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SHORT COMMUNICATION

Evaluation of the oxidative status of periparturient mares supplemented with high amount of α-tocopherol

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ABSTRACT

Aim of the study was to investigate the oxidative status during peripartum period in mares fed high amount of dietary α-tocopherol. α-Tocopherol, ferric reducing ability of plasma (FRAP) and reactive oxygen metabolites (d-ROMs) levels were measured in blood samples from 17 Thoroughbred mares at three intervals: (1) 20 days before the expected foaling date; (2) 12 h following parturition; (3) 7 days post-partum. The levels of α-tocopherol, d-ROMs and FRAP were retrospectively analysed in relation to the number of insemination services (Ins) after foaling performed per conception. The parameters α-tocopherol and d-ROMs evidenced minimal fluctuations during peripartum period while FRAP levels showed a linear decrease. The α-tocopherol did not show significant variations and was numerically higher in mares >10 years old while FRAP levels were significantly higher in older mares in the post-partum. Mares receiving three natural insemination services showed higher levels of FRAP in the pre-partum period ($p = .009$) and lower values of α-tocopherol ($p = .015$) in the postpartum compared to mares receiving one service. No d-ROMs level differences appeared among service classes. Oxidative stress is not present in periparturient mares fed 2.750 IU of supplementary α-tocopherol. Differences in redox metabolism are detectable between young and old mares. Plasma antioxidant potential is higher in older mares particularly in the post-partum period.

HIGHLIGHTS

- Oxidative stress is not present in periparturient mares fed 2.750 IU of supplementary α-tocopherol.
- Antioxidant potential of plasma is higher in mares older than 10 years compared to younger mares.
- The increased plasma antioxidant potential seems related to an increase in services per conception.

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Periparturient mare; oxidative stress; α-tocopherol; FRAP; d-ROMs

Introduction

Oxidative stress has been demonstrated to occur in late pregnancy and in the perinatal period in both dairy cattle and horse (Gorecka et al. 2002; Bernabucci et al. 2005; Jamali Emam Gheise et al. 2017). Moreover, oxidative stress occurrence has been demonstrated by El-Maaty et al. (2012) during foal heat in Arab mares at days 8 and 9 after parturition, affecting also fertility. Pregnancy is in fact a physiological state characterised by several metabolic and endocrine changes (Bazzano et al. 2014; Piccione et al. 2017) that increase oxygen requirement. The rising nutrient requirements of the foetus, especially in the last quarter of pregnancy, increases the metabolic demand of the mare. In the last weeks before parturition, leptin seems to regulate maternal nutrition decreasing feed intake and increasing energy expenditure, raising lipolysis and fatty acid oxidation and releasing heat from oxidation of substrates (Arfuso et al. 2016). Moreover, as a consequence of fetoplacental development, the hypothalamic–pituitary–adrenal axis increases the plasma glucocorticoid hormone (Piccione et al. 2017). The latter stimulates the mare's energy metabolism and nutrient partitioning to the conceptus (Barsnick and Toribio 2011; Bazzano et al. 2014) and consequently determines the raise of reactive oxygen

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species (ROS) production (Quijano et al. 2016). After parturition, the depression of the serum leptin leads to an increase of feed intake and to the increase of the insulin-like growth factor-1 level determining again a switch in metabolism (Bazzano et al. 2014), potentially leading to oxidative stress.

Considering the importance of oxidative stress in veterinary medicine, dietary antioxidants supplementation to animals has become more common in the last decades (Marlin and Dunnett 2007; Soffler 2007; Righi et al. 2016). Evidences have been provided that the supply of additional antioxidants in the diet may help to overcome the possible occurrence of oxidative damage during pregnancy and after delivery in mares (El-Maaty et al. 2013; Kotp et al. 2015). Among antioxidants, the most used by the feed industry is vitamin E, mainly as α-tocopherol (Renzi et al. 2005; Righi et al. 2016).

