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Research article

Relationship between postpartum uterine involution and biomarkers of inflammation and oxidative stress in clinically healthy mares (*Equus caballus*)

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ABSTRACT

To test the hypothesis that delayed/impaired uterine involution could be associated with oxinflammation, we studied the progression of the uterine involution in association with some biomarkers of inflammation and oxidative stress in clinically healthy mares (N = 26) during early postpartum. The examination of the reproductive tract was performed on Days 7 and 21 after foaling. Uterine involution was assessed considering: a) the increase of the gravid uterine horn diameter (GUHD) compared with diameter recorded before pregnancy during the previous breeding season; b) the level of endometrial edema (EE); c) the degree of accumulation of intrauterine fluid (IUFA); d) the status of the cervix (CS). Inflammation and oxidative stress were studied by measuring serum amyloid A (SAA), cortisol, DHEA, AOPP, protein carbonyl groups, malondialdheyde (MDA) and thiols in plasma on Days 7 and 21. By Day 21 after parturition, a significant improvement ($P < 0.01$) was observed for GUHD and EE; while IUFA increased in six animals. Plasma SAA and DHEA concentrations were higher when the clinical parameters indicated a lower degree of uterine involution. On Day 7, the cortisol/DHEA ratio was lower in animals with higher degree of EE. Plasma AOPP and MDA concentrations were significantly lower ($P < 0.05$) in animals with the lower GUHD. On Day 21, plasma MDA concentrations were significantly lower ($P < 0.05$) in animals with the lower IUFA. Our data suggest that a mild condition of inflammation and oxidative stress occur in mares with delayed/impaired uterine involution.

1. Introduction

Mares return rapidly to a fertile condition after foaling, and most of them ovulate within Day 20 postpartum (Morris and Allen, 2002; Sharma et al., 2010). However, breeding mares at the first postpartum heat (foal heat) achieve a lower conception rate (Woods et al., 1987). It is conceivable that a delayed/impaired uterine involution and a concurrent inflammatory status could impair embryonic development and eventually lead to embryonic loss (Woods et al., 1987; Ball, 1988).

The presence of a certain degree of inflammation during uterine involution is considered as physiological, as the endometrium reacts against invading microorganisms and the inflammatory process is important for cleaning the endometrium from tissue debris and microorganisms ascended during foaling (Jischa et al., 2008). The inflammatory response, however, should match the intensity of the insult and terminate as soon as the infection is controlled. If the inflammatory reaction fails to be switched-off, detrimental over-responses may occur, which may trigger a sneaky vicious circle leading to a chronic and systemic oxidative stress linked to mild chronic inflammation. The term "oxinflammation" has been recently coined to define this condition (Valacchi et al., 2018), which may lead to loss of immune reactivity and predispose to disease (Valacchi et al., 2018; Colitti et al., 2019). For example, in dairy cows a mild oxinflammatory-like condition can be observed in postpartum subjects with endometritis (Gabai et al., 2019), and a systemic oxidative stress was observed in animals affected by embryonic losses (Celi et al., 2011, 2012).

In the mare, the progress of uterine involution can be monitored by several clinical findings (Stanton, 2011). Gravid uterine horn diameter (GUHD) returns to its pre-gravid state around Day 23–24 post-partum (McKinnon et al., 1988; Lemes et al., 2017). Intrauterine fluid accumulation (IUFA) is considered as a clinical sign for mares susceptible of developing endometritis, as delayed uterine clearance or excessive fluid

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production are predisposing factors in the development of endometritis (Katila, 2016). In healthy subjects, uterine fluid content and discharge should be undetectable by Day 15 post-partum (Blanchard et al., 1989, 1991; Griffin and Ginther, 1991). Endometrial edema (EE) develops physiologically during estrus and disappears after ovulation. Prolonged EE results in continuation of inflammatory response (LeBlanc and Causey, 2009), and excessive EE without the presence of a large follicle can be indicative of endometritis (Diel de Amorim et al., 2016).

