to animal welfare.

Table 1 - Oxidative stress parameter values observed at 15-day intervals in the research group consisting of Thoroughbred and Haflinger horses (Mean ± SD).

Variable	Group 1 (0-15 days)	Group 2 (16-30 days)	Group 3 (31-45 days)	*P* value
MDA (nmol/mL)	1.90 ± 0.06 ^{ab}	1.86 ± 0.50 ^{b}	1.92 ± 0.06 ^{a}	0.022
SOD (U/mL)	15.59 ± 0.75 ^{ab}	16.05 ± 0.52 ^{a}	15.13 ± 0.92 ^{b}	0.018
CAT (IU/mL)	16.39 ± 1.88 ^{b}	18.45 ± 1.68 ^{a}	16.00 ± 2.31 ^{b}	0.010
GPx (U/L)	0.19 ± 0.03 ^{ab}	0.21 ± 0.03 ^{a}	0.18 ± 0.02 ^{b}	0.008
IMA (ΔABSU)	0.18 ± 0.02 ^{a}	0.12 ± 0.01 ^{b}	0.17 ± 0.03 ^{a}	<0.001

The influence of differences between observation periods is shown by the P values; different superscript letters within each row indicate differences between groups (P < 0.05). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, IMA: Ischemia-modified albumin

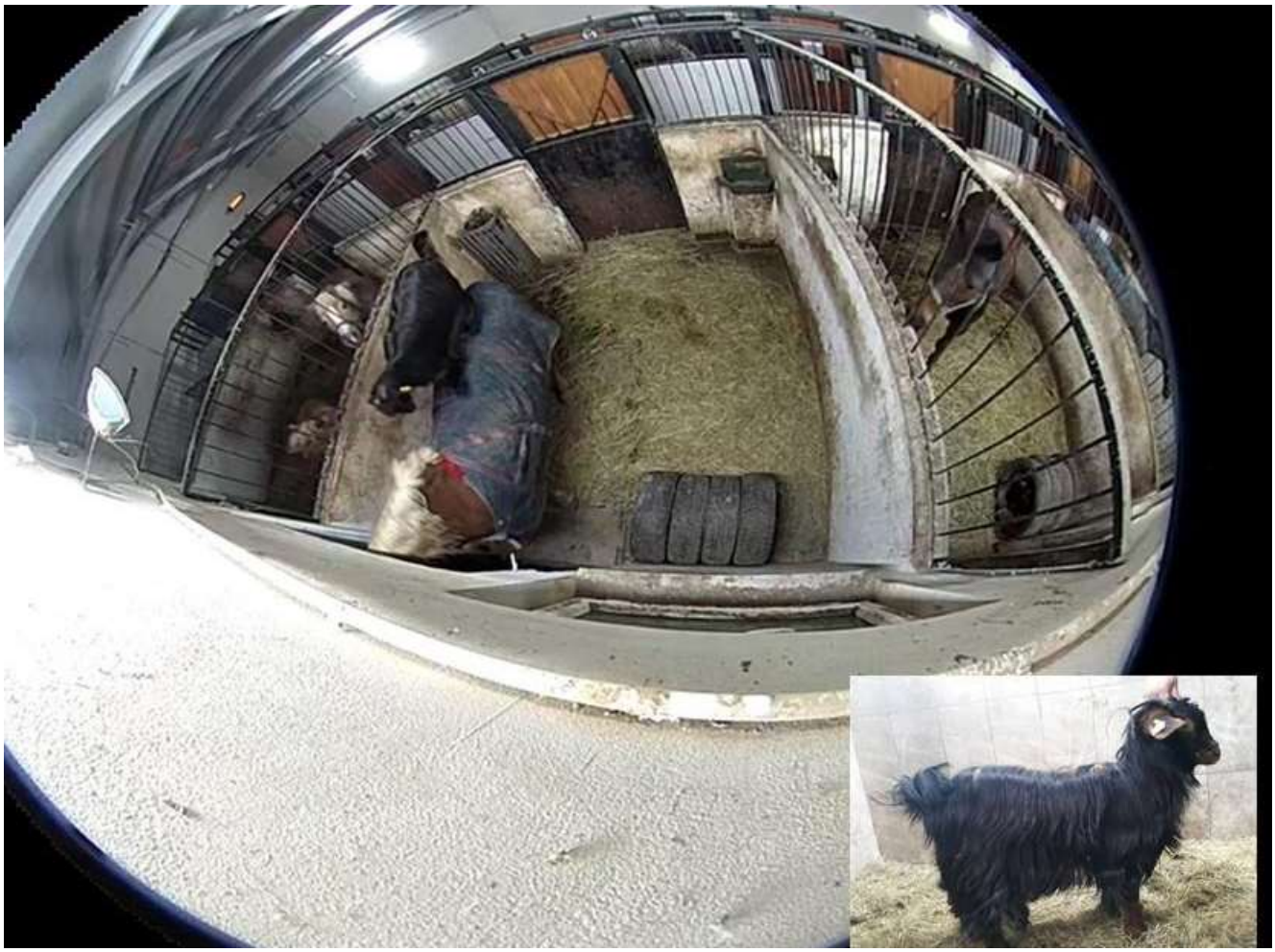

Figure 3 - A goat has ridden a horse.

CONCLUSION

When the data collected after the goats were placed near to the horses was analyzed, it was discovered that MDA and IMA levels fell while CAT, GPx, and SOD activities increased. These oxidative stress parameter results show that goats have a positive effect on the animal welfare of horses. Because horses are sensitive to their surroundings, their

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environment might induce stress parameter values that produce significantly diverse effects for them. So, there is a need for research that will house horses with various animals in acceptable animal welfare circumstances, analyze their stress metrics, and maintain a high level of welfare.

DECLARATIONS

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Ethical statement for publication

All authors have reviewed the ethical issues.

Authors' contribution

Both authors contributed to the field studies, data collection, and experiment design stages of the research. While B. Apaydin Yildirim did their saliva analysis, F. Yildirim analyzed the data statistically. Both authors contributed to the article's writing and read and approved the final article.