Close to parturition, the α-tocopherol demand increases due to the enhanced mammary secretion during colostrogenesis (Bondo and Jensen 2011). According to the study of Schweigert and Gottwald (1999), the observed concentration of α-tocopherol in colostrum was in fact 5.7 times higher than in mature milk (day 21 after parturition). The lactation period is metabolically stressful for the dam, both because of the high milk production (10–30 L milk/d) (Doreau and Martuzzi 2006), and the new pregnancy with a gradual increase of the requirement of α-tocopherol and other nutrients (Hargreaves 2002). The current α-tocopherol requirement for lactation is established at 80 IU of α-tocopherol/kg DM assuming a dietary intake of 2.5% of the body weight (BW) or 2 IU/kg BW (NRC 2007). However, the most progressive feedstuff industry is recommending higher doses of this vitamin (supplementary doses). This study aimed to assess the oxidative status and the availability of endogenous and exogenous circulating antioxidants in mares receiving high doses of α-tocopherol in the peripartum period. A further objective of the study was to provide preliminary data on the possible relation between redox status and fertility in the postpartum period.

Materials and methods

This study was carried out in accordance with the Italian Legislation on animal care (DL 26 04/03/2014).

The study was conducted on 17 Thoroughbred mares aged on average 10 years (4–16 years; nine younger than 10 years – Y – and eight older than 10 years – O), and weighing a mean of 531 kg (441–607 kg). The animals were permanently housed in a stud farm in the province of Varese (Northern Italy, 45°42′40.1″N 8°44′32.8″E) and were inseminated in the previous breeding season (period ranging from late February to middle June) by stallions housed in the same structure. The mares were kept in group in a paddock starting from 2 months before parturition and were separated 3 times/day to be individually fed the concentrate. After foaling, the same mares were housed in paddocks in smaller groups. The mares were individually administered the same concentrate adopted in the pre-partum through the same modalities.

During the observation period, mares were fed daily approximately 11 kg of first cut alfalfa hay (13.8% moisture; 16.3% crude protein, 41.9% NDF, 32.4% ADF, 8.1% ADL, 8.1% ash on dry matter basis). Hay was provided loosely in a number of sites in the paddock equal to the number of mares and was almost completely consumed individually by the animals. Each mare received 5.5 kg of concentrate (15.2% crude protein, 3.1% ether extract, 8.3% ash on dry matter basis) providing 500 IU/kg DM of α-tocopherol (DL-α-tocopherol acetate), for a total daily amount of 2.750 IU of this vitamin, in analogy to Bondo and Jensen (2011). The following natural insemination services (Ins) were then performed in correspondence of natural heats. Based on the mare, four stallions assessed for health conditions and fertility by the veterinarian staff of the stud, were employed.

Data regarding temperature and humidity were collected daily through the local meteorological station, in order to assess the potential occurrence of heat/oxidative stress of environmental origin.

At 20 (13–41 d) days before the expected foaling date (T1), 12 h (0–3 d) after delivery (T2) and 7 (6–12 d) days post-partum (T3), blood samples were collected from the jugular vein into 10 mL sterile blood tubes (Vacutainer Systems, Becton Dickinson, NJ, USA), containing lithium heparin as anticoagulant. All samples were collected during the second individual daily meal. Tubes were then centrifuged at 3.000 rpm for 10 min (Centrifichem System 600, Baker Instruments Corporation, PA, USA) and the plasma obtained was stored at −80 °C. Samples were tested for α-tocopherol, ferric reducing ability of plasma (FRAP) and reactive oxygen metabolites (d-ROMs) determination.

Plasma α-tocopherol level was determined as described by Renzi et al. (2005) using high-performance liquid chromatography – HPLC (C18 column, reversed phase, Thermoquest Corporation, San Jose, CA, USA) equipped with an UV detector (Micro UV-Vis 20RS, Carlo Erba, Italy) settled at 294 nm.

The antioxidant power of plasma was tested by FRAP measurement according to Benzie and Strain

(1996) using a microplate reader (Spectra Shell Microplate, SLT Spectra, Milan, Italy).

The amount of circulating reactive oxygen metabolites was determined by the d-ROMs test. In particular, a portable free radicals determination system (D-Roms test, Diacron, Grosseto, Italy) was used and the prepared solutions were read with spectrophotometer (Spectra Shell Microplate, SLT Spectra, Milan, Italy) set at 505 nm.

Statistical analysis

Data normal distribution was confirmed using the Kolmogorov–Smirnov normality test. Statistical analyses were conducted using the software SPSS for Windows, Version 21 (IBM SPSS Inc., Chicago, IL, USA). Data on α-tocopherol, d-ROMs and FRAP levels were analysed through the Mixed Model Procedure of the general linear model, using mare as random effect, days relative to foaling as covariate and interval (I), age class (C: younger – Y – or older – O – than 10 years), and number of services class following foaling (1–3) as fixed factors. The interaction between the interval and the age class, and between interval and number of services class were also tested.