Advance Oxidized Protein Products (AOPP) can be considered a biomarker of oxinflammation, as AOPP concentrations in peripheral blood reflect the occurrence of chronic inflammation and abnormal immune response (Celi and Gabai, 2015; Cristani et al., 2016; Gabai et al., 2019). AOPP increase following neutrophil activation during infections, and represent a useful marker of the enzyme myeloperoxidase (MPO) and HOCl (Celi and Gabai, 2015). Other biomarkers can give complementary information about oxidative stress. Protein carbonyls are indicators of protein oxidation (Celi and Gabai, 2015), while malondialdheyde (MDA) is a widely recognized biomarker of lipid peroxidation (Esterbauer et al., 1991; Tsikas, 2017), which has been often associated to several diseases, although it is still unclear whether MDA is an active player in the pathological mechanisms or it is a mere consequence of the pathological processes (Ayala et al., 2014; Gaschler and Stockwell, 2017). To assess the antioxidant status, measurement of thiol groups can be used (Jansen and Ruskovska, 2015). Thiols are organosulfur compounds containing sulfhydryl sidechain groups that act as antioxidants, stabilizing free radicals by accepting their unpaired electron. Glutathione (GSH) is the most important endogenous antioxidant (McLeay et al., 2017).

Serum amyloid A (SAA) is the major acute phase protein (APP) in the horse (Witkowska-Piłaszewicz et al., 2019). The synthesis of SAA takes place mainly in the liver under the stimulation of inflammatory cytokines (IL-1, IL-6, TNFα), glucocorticoids and growth factors. In the horse, SAA synthesis occurs also in the endometrium (Christoffersen et al., 2010). It is involved in the early response to tissue injury, infection, trauma, toxins and neoplasia (Hultén et al., 1997; Pepys et al., 1989; Urieli-Shoval et al., 2000). It is a rapid-responding and very sensitive biomarker of acute inflammation (Pepys et al., 1989; Stoneham et al., 2001). Although SAA is not associated to naturally occurring subclinical endometritis (Witkowska-Piłaszewicz et al., 2019), it may serve to monitor acute inflammatory episodes (Christoffersen et al., 2010).

During inflammation, impaired hypothalamus-pituitary-adrenal (HPA) axis responsiveness has been shown in numerous animal models and human inflammatory diseases (Silverman and Sternberg, 2012). HPA axis activation occurs during acute inflammation (Ayala et al., 2012; Mair et al., 2014), while cortisol secretion can be differently regulated by chronic inflammation (Mills et al., 1997; Ayala et al., 2012; Silverman and Sternberg, 2012). Based on studies performed mainly on humans and laboratory animals, the adrenal androgen dehydroepiandrosterone (DHEA) plays an anti-glucocorticoid role, as it can inhibit proinflammatory cytokines (Prall and Muehlenbein, 2018; Straub et al., 1998; Daynes et al., 1993). To the best of our knowledge, few studies investigated the relationship between inflammation and DHEA in the horse. A significant increase in DHEA-S was observed in septic compared with healthy and sick non-septic newborn foals (Dembek et al., 2017), and plasma DHEA concentrations were higher in foal affected by neonatal maladjustment syndrome or other neonatal pathologies (Aleman et al., 2013). However, associations between plasma DHEA concentrations and inflammation have been found in other ungulate species, such as the postpartum dairy cows with metritis, where it was hypothesized that DHEA may be an anti-inflammatory signal during prolonged inflammation, and a biomarker or prognostic indicator for evaluating disease severity (Gundlach et al., 2017). A more accurate evaluation of the HPA axis activation in stressful or inflammatory situations may be achieved by analyzing the cortisol/DHEA ratio. For example, in cows the cortisol/DHEA ratio was higher in lame than sound cows (Almeida et al., 2008; O'Driscoll et al., 2015), and increased numerically with increasing severity of sole hemorrhage (O'Driscoll et al., 2017).

