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ORIGINAL ARTICLE

Impact of Antioxidant–Oxidant Status at Mating on Reproductive Performance of Gray Shirazi Ewes

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Abstract

This study aimed to evaluate changes in oxidant–antioxidant status at mating, mid-pregnancy, and early lactation in Gray Shirazi ewes and to investigate their associations with pregnancy outcome categories, lamb birth weight, parity, and body condition score (BCS). Sixty healthy primiparous and multiparous Gray Shirazi ewes were randomly selected and monitored from mating to early lactation (September 2021–April 2022). Blood samples were collected at mating, mid-pregnancy, and early lactation. Serum concentrations of superoxide dismutase (SOD), glutathione peroxidase (GPX), nitric oxide (NO), total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured using commercial kits. Data were analyzed using repeated-measures and one-way ANOVA followed by LSD post-hoc tests, as well as Pearson correlation analysis ($P < 0.05$). SOD activity was significantly higher during early lactation than at the other stages ($P < 0.05$), whereas GPX activity was lowest during pregnancy. TAC levels were significantly lower in early lactation than at the other sampling stages. MDA concentrations were highest at mating ($P < 0.05$), while NO levels did not differ among stages. At mating, non-pregnant ewes showed significantly higher MDA and NO levels than ewes that later produced single or twin lambs. No significant correlations were observed between oxidative markers and lamb birth weight. However, MDA levels were negatively correlated with BCS during pregnancy ($r = -0.301$, $P < 0.05$). Oxidant–antioxidant status in Gray Shirazi ewes varies across reproductive stages, reflecting physiological and metabolic adaptations. Certain oxidative markers, particularly MDA, TAC, and NO, showed associations with pregnancy outcomes. Monitoring oxidative status at different reproductive stages may contribute to improved management of oxidative stress and reproductive performance in ewes.

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Introduction

Sheep husbandry and breeding have a long history worldwide, particularly in Iran. Among domesticated animals, sheep are notable for their versatility, as their meat, milk, wool, skin, and nearly all parts of their bodies are utilized by humans. Oxidative stress represents an active area of research in veterinary medicine. This phenomenon has been studied in the context of many diseases, including sepsis, mastitis, enteritis, pneumonia, and joint disease (1). However, limited studies have been conducted in ruminants, particularly sheep and goats, and most of these have been isolated investigations focusing on mastitis, pneumonia, retained placenta, and metabolic diseases around parturition (2). Studies on oxidative stress in small ruminants are still in their early stages and remain underdeveloped. In addition, further research is needed to identify more accurate and standardized markers for assessing oxidative stress in veterinary medicine, although several methods currently exist (1). Some studies in dairy cows indicate the occurrence of periparturient oxidative stress, which is influenced by nutrition and body condition score (BCS) and is associated with metabolic diseases (2). Peripartum oxidative stress has also been reported in ewes (3). However, the periparturient period is characterized by rapid and intense metabolic changes and increased nutritional requirements in the animal. based on existing studies, ewes may also be affected by oxidant or antioxidant factors during other reproductive/production stages, which are associated with metabolic changes and lower nutritional requirements (3). In the study by Mohebbi-Fani et al. (2012), changes in oxidant/antioxidant factors were also observed during mating and pregnancy, indicating that the mating stage poses a greater challenge in terms of oxidative stress, even compared with the pregnancy period (4). Reactive oxygen species are produced in steroid-producing cells and mononuclear phagocytes in the corpus luteum, and their increase plays a role in luteolysis (5). In addition, reactive oxygen radicals are naturally produced during embryonic metabolism; however, their increase during oxidative stress may be associated with impaired development and embryonic death (6). On the other hand, twinning is considered a desirable trait in ewes and can provides economic benefits in the breeding and maintenance of different breeds of this species. Various factors influence the reproductive efficiency of ewes and increase lambing rates, most of which are related to the conditions of the ewes around mating period (7). These factors include ewe weight at mating, nutritional status, maternal age, climate, and season (8, 9). Insufficient dietary energy intake can lead to increased reactive oxygen species (ROS) production by reducing proton leak from mitochondria (10). Defects in electron transport in mitochondria, resulting in the production of superoxide and hydrogen peroxide, are the leading causes of oxidative stress in animals (11). Given that the exact mechanisms underlying the effects and changes in oxidant/antioxidant factors during the reproductive process of ewes, especially Gray Shirazi ewes, remain unclear, more extensive studies in this field are necessary to improve reproductive efficiency and reduce economic losses. Therefore, the present study aimed to evaluate changes in oxidant/antioxidant status at mating, mid-pregnancy, and early lactation in Gray Shirazi ewes, and to investigate their associations with pregnancy outcome categories, lamb birth weight, parity, and body condition score.

