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Changes in serum PON1 activity, gene expression and their association with lipid profile parameters in healthy Darehshori neonatal foals: a preliminary study

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Abstract: Paraoxonase 1 (PON1) contributes to the antioxidant mechanisms that prevent lipoprotein oxidation and act as a negative acute-phase protein. This study aimed to evaluate changes in serum PON1 activity, the leukocyte *PON1* gene expression, as well as lipid profile tests (LDL, HDL, triglyceride, and cholesterol) in 16 healthy Darehshori newborn foals up to 60 days of life. **B**lood samples were collected at 1, 7, 15, 30, and 60 days used for detecting biochemical parameters, PON1 activity, and, and its gene expression, using real-time RT-PCR. Low levels of PON1 activity were observed at birth, which increased significantly with increasing age at days 7, 15, 30, and 60. In addition, HDL levels were significantly increased at 15, 30, and 60 days in male foals, and in contrast, LDL and cholesterol levels were significantly reduced on days 15, 30, and 60 compared to the first day of life in newborn foals. For the first time, *PON1* mRNA was detected in foal leukocytes and its gene expression showed a significant decrease compared to the birthday. The positive correlations between *PON1* expression/HDL, LDL, and cholesterol in the first week of life and between PON1 enzyme activity/ HDL, LDL, and cholesterol in different samples and sexes may indicate an adaptive increase in antioxidant defenses in response to oxidative stress. The authors suggest that *PON1* expression in leukocytes may be related to the enzyme's ability to reduce intracellular oxidant levels.

Key words: Horse, neonatal foals, paraoxonase, lipid profile, gene expression

1. Introduction

The health of newborn foals may be affected by the metabolic and nutritional status of the mare during pregnancy, parturition, and postpartum periods [1]. It is well known that oxidant/antioxidant balance has a comprehensive influence on the health of farm animals [2]. The antioxidant defense system has protective effects against proinflammatory mediators and oxidative stress [3]. In this regard, paraoxonase-1 (PON1) is found in a variety of mammalian species that act as antiinflammatory and antioxidant enzymes and mainly hydrolyze organophosphates [4]. Paraoxonase has a wide range of substrates and different catalytic activities, including lactonase and arylesterase, and acts as a negative acute-phase protein (APP) [5–7]. *PON1* is primarily expressed in the liver [5,6]. PON1 prevents high-density lipoprotein cholesterol (HDL-C) oxidation by hydrolyzing specific lipid peroxides [7]. In turn, HDL is necessary for PON1 secretion, distribution, stability, and activity. Therefore, any changes in the structure and composition of HDL could explain the reduced activity and hepatic gene expression of *PON1* [7,8]. Serum PON1 concentration has been regulated by several factors, such as genes, age, sex, lifestyle, and pharmaceutical interventions [9]. Additionally, PON1 has been demonstrated to protect against prenatal and postnatal organophosphate (OP) toxicity in animal studies. [10]. Generally, low serum PON1 is related to different diseases such as atherosclerosis, diabetes, cancer, chronic renal failure, and hepatic disorders. Serum PON1 activity decreases in inflammatory conditions and oxidative stress, too. Therefore, the activity of this enzyme could be considered a potential biomarker for many pathological conditions and also as a part of the innate immune system [4, 11]. PON1 activities were also investigated in diseased animals [11,12]. It was indicated that measurement of PON1 activity might be useful to evaluate the progression of inflammatory conditions in horses [7,13]. Recently, paraoxon-based methods have been validated to measure serum PON1 activity of equine serum samples, and reference intervals have been established for this species. It was also suggested that the assessment of PON1 activity is useful to correctly classify healthy and sick horses [14].

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Even though the fact that PON1 plays a significant role in clinical diagnostic work in horses, there has been no research on the changes in serum PON1 activity and gene expression in healthy newborn foals. Van Lenten et al. [15] pointed out the presence of the *PON1* gene in the liver of laboratory animals. Ruggerone et al. [14] suggest that serum PON1 is inactivated during acute phase response and its hepatic gene expression is inhibited as well. The foal liver biopsy, on the other hand, necessitates anesthesia and laparoscopic or ultrasonography guides, which are costly, time-consuming, and potentially life-threatening [16]. Because liver biopsy is a very invasive method, determining paraoxonase gene expression in the circulating leukocytes can be considered a safer strategy. Moreover, it is indicated that PON2, another member of the paraoxonase family, is involved in reducing the intracellular oxidative stress of macrophages [17]. But research on *PON1* expression in leukocytes is still not available. Therefore, the purpose of the present study was to investigate the *PON1* gene expression in leukocytes, lipid profile, and serum PON1 activity in newborn foals for the first time.