In this study, we hypothesized that a delayed/impaired uterine involution could be associated with oxinflammation. To test this hypothesis, we monitored the progression of the uterine involution in association with selected biomarkers of inflammation and oxidative stress in clinically healthy mares during early postpartum.

2. Materials and methods

2.1. Ethical issues

Clinical observations and biological samples used in this work were collected during routinely clinical procedures not performed for experimental purposes. According to the Italian law for the protection of experimental animals (Law Decree n. 26 issued on 4 March 2014, art. 2), the approval by an ethical committee is not required under the circumstances that this trial was carried out.

2.2. Animals and reproductive management

The trial was conducted between February and May 2018 in three different breeding farms located in Northeastern Italy. Twenty-six clinically healthy Standardbred mares aging between 5 and 21 years (mean ± s.e.m.: 10.3 ± 0.7 years) were enrolled in the study. Since Day 30 before parturition, animals were fed with a lactation diet consisting in 8 kg/day hay and 4 kg/day of a commercial fodder (Impact®Professional Mare and Foal Horse Feed, Purina®; 16.00 % Crude Protein, 6.00 % Crude Fat and 12.50 % Crude Fiber; Donoghue et al., 1990).

Animals underwent the same clinical management consisting in the ultrasonographic (ExaGo, 7.5 MHz linear probe, Multimage srl, Italy) examination of the reproductive tract performed on Days 7 and 21 after parturition. The exam considered the uterine characteristics, and the presence of a corpus luteum and follicles. Uterine involution was assessed *ex post* considering: a) the increase of the gravid uterine horn diameter (GUHD) compared with diameter recorded before pregnancy; b) the level of endometrial edema (EE); c) the accumulation of intrauterine fluid (IUFA); d) the status of the cervix (CS). GUHD, EE and IUFA are known to be influenced by the progress of involution (Stanton, 2011), while the presence of a damaged cervix was considered as a potential additional risk factor for developing endometritis. The clinical parameters were assessed by the same skilled clinician, and graded as described in Table 1. Mares were not inseminated before Day 21 after parturition.

2.3. Laboratory analyses

Blood samples were collected from the jugular vein on Days 7 and 21 in tubes with lithium heparin as anticoagulant. The samples were then centrifuged (3500 x g, 20 min, 4 °C), and the plasma was aliquoted in Eppendorf (1.5 ml) and stored at -20 °C until used for analyses.

Plasma SAA was determined by an immunoturbidimetric method (EIKEN SAA TIA Eiken Chemical Company, Tokyo, Japan) adapted for the horse (Jacobsen et al., 2006). Cortisol and DHEA concentrations were measured by a microtiter radioimmunoassay (Marinelli et al., 2007). Plasma protein concentrations were measured by the BCA method (BCA Protein assay kit; Pierce Biotechnology, Rockford, IL, USA), following the manufacturer instructions. Spectrophotometric methods were used to measure plasma AOPP (Witko-Sarsat et al., 1996) and protein carbonyl groups (Levine et al., 1990). MDA measurement was performed by the TBARs assay described by Yoshida et al. (2005). Plasma thiols were measured by the Ellman's reagent (Sigma-Aldrich, St. Louis, MO, USA) method (Hu, 1994; Mahmoud et al., 2013).

2.4. Statistical analysis

All statistical tests were performed by the SPSS 24.0 software. The Crosstabs procedure and Chi-square test were used to study the evolution of the clinical parameters from Day 7 to Day 21. The Kolmogorov-

Table 1. Description and grading of the ultrasonographic parameters used for the clinical evaluation of the uterine involution.