Results and discussion

Even if the α-tocopherol supplementation adopted in the observed stud farm exceeded the NRC recommendation (which is of 1 IU/kg of body weight) of about five times, evidences are provided for a beneficial effect of vitamin E supplementation in mares. Bondo and Jensen (2011) and Hoffman et al. (1999) administered daily supplementation of 2500 IU of vitamin E during peripartum period, reporting a raised plasma level of α-tocopherol in both the mare milk and foals' blood (Hoffman et al. 1999; Bondo and Jensen 2011).

The foaling period ranged from January 23rd to May 17th, and the observation period lasted from the end of December until the end of June. Based on the thermal-humidity index calculation, no occurrence of heat-stress was detected.

Overall, α-tocopherol and d-ROMs analysis conducted at the three time intervals (T1, T2, T3) did not show significant variations while FRAP levels were different ($p = .032$) between T1 (320.9 mM) and T3 (243.1 mM), with a linear decrease along the peripartum (Table 1). Plasma α-tocopherol levels appeared quite low in relation to other studies. However, according to Steiss et al. (1994), Thoroughbreds usually show lower concentrations of plasma α-tocopherol than other breeds.

Table 1. Plasmatic levels of α-tocoferol, d-ROMs and FRAP at day 20 pre-partum (T1), at 12 h after foaling (T2), and at day 7 post-partum (T3).

	T1	T2	T3	SEM	*p* Value
α-tocoferol, mg/dL	0.82	0.82	0.74	0.09	.930
d-ROMs, μmol/L	347.20	337.00	341.70	13.97	.938
FRAP, μmol/L	320.90^{b}	280.50^{ab}	243.10^{a}	25.58	.032

Values are reported as least square means.
a,b: $p < 0.05$.
d-ROMs: reactive oxygen metabolites; FRAP: ferric reducing ability of plasma.

FRAP is expression of the sum of the non-enzymatic antioxidant activities of uric acid, ascorbic acid and proteins – that have been estimated in humans to contribute for the 60%, 15% and 10%, respectively to the total value – as well as of α-tocopherol, bilirubin and others components – each contributing for about 5% to the total FRAP value – in plasma (Benzie and Strain 1996). The reduction of its levels during the observation period probably indicates a general consumption of endogenous antioxidant molecules in the peripartum. These results are in agreement with those observed by other authors in mares (Gorecka et al. 2002; Sgorbini et al. 2015) and in cattle (Castillo et al. 2005; Marlin and Dunnett 2007). Interestingly, a numerical decrease of α-tocopherol – a minor contributor to FRAP – was detectable in the postpartum. However, this variation was not significant, probably in relation to its abundant dietary supply. A further, partial explanation of α-tocopherol level constancy is provided by Hargreaves (2002), Kuhl et al. (2012), Schweigert and Gottwald (1999). These authors observed that placentation in the horse does not allow the transfer of fat-soluble vitamins, including α-tocopherol, from the pregnant mare to its foetus and therefore a decrease in serum α-tocopherol before parturition is not detectable.

The α-tocopherol was numerically higher in O than in Y mares, with an increasing difference from T1 to T3, even if not significant ($p = .105$). Whereas, a significant trend was observed for FRAP levels, that resulted constantly higher in O mares at all the intervals. In particular, differences in FRAP levels between age classes was relevant at 7 days after foaling ($p = .040$). Differently, d-ROMs levels did not show any significant difference between the two age classes and through the considered periods (Table 2). These findings could be related to a higher metabolic rate and physical activity of younger subjects (Blakley and Bell 1994; McGowan 2016). These results are partially in contrast to findings from Gorecka et al. (2002) that

Table 2. Plasmatic levels of α-tocoferol, d-ROMs and FRAP at day 20 pre-partum (T1), at 12 h after foaling (T2), and at day 7 post-partum (T3) expressed in relation to age classes (9 younger: Y < 10 years; 8 older: O > 10 years).