2. Materials and methods

2.1. Animals and experimental design

The experimental procedures were approved by the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of animals)Date: 8.9.2020, No: 190.2218). Thirty-two pregnant mares were monitored closely until parturition, and 16 healthy newborn Darehshori foals (8 females and 8 males) were selected. All foals were born under staff supervision and remained with mares throughout the experiment. All of the foals in this study were fed and kept in similar conditions. The newborn foals received adequate colostrum. The study was designed to last 60 days.

2.2. Sample collection

Blood collection was carried out by jugular venipuncture after birth as well as 7, 15, 30, and 60 days of age, using tubes containing sodium heparin for plasma and a nonanticoagulant tube for serum. The samples were centrifuged at 4000 rpm for 10 min. Serum was collected to determine the lipid profile and PON1 activity and stored at –20 °C until use. The plasma was discarded and a buffy coat was used to evaluate *PON1* gene expression.

2.3. Measurement of serum PON1 activity

Serum PON1 activity was assessed using a commercial kit (ZellBio, Ulm, Germany, catalog number: ZB-PON-48A) according to the manufacturer's instructions. The hydrolysis of paraoxon to p-nitrophenol produces a yellow color. The wavelength was monitored at 0 and 2 min at 412 nm by a colorimeter. Enzyme activity was calculated according to the following formula:

Enzyme activity (U/mL) = OD / 0.0008.

2.4. Lipid profile assay

Triglyceride (TG) and total cholesterol (TC) concentrations were measured using an enzymatic colorimetric assay (Pars Azmoon kits, Tehran, Iran, catalog numbers: GPO-PAP, CHOD-PAP) by an automatic serum auto-analyzer (BT3000, Italy). The level of HDL-C was assayed by using enzymatic kits (Bionic Diagnostic Kit, Tehran, Iran, catalog number: 20.04 LTS) according to the colorimetric method based on standard manual kit procedures with the utilization of an auto analyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedwald et al. [18].

2.5. RNA isolation and cDNA synthesis

Total RNA was extracted from the collected blood samples using the RNX-Plus solution according to the company manual (Sinaclone, Iran, catalog number: RN7713C). The quantity and quality of the purified RNAs were verified by a nanodrop spectrophotometer and electrophoresis using 1% agarose gel, respectively. Approximately 50 ng of RNA was used for the complementary DNA (cDNA) synthesis using a cDNA synthesis kit (Vivantis, Malaysia, catalog number: cDsk01-050) according to its manufacturer's protocol. Synthesized cDNA was kept at –20 °C until it was used.

2.6. Primer design and qRT-PCR experiment

The specific primers were designed with the GeneRunner software, tested with the NCBI Primer Blast, and synthesized by CinnaGen (CinnaGen Co, Tehran, Iran). The full properties of the designed primer are accessible in Table 1. The GAPDH gene was used as an internal control gene for relative gene expression analysis [19]. Real-time RT-PCR was performed. Each reaction contained 0.5 µL of cDNA, 0.5 µL of 0.4 µM solutions of each of the forward and reverse primers and 5 µL of 2 × SYBR green master mix in a total volume of 10 µL. Then, all strip tubes were entered into the StepOne Real-Time PCR (Applied Biosystems, Carlsbad, CA, USA). For PCR amplification, the following steps were undertaken: initial denaturation at 95 °C for 15 min, 40 cycles at 95 °C for 10 s, 55 °C for 30 s, then 72 °C for 30 s. Finally, the fold changes were calculated using the $2^{-\Delta\Delta CT}$ method by Livak and Schmittgen [20].

2.7. Statistical analysis

The data is normalized via the Shapiro–Wilk test and SigmaPlot 14 version (Systat Software Inc.) program was used to analyze the data (mean ± SEM) using repeated measure one-way analysis of variance (RM ANOVA) and Pearson correlation at the $p < 0.05$ level. Dunnett's method, Tukey's and Holm–Sidak tests were also used for multiple comparisons of data.