Clinical Parameter	Grade 0	Grade 1	Grade 2
GUHD[1]	GUHD Increase ≤10%	10% < GUHD Increase <40%	GUHD Increase ≥40%
IUFA	Fluid not detectable	Fluid layer <2 mm	Fluid layer ≥2 mm
EE	Endometrial folds not detectable	Endometrial folds in radial pattern	Marked presence of large distended endometrial folds
CS	Intact		Damaged

GUHD: Gravid Uterine Horn Diameter; IUFA: Intra-Uterine Fluid Accumulation; EE: Endometrial Edema; CS: Cervix Status.
[1] GUHD increase was assessed by comparing the GUHD measured on either Day 7 and Day 21 with the pre-pregnancy diameter recorded during the previous breeding season.

Smirnov test with a Lilliefors significance level was used for testing normality. As the normalization of the SAA plasma concentrations was not possible (Figure 1 A), the effects of the clinical parameters on SAA were studied by the non-parametric Kruskal-Wallis one-way ANOVA and pairwise multiple comparisons. Plasma MDA concentrations were not normally distributed and were Log transformed. The concentrations of biomarkers were analyzed by the Mixed Model option of SPSS 24.0, which included the fixed effects of GUHD, IUFA, EE, Day post-partum (D), and the interactions IUFA*D and EE*D. The animal was the random effect. Data are displayed as estimated marginal means