Data availability

The data used and analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors have not declared any conflict of interest.

REFERENCES

Arndt SS, Goerlich VC and Van Der Staay FJ (2022). A dynamic concept of animal welfare: the role of appetitive and adverse internal and external factors and the animal's ability to adapt to them. Frontiers in Animal Science, 3: 908513. https://doi.org/10.3389/fanim.2022.908513

Bar–Or D, Lau E and Winkler JV (2000). A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. The Journal of Emergency Medicine, 19: 311-315. https://doi.org/10.1016/s0736-4679(00)00255-9

Gill W, Meadows DG and Neel VB (2005). Understanding horse behaviour, Agricultural Extension service, Institute of Agriculture, 4-H Member Guide, Unit 8, Grade 12, Tennessee. https://www.shelbycountytn.gov/DocumentCenter/View/1177/Understanding-Horse-Behavior?bidId=

Goth L (1991). A simple method for determenation of serum catalase activity and revision of serum catalase activity and revision of reference range. Clinica Chimica Acta, 196: 143–152. https://doi.org/10.1016/0009-8981(91)90067-m

Hanis F, Chung ELT, Kamalludin MH and Idrus Z (2020). The influence of stable management and feeding practices on the abnormal behaviors among stabled horses in Malaysia. Journal of Equine Veterinary Science, 94: 103-230. https://doi.org/10.1016/j.jevs.2020.103230

Hockenhull J and Creighton E (2010). Unwanted oral investigative behaviour in horses: A note on the relationship between mugging behaviour, hand-feeding titbits and clicker training. Applied Animal Behaviour Science, 127(3-4): 104-107. https://doi.org/10.1016/j.applanim.2010.08.008

Kappeler P and Kraus C (2010). Levels and mechanisms of behavioural variability. Animal Behaviour: Evolution and Mechanisms, Springer-Verlag Berlin Heidelberg, 655-684. https://doi.org/10.1007/978-3-642-02624-9_21

Landsberg GM and Denenberg S (2019). Behavior problems in horses, MSD Veterinary Manual, Merck Sharp, and Dohme Corp., a subsidiary of Merck and Co., Inc., Kenilworth, NJ, USA. https://www.msdvetmanual.com/horse-owners/behavior-of-horses/behavior-problems-in-horses