3. Results

In both sexes, PON1 activity increased significantly from birth onwards, and conversely, leukocyte *PON1* gene

expression and serum levels of LDL and cholesterol were reduced on days 15, 30, and 60 compared to the first day of life ($p < 0.05$) (Table 2). The comparison of lipid profiles (cholesterol, LDL, HDL, and triglycerides), PON1 activity, and *PON1* gene expression rate in female and male foals is described in (Table 3). At the age of two months, positive correlations occurred between TG/PON1 activity ($r = +0.689$, $p = 0.019$) and TG/*PON1* expression ($r = +0.74$, $p = 0.009$). The relationship between TG/PON1 activity had already occurred on the day of birth ($r = +0.671$, $p = 0.0239$). From the first week onwards, a positive correlation occurred between cholesterol/HDL, cholesterol/LDL, LDL/HDL, and triglyceride/HDL (Table 4).

Serum PON1 activity was low in female foals at birth and increased significantly at 7, 15, 30, and 60 days of age compared to the birthday ($p = 0.005$, $p = 0.001$, $p = 0.002$, and $p = 0.002$, respectively). Similarly, serum PON1 activity of male foals increased with age and exhibited higher levels at 7, 15, 30, and 60 days of age ($p < 0.001$) compared to the first day of life. The age-associated increase in serum PON1 activity was higher in male foals at 30 and 60 days than in females. However, this increase was not statistically significant (Table 3 and Figure 1).

The decreasing trend of gene expression in female foals continued up to 60 days of life, and only a significant increase was observed at 30 days compared to 15 days ($p = 0.036$). There were also significant reductions in cholesterol and LDL concentrations that occurred in 30 and 60 days compared to the first day of female life (Table 3 and Figure 2). Positive correlations were found between *PON1* expression/cholesterol, LDL and HDL at 7 days ($r = +0.706$, $p = 0.0151$; $r = +0.688$, $p = 0.0193$ & $r = +0.662$, $p = 0.0265$) and negative ones with triglyceride at 60 days of age ($r = -0.643$, $p = 0.033$) (Table 4).

On the first day of female life, a positive and negative correlation were recorded between cholesterol/LDL ($r = +0.996$, $p = 0.000127$) and TG/HDL ($r = -0.9$, $p = 0.037$), respectively. Afterward, paraoxonase activity/HDL experienced a negative correlation ($r = -0.901$, $p = 0.0364$) at 7 days of age, but other correlations (HDL/LDL, HDL/TG, and TG/cholesterol) were positive at this time and also at 15 days of age ($p < 0.05$). In the first month of life, only a positive correlation occurred between LDL/cholesterol ($r = +0.97$, $p = 0.000631$) (Table 4).

In male foals, HDL increased at 15, 30, and 60 days, and in contrast, serum LDL was reduced at 60 days

Table 1. Sequences of oligonucleotide primers used for qRT-PCR analysis.

Gene	Primers	Sequence	Product size (bp)	Accession number
GAPDH	Forward	5-GGTCGGAGTAAACGGATTTGGC-3	130	XR_546436.1
	Reverse	5- CTGAAACATGTAGACCATGTAG -3		
PON1	Forward	5'- GTTATATTTGGGTTTAGCATGG-3'	125	XM_001493403.6
	Reverse	5'- CATAGACATACTTGCCATCAGG-3'		

bp: Base pair.

Table 2. Serum lipid profiles, PON1 activity and, its leukocyte gene expression (mean ± SEM) in Darehshori newborn foals up to 60 days of age.

Days of sampling	**Parameters**					
	PON1 mRNA (fold change)	**PON1 activity** (U/mL)	**LDL (mg/dL)**	**HDL (mg/dL)**	**TG (mg/dL)**	**Cholesterol (mg/dL)**
1	0.807 ± 0.102	23.23 ± 3.02	213.57 ± 36.93	56.72 ± 3.29	85.54 ± 15.62	297.45 ± 38.76
7	**0.25 ± 0.043[a, c]**	**53.16 ± 4.23[a]**	152.27 ± 34.36	70.63 ± 5.26	89 ± 14.72	240.36 ± 40.14
15	**0.26 ± 0.029[a, c]**	**60.33 ± 4.25[a]**	**125.3 ± 18.04[a]**	67.72 ± 4.96	68.63 ± 14.82	**202.63 ± 23.4[a]**
30	**0.255 ± 0.02[a, c]**	**61.55 ± 4.93[a]**	**108.52 ± 12.54[a]**	62.63 ± 2.57	**52.81 ± 7.58[b]**	**181.72 ± 14.87[a]**
60	**0.15 ± 0.024[a]**	**64.34 ± 4.56[a]**	**87.85 ± 6.79[a]**	66.27 ± 4.22	**43.18 ± 6.62[b]**	**165 ± 9.24[a]**