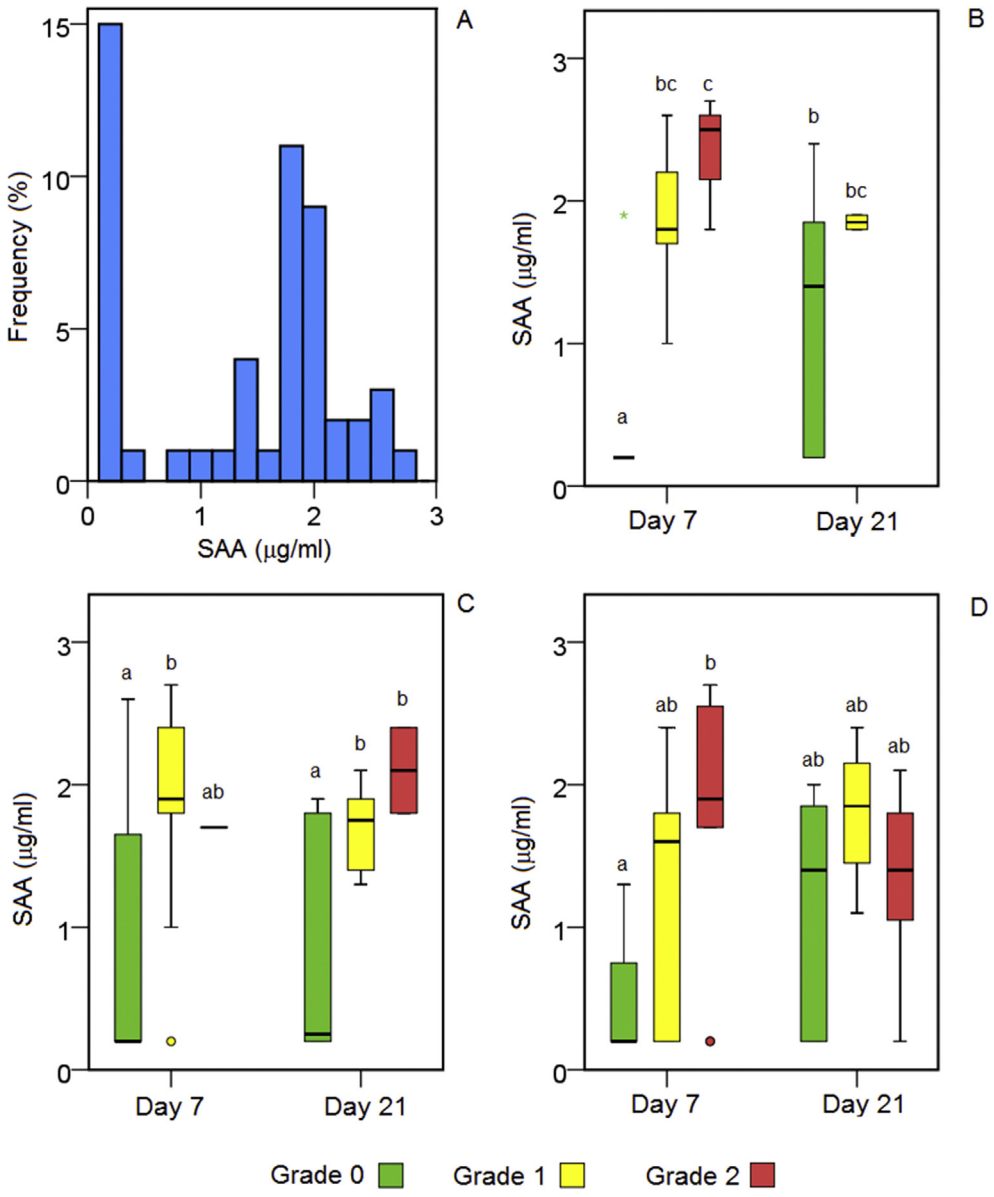

Figure 1. Serum amyloid A (SAA) concentrations in the peripheral blood of postpartum mares (N = 26) in relation to the evaluation of the clinical parameters (A: value distribution; B: Gravid Uterine Horn Diameter; C: Intra-Uterine Fluid Accumulation; D: Endometrial Edema). Different superscript letters indicate statistically significant differences ($p < 0.05$) between groups (Kruskal-Wallis ANOVA, SPSS 24.0).

(±s.e.m.). The Bonferroni test was used to compare differences between means. Differences were considered as statistically significant if $P < 0.05$.

3. Results

3.1. Clinical findings and assessment of uterine involution

The evolution of the clinical parameters is shown in Table 2. By day 21 after parturition, a significant improvement was observed for GUHD (Grade 0 observed in 24 mares; $P < 0.001$) and EE (Grade 0 observed in 15 mares; $P < 0.002$). IUFA worsened to Grade 1 or 2 in six animals. The cervix was intact in 23 mares on Day 7, and was classified as damaged on Day 21 in one animal only. Therefore, the parameter CS was not considered further as a classification variable. On Day 21, all mares borne a fully developed corpus luteum.

3.2. Hormones and biomarkers of inflammation and oxidative stress

Plasma SAA was not normally distributed (Figure 1 A) and plasma SAA concentrations were not different between Day 7 and Day 21 after parturition. SAA plasma concentrations were higher when the clinical parameters suggested a lower degree of uterine involution, and significant effects ($P < 0.05$) of GUHD, IUFA and EE on SAA plasma concentrations were observed on Day 7. Only IUFA showed a significant effect on plasma SAA ($P < 0.05$) on Day 21 (Figure 1 B, C, D).

Plasma AOPP and MDA concentrations were significantly lower ($P < 0.05$) in animals with GUHD Grade 0 (Table 3). Plasma CT and MDA concentrations increased from Day 7 to Day 21 ($P < 0.05$; Table 3). Plasma MDA concentrations were significantly lower ($P < 0.05$) in animals with the lower IUFA on Day 21 (Table 3).

Plasma cortisol was not affected by any of the clinical parameters considered, but increased from Day 7 to Day 21 ($P < 0.05$; Table 4). Plasma DHEA was affected by GUHD, IUFA and EE ($P < 0.05$). Differences were more pronounced in animals with lower degree of uterine involution and on Day 21 (Table 4). The cortisol/DHEA ratio was lower in animals with the higher degree of EE and on Day 7 (Table 4).

4. Discussion

The observation that the degree of uterine involution, as described by IUFA, EE and GUHD, affected some biomarkers of inflammation and oxidative stress led to the hypothesis that delayed/impaired involution can be associated with a mild degree of systemic inflammation combined to oxidative stress in the postpartum mare, recently named "oxinflammation" (Valacchi et al., 2018; Colitti et al., 2019).