	T1		T2		T3		Overall			*p* Value		
Item	Y	O	Y	O	Y	O	Y	O	SEM	C[a]	I[a]	C * I[b]
α-tocopherol, mg/dL	0.81	0.83	0.74	0.96	0.51	1.14	0.69	0.97	0.09	.105	.983	.394
d-ROMs, µmol/L	384.90	296.10	334.60	334.40	340.50	349.90	353.30	326.80	13.97	.357	.936	.400
FRAP, µmol/L	262.70	394.00	235.90	301.40	186.60[c]	305.10[d]	228.40	333.50	25.58	.040	.369	.866

Values are reported as least square means. SEM: standard error of the mean; d-ROMs: reactive oxygen metabolites; FRAP: ferric reducing ability of plasma.
[a]C: class; I: interval of time (period).
[b]C * I: interaction between class per interval.
[c,d]$p < 0.05$.

Table 3. Plasmatic levels of vitamin E, d-ROMs and FRAP at day 20 pre-partum (T1), at 12 h after foaling (T2), and at day 7 post-partum (T3) expressed retrospectively based on the number of insemination services following foaling (1, 2, 3) carried out to obtain a pregnancy.

	T1			T2			T3				*p* Value		
Item	1	2	3	1	2	3	1	2	3	SEM	C[a]	I[a]	C * I[b]
α-tocopherol, mg/dL	0.57	0.98	1.35	0.81	0.74	0.90	0.68[d]	1.99[e]	0.270[c]	0.09	.015	.501	.000
d-ROMs, µmol/L	365.40	324.90	319.00	330.90	346.00	356.60	338.20	370.90	348.60	13.97	.987	.910	.896
FRAP, µmol/L	119.40[c]	350.20[cd]	481.00[d]	249.70	291.00	345.20	175.40	309.20	313.00	25.58	.009	.325	.666

SEM: standard error of the mean; d-ROMs: reactive oxygen metabolites; FRAP: ferric reducing ability of plasma.
Values are reported as least square means.
[a]C: services class; I: interval of time (period).
[b]C * I: interaction between services class and interval.
[c,d,e]$p < 0.05$.

demonstrated no apparent effect of age on the total antioxidant status of mares during peripartum period. However, age-related effects associated with vitamin nutrition and oxidant/antioxidant equilibrium in periparturient mares, have been documented to a limited extent.

Table 3 reports data on α-tocopherol, d-ROMs and FRAP levels retrospectively averaged on the base of the number of Ins carried out to obtain pregnancy after the studied peripartum, parameter considered as indicator of the mares' reproductive efficiency. No age effect was found on the conception rate (data not shown). Noticeable differences among the number of services classes were found concerning α-tocopherol levels in the postpartum period but this observation was not confirmed at the other intervals. In fact, mares which required three Ins showed the lowest levels compared to the others ($p = .015$). In the prepartum period, FRAP levels appeared different among number of services classes with the highest values in the mares inseminated three times ($p < .05$). This trend persisted in the subsequent periods, with increasing values of FRAP from the 'one service' group to the 'three services' group. Regarding d-ROMs levels, no trend or differences among classes or periods were observed mainly because the considered subjects were healthy animals.

The slight numerical decline of α-tocopherol after parturition could be explained by the onset of lactation in the observed mares (Schweigert and Gottwald 1999). Overall, the results on d-ROMs, FRAP and α-tocopherol indicate the ability of mare's organism in maintaining the reducing-oxidative homeostasis in the tested conditions.

Concerning the mare reproductive efficiency, several studies conducted on mares with fertility disorders evidenced increased serum concentration of ROS, along with increased antioxidant potential of plasma. Little information is available in literature on the possible physiological role of ROS on reproduction (Kirschvink et al. 2008; Tvrdá et al. 2011; Wong et al. 2012; Lushchak 2014; Bresciani et al. 2017). An elevated production of ROS is generally due to the 'respiratory burst' occurring during inflammation and pathological phenomenon.

Mares requiring three inseminations had lower vitamin E in the post-partum period, higher FRAP levels during the pre-partum and no changes in d-ROMs value during all the experimental period. Contemporary, the higher FRAP values, as previously reported, were found in the older mares. Consequently, it cannot be excluded that the lower fertility can be related to age.

Conclusions

The present study shows that oxidative stress does not occur in periparturient healthy mares receiving a

supplement of 2.750 IU of α-tocopherol in the diet and differences in redox metabolism are detectable between Y and O mares. During the peripartum period, the antioxidant potential of plasma appears to be higher, along with higher plasma level of α-tocopherol, in the O mares considered in the present investigation. The increased peripartum antioxidant potential of plasma seems related to lower fertility but based on the results, a role of the age could also be hypothesised.

Disclosure statement

No potential conflict of interest was reported by the authors.

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