[a] Significant to the birthday ($p < 0.05$).
[b] Significant to the 7th day ($p < 0.05$).
[c] Significant to the 60th day ($p < 0.05$).

Table 3. Serum lipid profile, PON1 activity and, its leukocyte gene expression in female and male foals (mean ± SEM) from birth up to the 60th day of life.

Days of sampling	Sex of foals	Parameters					
		PON1 mRNA (fold change)	PON1 activity (U/mL)	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)	Cholesterol (mg/dL)
1	Female	0.91 ± 0.086	29.087 ± 5.12	**261.1 ± 73.37**[a, A]	64.8 ± 3.52	122.4 ± 25.8	362.6 ± 69.3
	Male	0.72 ± 0.172	13.36 ± 4.39	173.93 ± 26.87	50 ± 3.41	54.83 ± 5.81	243.16 ± 31.7
7	Female	**0.307 ± 0.09**[a]	**53.29 ± 6.13**[a]	174.68 ± 74.37	79 ± 9.44	86.4 ± 18.84	270.2 ± 86.8
	Male	**0.205 ± 0.022**[a]	**53.06 ± 6.37**[a]	133.6 ± 22.09	63.66 ± 4.56	91.16 ± 23.5	215.5 ± 24.6
15	Female	0.24 ± 0.029	**60.37 ± 7.57**[a]	114.96 ± 37.92	68 ± 9.3	59.2 ± 13.91	194.8 ± 49.75
	Male	**0.277 ± 0.05**[a, c]	**58.63 ± 5.25**[a]	133.91 ± 14.2	**67.5 ± 5.7**[a]	76.5 ± 25.49	209.16 ± 18.4
30	Female	**0.282 ± 0.026**[b]	**56.6 ± 6.56**[a]	**75.2 ± 8.67**[a]	57.6 ± 2.06	48 ± 7.68	**142.4 ± 10.6**[a, A]
	Male	**0.233 ± 0.029**[a, d]	**82.35 ± 22.5**[a]	136.3 ± 13.69	**66.83 ± 3.7**[a]	56.83 ± 12.8	214.5 ± 16.2
60	Female	**0.21 ± 0.028**[a, A]	**57.38 ± 4.7**[a]	**73.58 ± 9.4**[a]	61.6 ± 3.61	**25.4 ± 3.02**[aA]	**146 ± 7.19**[a, A]
	Male	**0.098 ± 0.024**[a]	**68.14 ± 7.82**[a]	**99.75 ± 6.93**[a]	**70.16 ± 7.1**[a]	58 ± 7.76	180.83 ± 12.97

[a] Significant to the birthday ($p < 0.05$).
[b] Significant to the 15th day ($p < 0.05$).
[c] Significant to the 30th day ($p < 0.05$).
[d] Significant to the 60th day ($p < 0.05$).
[A] Significant to another sex on the same day ($p < 0.05$).

compared to the birthday ($p < 0.05$). A positive correlation was recorded between LDL/cholesterol ($r = +0.952$, $p = 0.0034$) on day 1 and also between LDL/paraoxonase activity ($r = +0.9$, $p = 0.0146$), LDL/cholesterol ($r = +0.932$, $p = 0.0067$) and HDL/TG ($r = +0.87$, $p = 0.0243$) on day 7. On the 15th day of their lives, positive correlations were found between paraoxonase activity/cholesterol ($r = +0.983$, $p = 0.00042$) and HDL/TG ($r = +0.861$, $p = 0.0278$). It was found that serum PON1 activity was positively correlated with cholesterol ($r = +0.878$, $p = 0.0214$) and LDL ($r = +0.902$, $p = 0.0139$) and also LDL/cholesterol ($r = +0.943$, $p = 0.00476$) on the 30th day. On the 60th day of life, cholesterol was positively correlated with HDL ($r = +0.914$, $p = 0.0108$) and LDL ($r = +0.828$, $p = 0.042$). A positive correlation was found between *PON1* expression/HDL and triglyceride at 7 days ($r = +0.849$, $p = 0.0326$ and $r = +0.834$, $p = 0.0389$, respectively) (Tables 3 and 4).