Animals enrolled in this study were clinically healthy and did not display conditions of acute inflammation in proximity of the days of clinical examinations, as suggested by SAA concentrations, which did not show the huge rise in plasma typical of an acute phase response (Christoffersen et al., 2010) on Days 7 and 21. GUHD returned to Grade 0 in most mares, although some of them still showed some degree of IUFA and EE on Day 21.

Plasma SAA concentrations observed at both Day 7 and Day 21 were compatible with those reported for healthy horses (Witkowska-Piłaszewicz et al., 2019) and comparable with those reported by Christoffersen et al. (2010) before and 96 h after induced endometritis. It is worth noting, however, that SAA concentrations observed on Day 7 were higher in mares with delayed/impaired uterine involution as assessed by GUHD, IUFA and EE. On Day 21, on the other hand, plasma SAA concentrations were generally higher only when IUFA were Grade 1 or Grade 2, while no close associations between SAA concentrations and GUHD and EE were observed. As all mares bore a fully developed corpus luteum on Day 21, it is unlikely that SAA concentrations could be affected by the stage of the estrous cycle. As excessive uterine fluid production can be predisposing factors for endometritis development (Katila, 2016), this finding might suggest a mild inflammatory condition associated with IUFA.

The higher concentrations of plasma AOPP and MDA observed in animals with Grade 1 and Grade 2 GUHD suggested a mild condition of oxidative stress in animals with delayed uterine involution. In addition, MDA was higher in Grade 1 and 2 IUFA on Day 21, suggesting also an association between a mild degree of uterine inflammation and oxidative stress. Indeed, our results are in line with those of Yaralioglu-Gurgoze et al. (2005), who found plasma MDA increased in mares affected with endometritis compared to healthy mares. AOPP may be considered a biomarker of oxidative stress linked to phagocyte activity (Celi and Gabai, 2015). Therefore, the higher plasma AOPP concentrations observed in mares with clinical sign of delayed uterine involution may depend on a general inflammatory status that activates peripheral neutrophils. However, the degree of oxidative stress and inflammation observed in postpartum mares is lower than what has been observed in postpartum cows affected by endometritis (Gabai et al., 2019). In this context, it is not surprising that protein carbonyls were not affected by any of the clinical parameters, as they are not specific products of the neutrophil oxidative burst and a variety of oxidation mechanisms can lead to their formation (Celi and Gabai, 2015).

The delay in uterine involution did not affect thiol group concentrations, suggesting that the animals were in a favorable redox status. This finding is compatible with the fact that all animals were clinically healthy, and the inflammatory and oxidative stress conditions were only lightly altered (Jansen and Ruskovska, 2015). In addition, good management and anti-oxidant supplementation may have a role in maintaining a high level of circulating thiols even in the presence of subclinical inflammatory conditions. Indeed, anti-oxidant supplementation in human athletes can contrast endogenous anti-oxidant depletion (McLeay et al., 2017).

Table 2. Evolution of the clinical parameters from Day 7 to Day 21 after parturition in the 26 mares (count).

					Chi-square test	
Parameter		Grade 0	Grade 1	Grade 2	X^2	P
GUHD	Day 7	9	14	3	18.818	0.001
	Day 21	24	2	0		
IUFA	Day 7	11	13	2	0.751	0.687
	Day 21	14	10	2		
EE	Day 7	3	11	12	12.582	0.002
	Day 21	15	4	7		
		Intact	Damaged			
CS	Day 7	23	3		1.083	0.298
	Day 21	25	1			

GUHD: Gravid Uterine Horn Diameter; IUFA: Intra-Uterine Fluid Accumulation; EE: Endometrial Edema; CS: cervix Status.