Leme DP, Beatriz A, Parsekian H, Kanaan V and Jose M (2014). Management, health, and abnormal behaviors of horses: A survey in small equestrian centers in Brazil. Journal of Veterinary Behavior, 9: 114-118, http://dx.doi.org/10.1016/j.jveb.2014.01.004

Lesimple C, Reverchon-Billot L, Galloux P, Stomp M, Boichot L, Coste C and Hausberger M (2020). Free movement: A key for welfare improvement in sport horses?. Applied Animal Behaviour Science, 225: 104972. https://doi.org/10.1016/j.applanim.2020.104972

Maciejczyk M, Bielas M, Zalewska A and Gerreth K (2021). Salivary biomarkers of oxidative stress and inflammation in stroke patients: from basic research to clinical practice. Oxidative Medicine and Cellular Longevity, 1-22. https://doi.org/10.1155/2021/5545330

Matkovics B, Szabo L and Varga IS (1988). Determination of enzyme activities in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika, 15: 248–250. https://cir.nii.ac.jp/crid/1572824500566167680

McAfee LM, Mills DS and Cooper JJ (2002). The use of mirrors for the control of stereotypic weaving behaviour in the stabled horse. Applied Animal Behaviour Science, 78: 159-173. https://doi.org/10.1016/S0168-1591(02)00086-2

McCord JM (2000). The evalution of free ardicals and oxidative stres. The American Journal of Medicine, 108 (8), 652-659. https://doi.org/10.1016/S0002-9343(00)00412-5

Mellor DJ (2015). Positive animal welfare states and encouraging environment-focused and animal-to-animal interactive behaviours. New Zealand Veterinary Journal, 63(1): 9-16. https://doi.org/10.1080/00480169.2014.926800

Munoz AL, Ainardi CF, Rehhof VC, Cruces LJ, Ortiz RR and Briones LM (2014). Prevalence of stereotypies in thoroughbred race horses at Club Hípico Concepcion, Chile. Revista MVZ Córdoba, 19: 4259-4268. http://www.scielo.org.co/pdf/mvz/v19n3/v19n3a07.pdf

Munoz L, Leon C, Cruces J, Ortiz R and Briones M (2018). Locomotor stereotypies and racing performance in thoroughbred horses. Journal of Veterinary Behavior, 25: 24-27. https://doi.org/10.1016/j.jveb.2018.03.006

Normando S, Canali E, Ferrante V and Verga M (2002). Behavioral problems in Italian saddle horses. Journal of Equine Veterinary Science, 22: 117-120. https://doi.org/10.1016/S0737-0806(02)70123-8

Ono K, Morimoto Y, Inoue H, Masuda W, Tanaka T and Inenaga K (2006). Relationship of the unstimulated whole saliva flow rate and salivary gland size estimated by magnetic resonance image in healthy young humans. Archives of Oral Biology, 51(4): 345-349. https://doi.org/10.1016/j.archoralbio.2005.09.001

Özbeyaz C and Akçapınar H (2006). Horse breeding [English], Ankara University, Faculty of Veterinary Medicine, Animal Science, Ankara, 2006. https://acikders.ankara.edu.tr/course/

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M and et al. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Journal of Cerebral Blood Flow & Metabolism, 40(9): 1769-1777. https://doi.org/10.1177/0271678X2094382

Streckfus CF and Bigler LR (2002). Saliva as a diagnostic fluid. Oral Diseases, 8(2): 69-76. https://doi.org/10.1034/j.1601-0825.2002.1o834.x

Sun Y, Oberley LW and Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34: 497-500. https://doi.org/10.1093/clinchem/34.3.497

Wei PF, Ho KY, Ho YP, Wu YM, Yang YH and Tsai CC (2004). The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1β in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases. Journal of Periodontal Research, 39: 287-293. https://doi.org/10.1111/j.1600-0765.2004.00744.x

Yoshioka T, Kawada K and Shimada T (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American Journal of Obstetrics and Gynecology, 135(3): 372-376. https://doi.org/10.1016/0002-9378(79)90708-7

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