The comparison between sexes revealed that female serum LDL concentration was significantly higher than that of male foals ($p = 0.001$) up to the 30th day of life, and after that, LDL and cholesterol levels were significantly reduced compared to males ($p < 0.001$). Similarly, in the second month, serum cholesterol and triglyceride concentrations of female foals were lower than those of males ($p = 0.008$ and $p = 0.015$, respectively). The reduction in the relative expression of *PON1* from birth to 60 days was significantly greater in males than female foals, so that on day 60 it reached 0.0988 ± 0.0242 compared to 0.211 ± 0.028 for females ($p = 0.023$).

4. Discussion

PON1 has been shown to be involved in oxidative stress, lipid metabolism, and immunity [4]. Variations in PON1 activity have been observed in many pathologic conditions [13,14]. Despite extensive research to assay PON1 activity in different species, it has not been evaluated in newborn foals yet. Therefore, in the present study, the authors attempted to investigate changes in leukocyte *PON1* gene expression, PON1 activity, and lipid profile in healthy newborn foals up to 60 days of age in the current study.

According to the obtained results, PON1 activity was at a low level on the first day of life and increased significantly up to the 60th day. Interestingly, antioxidant defense and PON1 activity of newborn babies are at a lower level too, which increases with age and reaches an adult level at 6 months to 2 years [21,22]. These findings are consistent with those mentioned by Giordano et al. [23].

Low PON1 activity has been reported in other newborn species, and Ruggerone et al. [14] believe that the lower PON1 activity in neonates could be associated with hepatic immaturity, differences in lipid metabolism, and more susceptibility to oxidative stress. According to Matyas and Zaharie [22], the cause of increasing PON1 activity with age could be associated with the enhancement of antioxidant abilities. In the present study, similar to that

Table 4. The summary of significant correlations between different parameters.

	***PON1* gene expression**	**PON1 activity** (U/mL)	**LDL (mg/dL)**	**HDL (mg/dL)**	**Triglyceride (mg/dL)**	**Cholesterol (mg/dL)**
***PON1* gene expression**			r = +0.688, P = 0.0193 **BS**, Day 7 & R = +0.835, P = 0.048 **M**, Day 30	r = +0.662, P = 0.0265 **BS**, r = +0.849, P = 0.0326 **M**, Day 7	r= –0.643, P = 0.033 **BS**, Day 60 & r = +0.834, P = 0.0389 **M,** Day 7	r = +0.706, P = 0.015 **BS**, Day 7
PON1 activity (U/mL)			r= +0.9, P = 0.01 **M**, Day 7 & r = +0.902, P = 0.01 **M,** 30 Days	r = –0.901, P = 0.03 **F,** Day 15		r = +0.983, P = 0.0004 & r = +0.878, P = 0.02, **M**, 15 & 30 Days
LDL (mg/dL)						r = +0.97, P = 0.0006 **F**, 30 days, r = +0.97, P = 0.0006 & r = +0.932, P = 0.006 & r = +0.943, P = 0.004 & r = +0.828, P = 0.042 **M**, Days 1, 7 & 30 & 60
HDL (mg/dL)			+**BS** (> 7 days) + **F**, 7 & 15 Days		+**F**, 7 & 15 Days & r = +0.87, P = 0.02 & r = +0.861, P = 0.02, **M**, 7 & 15 Days	r = +0.914, P = 0.0108 **M**, 60 Days
TG (mg/dL)		r = +0.671, P = 0.0239 & r = +0.689, P = 0.019 **BS,** 1 & 60 Days		+ (>7 days) r = –0.9, P = 0.037 **F,** Day 1		+ **F,** 7 & 15 Days
Cholesterol (mg/dL)			+ (>7 days) r = +0.996, P = 0.0001 **F,** Day 1	+ (>7 days)		

BS: Both sexes, **F**: Female foals, **M**: Male foals.