Table 3. Advanced Oxidation Protein Products (AOPP), total carbonyl groups (CT), malondialdheyde (MDA) and thiol concentrations in the peripheral blood of postpartum mares (N = 26) in relation to the evaluation of the clinical parameters. Data are expressed as mean ± s.e.m..

Clinical Evaluation		AOPP (nmol/mg protein)		CT (nmol/mg/protein)		MDA (nmol/ml)		Thiol (nmol/ml)	
		Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
IUFA	Grade 0	0.59 ± 0.08	0.97 ± 0.12	0.49 ± 0.10	0.52 ± 0.13	2.7 ± 1.1	3.4 ± 1.2^{a}	331 ± 23	327 ± 23
	Grade 1	0.53 ± 0.07	0.77 ± 0.12	0.34 ± 0.09	0.58 ± 0.12	3.2 ± 1.1	5.5 ± 1.2^{b}	286 ± 19	343 ± 21
	Grade 2	0.51 ± 0.15	0.40 ± 0.22	0.21 ± 0.20	0.67 ± 0.21	2.9 ± 1.2	4.9 ± 1.3 ^{ab}	350 ± 44	399 ± 32
EE	Grade 0	0.70 ± 0.13	0.74 ± 0.13	0.25 ± 0.17	0.58 ± 0.13	2.6 ± 1.2	4.5 ± 1.2	299 ± 38	362 ± 23
	Grade 1	0.51 ± 0.08	0.80 ± 0.16	0.40 ± 0.11	0.53 ± 0.16	3.1 ± 1.1	4.8 ± 1.2	329 ± 24	343 ± 25
	Grade 2	0.46 ± 0.07	0.60 ± 0.13	0.40 ± 0.09	0.69 ± 0.14	3.2 ± 1.1	4.2 ± 1.2	339 ± 21	364 ± 23
GUHD	Grade 0	0.45 ± 0.06^{a}		0.41 ± 0.07		2.3 ± 1.1^{a}		311 ± 14	
	Grade 1	0.71 ± 0.07^{b}		0.45 ± 0.08		4.4 ± 1.1^{b}		353 ± 16	
	Grade 2	0.74 ± 0.14^{b}		0.54 ± 0.18		4.7 ± 1.2^{b}		354 ± 40	
D		0.55 ± 0.07	0.71 ± 0.10	0.35 ± 0.09	0.59 ± 0.11*	2.9 ± 1.1	4.5 ± 1.1*	322 ± 21	356 ± 19

IUFA: Intra-Uterine Fluid Accumulation; EE: Endometrial Edema; GUHD: Gravid Uterine Horn Diameter; D: Day after parturition.
Different superscript letters within the same day indicate statistically significant differences (Bonferroni test; $p < 0.05$). The asterisk (*) indicates significantly different means between Day 7 and Day 21.

Table 4. Cortisol and DHEA concentrations and cortisol/DHEA ratio in the peripheral blood of postpartum mares (N = 26) in relation to the evaluation of the clinical parameters. Data are expressed as mean ± s.e.m..

Clinical Evaluation		Cortisol (ng/ml)		DHEA (pg/ml)		Cortisol/DHEA Ratio	
		Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
IUFA	Grade 0	10.8 ± 1.7	15.0 ± 1.7	638 ± 40	549 ± 59^{a}	18 ± 3	34 ± 5
	Grade 1	12.9 ± 1.5	14.3 ± 1.6	583 ± 34	518 ± 55^{a}	24 ± 3	32 ± 5
	Grade 2	9.9 ± 3.3	15.2 ± 2.3	656 ± 76	784 ± 102^{b}	15 ± 6	26 ± 8
EE	Grade 0	13.0 ± 2.9	14.4 ± 1.7	596 ± 65	504 ± 62^{a}	23 ± 6	35 ± 5^{b}
	Grade 1	10.7 ± 1.8	16.1 ± 1.8	571 ± 41	467 ± 75^{a}	20 ± 3	40 ± 6^{b} **
	Grade 2	10.0 ± 1.6	14.0 ± 1.7	712 ± 36	881 ± 64^{b} *	13 ± 3	17 ± 5^{a}
GUHD	Grade 0	12.2 ± 1.1		575 ± 31^{a}		24 ± 3	
	Grade 1	13.6 ± 1.2		545 ± 35^{a}		28 ± 3	
	Grade 2	13.2 ± 3.0		745 ± 72^{b}		22 ± 6	
D		11.2 ± 1.6	14.8 ± 1.4 *	626 ± 35	617 ± 48	19 ± 3	31 ± 4 *

IUFA: Intra-Uterine Fluid Accumulation; EE: Endometrial Edema; GUHD: Gravid Uterine Horn Diameter; D: Day after parturition.
Different superscript letters within the same day indicate statistically significant differences (Bonferroni test; $p < 0.05$). The asterisks indicates significantly different means between Day 7 and Day 21 (*$P < 0.05$; **$P < 0.01$).

Although the HPA axis activation occurs during inflammation (Ayala et al., 2012; Mair et al., 2014), plasma cortisol cannot be accounted for a specific indicator of inflammation, as several stimuli such as restraint and manipulation can trigger cortisol release (Monk et al., 2014). In our study, the increase in plasma cortisol observed on Day 21 could be related to the presence of bigger and more demanding foals in terms of milk consumption and psycho-physical energy expenditure by the mother. This augmented metabolic demand may also explain the increase in protein carbonyls observed between Day 7 and 21. Indeed, an increase in oxidative stress after parturition related to increased milk production can be observed in early lactating cows (Castillo et al., 2005).

In this study, DHEA concentrations are consistently higher in animals with the lower degree of uterine involution (GUHD, IUFA and EE Grade 2), in particular on Day 21. This finding is compatible with a certain degree of systemic inflammation in conditions of delayed uterine involution. As a matter of fact, a significant increase in DHEA-S was observed in septic compared with healthy and sick non-septic newborn foals (Dembek et al., 2017), and plasma DHEA concentrations were higher in foal affected by Neonatal maladjustment syndrome or other neonatal pathologies (Aleman et al., 2013). Also in postpartum dairy cows, plasma DHEA concentrations were higher in cows with metritis, and it was hypothesized that DHEA could represent an anti-inflammatory signal during prolonged inflammation, and a biomarker or prognostic indicator for evaluating disease severity (Gundlach et al., 2017).

Conversely, the observation that cortisol/DHEA ratio was lower in Grade 2 EE is not in agreement with other studies. In other ungulates such as the dairy cow, Almeida et al. (2008) and O'Driscoll et al. (2015) found that cortisol/DHEA ratio was higher in lame than sound cows, although not significantly in the study by O'Driscoll et al. (2015). In another study, cortisol/DHEA ratio increased numerically with increasing severity of sole hemorrhage in cows (O'Driscoll et al., 2017). It is important to consider that stressors are different in nature (inflammatory, physical or social) and persistency, and may affect cortisol and DHEA release in different ways leading to different stress phenotypes. Finally, higher DHEA plasma concentrations in animals with lower degree of uterine involution may depend upon reduced intracrine DHEA metabolism. To the best of our knowledge, no studies about endometrial DHEA conversion in androgens/estrogens are present in the literature. However, circulating DHEA and DHEA-S can be utilized as androgen/estrogen precursors by the human endometrium, and this conversion can be differently regulated in both normal and pathologic endometrium (Gibson et al., 2018).

5. Conclusion

Our data suggested that a mild condition of inflammation and oxidative stress occur in mares with signs of delayed/impaired uterine involution.

Declarations

Author contribution statement

M. Falomo: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

B. Del Re and M. Rossi: Performed the experiments; Wrote the paper.

E. Giaretta and L. Da Dalt: Contributed reagents, materials, analysis tools or data; Wrote the paper.

G. Gabai: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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