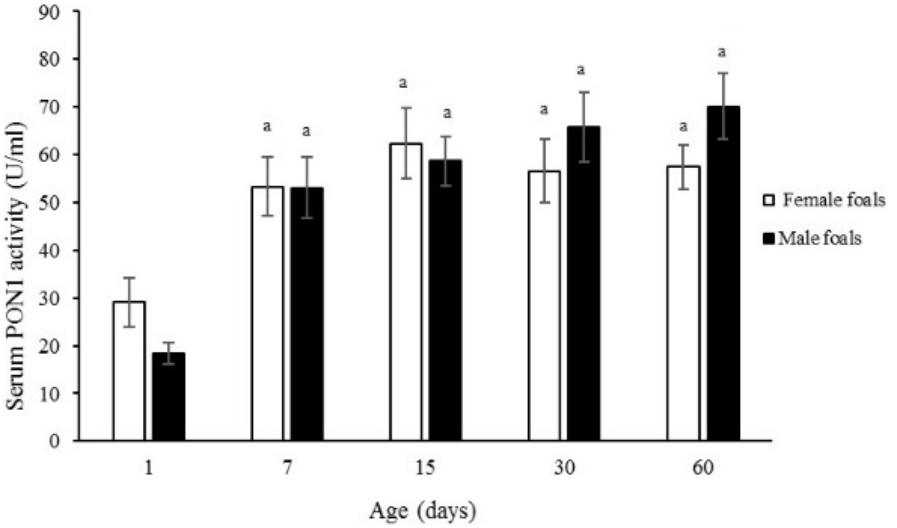

Figure 1. Changes in serum PON1 activity in female and male foals from birth up to the 60th day of life.
[a] Significant to day 1 ($p < 0.05$).

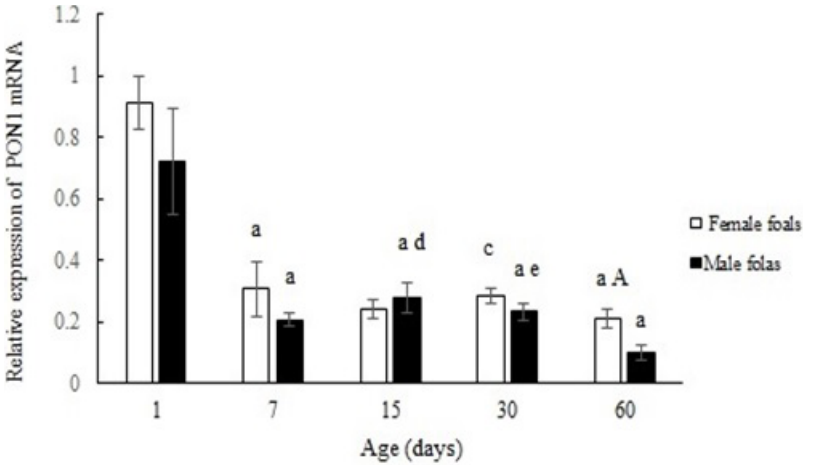

Figure 2. Changes in mRNA expression levels of *PON1* in female and male foals from birth up to the 60th day of life.
[a] Significant to the birthday ($p < 0.05$).
[c] Significant to the 15th day ($p < 0.05$).
[d] Significant to the 30th day ($p < 0.05$).
[e] Significant to the 60th day ($p < 0.05$).
[A] Significant to another sex on the same day ($p < 0.05$).

in the study of Ruggerone et al. [14]. No significant sex-dependent difference was observed in PON1 activity on the first day of life. Contrary to this finding, *PON1* gene expression was significantly decreased up to 60 days of age, and the sex-related differences in *PON1* gene expression were shown to that female foals have a higher level than males on 60 days of age ($p = 0.023$). Thomàs-Moyà et al. [24] emphasized that sex hormones can regulate and stabilize PON1 activity at a higher level in young female rats, which reduces with increasing age. Previous studies have shown that inflammation, oxidative stress, hypolipidemic drugs, and polyphenols can modulate liver *PON1* expression [25,26]. *PON1* gene expression has also been reported in the mouse kidney, lung, heart, and small intestine. Therefore, PON1 has less tissue distribution compared to PON2, which is exclusively recognized as an intracellular antioxidant and expressed ubiquitously in tissues [27]. So, the correlation between hepatic *PON1* mRNA and serum PON1 activity has been studied in laboratory animals, but there is no report about their relationship in foals. Pearce

et al. [16] considered that liver biopsy is so life-threatening for newborn foals, which is why the authors decided to determine the leukocyte *PON1* mRNA. This study indicated the presence of *PON1* mRNA in foal leukocytes for the first time, which contrasts with Rosenblat et al.'s [28] findings. They reported the presence of PON2 and PON3 mRNA but not PON1 in murine macrophages and proposed that oxidative stress can regulate *PON* gene expression and its activities in macrophages and the liver. The obtained results contradict a previous study by Mackness et al. [27], which found that human *PON1* expression was restricted to the liver, kidney, and colon. It was also confirmed that the PON family can improve macrophage function by reducing mitochondrial oxidant formation and preventing the induction of apoptosis [17]. The authors suggest that *PON1* expression in leukocytes may be related to the enzyme's ability to reduce intracellular oxidant levels.

In the first week of life, a positive correlation was observed between *PON1* gene expression/cholesterol ($r = +0.706$, $p = 0.0151$), *PON1* gene expression/HDL ($r = +0.662$, $p = 0.0265$) and *PON1* gene expression/LDL ($r = +0.688$, $p = 0.0193$), and then on the 60th day of life, a negative correlation was recorded between *PON1* gene expression/triglyceride ($r = -0.643$, $p = 0.033$) in both sexes. Antioxidant activity is very important for growing foals. Neuroaxonal dystrophy (NAD) and the related condition equine degenerative myeloencephalopathy (EDM) are examples of these conditions, which support a direct effect of antioxidant activities on horse central nervous system (CNS) health and function. It has been established that vitamin E prevents oxidized cholesterol (oxysterol) formation, lipid peroxidation, and demyelination [29, 30].

Our findings indicate an increase in PON1 activity and, conversely, lower *PON1* gene expression**,** LDL, cholesterol, and triglyceride concentrations on the 60th day of sampling. Numerous studies have reported a close physiological association between PON1 and HDL in plasma [8, 31]. PON1 is considered one of the major contributors to antioxidative HDL function [8]. In the present study, an increase in PON1 activity was accompanied by an increase in HDL concentration on days 15, 30, and 60 in male foals. Increased serum PON1 activity may be a result of increased synthesis of HDL. However, no positive correlation was observed between PON1 enzyme activity and HDL on all days. These data are inconsistent with previous studies by Dullaart et al. [32] and Teimouri and Nayeri [33] which documented serum PON1 activity correlated well with HDL concentration and can be explained by the limited duration of the present experiment. On the other hand, it has been shown that PON1 inactivation could happen due to high levels of triglycerides, LDL, and cholesterol [21, 33]. The possible mechanisms for PON inactivation due to oxidation of LDL are related to the irreversible reaction of oxidized lipids in Ox-LDL with the PON's free sulfhydryl [34]. In this study, an increase in PON1 activity was observed along with a progressive decrease in LDL, TG, and cholesterol levels compared to the first day of age. High levels of cholesterol, TG, and LDL in newborn foals could be related to colostrum and milk intake, which leads to quantitative changes in blood lipid profile values and lipoprotein composition as previously reported for newborn calves [35]. These results also suggest that during the early postnatal period, cholesterol spontaneously transfers from LDLs to HDLs, leading to a gradual increase in HDL levels. The mechanism by which colostrum affects lipoprotein status in various species is unproven, but it may be associated with the modifying effect of bioactive components such as insulin and insulin-like growth factor I on digestion and absorption of lipids, by possibly altering lipase activity or fatty acid-binding proteins [36].

In conclusion, this study shows the low activity of PON1 at birth, which increased with increasing age and could provide appropriate support for enhancing antioxidant supply in newborn foals. Changes in leukocyte *PON1* gene expression were recorded for the first time and positive correlations between *PON1* expression/HDL, LDL, and cholesterol and PON1 enzyme activity/HDL, LDL, and cholesterol in different samples and sexes were achieved, which may indicate an adaptive increase in antioxidant defenses in response to oxidative stress. However, in this study, there were some limitations to the use of Western blot quantification. Further studies are needed to investigate the protein expression profile of PON1 in leukocytes.

List of abbreviations

PON1: Paraoxonase- 1; HDL-C: High-density lipoprotein cholesterol; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol.

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