Materials and Methods

Animals

A total of 60 healthy Gray Shirazi ewes, both primiparous and multiparous, were randomly selected for this study. The study was conducted from September 2021 to April 2022. The animals were kept at the Aliabad Kamin Research Station, affiliated with the Fars Agricultural and Natural Resources Research and Training Center in the north of Fars Province. The animals were fed manually with a diet consisting of barley, straw, and alfalfa. Mineral and vitamin supplements were administered once a week at a rate equivalent to 1% of the total ration. Furthermore, lick bricks and bicarbonate were provided to the sheep to prevent possible acidosis and mineral deficiencies. The sheep were removed from their enclosures during daylight hours and exposed to sunlight. Shearing was conducted annually, specifically in May. The BCS of all ewes in this study was documented according to the methodology established by Russell et al. (1969) (12). The ewes were all equipped with ear tags to facilitate precise data collection.

Treatment

The synchronization of all ewes was achieved through ram effect in conjunction with an injection of PGF2α. In instances where estrus was not observed, a second dose of PGF2α was injected approximately eight days later. Neck bands were applied to facilitate the identification of the study ewes within the flock. The sampling process was conducted in three stages, commencing during the breeding season, which occurred in September and October. The initial stage occurred during mating, the second stage

transpired approximately 2 to 4 weeks prior to parturition (pregnancy was diagnosed by ultrasonography), and the final stage occurred 1 to 2 weeks post parturition.

Sampling and Laboratory Analyses

Blood samples were collected from the jugular vein into plain tubes (without anticoagulant), and serum was separated by centrifugation. Subsequently, serum samples were stored at -20°C until laboratory assessment. During parturition, the frequency of multiple births and stillbirths, and the weight of the lambs were recorded. The collected data were compiled for further analysis.

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the serum samples were measured using Randox laboratory kits (Randox Laboratories, UK) on a BT1500 autoanalyzer (Biotecnica Instruments, Italy). Furthermore, the levels of nitric oxide (NO), total antioxidant capacity (TAC), and malondialdehyde (MDA) in the serum samples were measured using Zelbio laboratory kits (ZellBio GmbH, Germany) by the colorimetric method, according to the manufacturer's instructions.

Statistical Analysis

The statistical analysis of the collected data was performed using SPSS version 26 software. To compare data across the different stages of pregnancy, a repeated measures analysis of variance (ANOVA) was used, followed by a least significant difference (LSD) post-hoc test. To compare the means of other groups at each stage, a one-way ANOVA was conducted, also followed by an LSD post-hoc test. Additionally, a correlation analysis was conducted among the variables using the Pearson correlation coefficient. Data are presented as mean ± standard deviation, and $p < 0.05$ was considered statistically significant.

Results

Antioxidant Status Across Sampling Stages

As illustrated in Table 1, the mean ± standard deviation of antioxidant concentrations at different sampling stages in Gray Shirazi ewes is presented. A comparison of serum antioxidant concentrations in ewes across different sampling stages demonstrated that the mean SOD in the third stage (beginning of lactation) was significantly higher than in the other stages ($P < 0.05$). This value for GPX in the second stage (pregnancy) sampling was significantly lower than in other stages ($p < 0.05$). In contrast to the SOD results, the mean serum TAC was significantly lower at the third sampling stage (early lactation) compared with the other stages ($P < 0.05$). in the case of MDA, this value demonstrated a substantial increase in the first stage sampling compared with the other stages ($p < 0.05$). However, no statistically significant differences were observed among the various sampling stages in serum NO levels in ewes ($p \geq 0.05$). The higher TAC observed at mating compared with later stages, together with increased MDA, may reflect a compensatory upregulation of antioxidant defenses in response to increased oxidative challenge rather than indication of oxidative balance. Elevated TAC does not necessarily preclude oxidative stress, particularly when lipid peroxidation markers such as MDA are also increased. The study included a total of 60 ewes: 35 were non-pregnant, 37 carried single lambs, and 8 carried twins.

Table 1. Mean ± standard deviation of the concentration of antioxidant factor at different sampling stages in Gray Shirazi Ewes

Sampling stage	NO (µM)	MDA (µM)	TAC (mM)	GPX (U/ml)	SOD (U/ml)
First (Mating)	6.69±2.11	19.28±5.63^{a}	0.11±0.05^{a}	443.87±27.73^{a}	0.17±0.05^{a}
Second (Pregnancy)	10.70±3.32	8.69±1.32^{b}	0.05±0.01^{b}	237.31±37.73^{b}	0.21±0.01^{a}
Third (Early lactation)	9.64±2.75	8.27±1.62^{b}	0.02±0.01^{b}	444.37±83.59^{a}	0.37±0.09^{b}

Different superscript letters within a column indicate significant differences between sampling stages ($p < 0.05$).

Comparison Based on Pregnancy Outcome

A comparison of ewes at different sampling stages across three groups revealed no significant difference in any of the sampling stages in terms of SOD and GPX levels ($p \geq 0.05$). The results of comparing TAC values at different sampling stages among the non-pregnant, singleton, and twin groups showed that during mid-pregnancy sampling, the average TAC in the group that was non-pregnant at the end was significantly lower than the other groups ($p<0.05$). Additionally, in the context of MDA, a statistically significant difference was observed among ewes with varying numbers of lambs in the initial sampling stage.

However, no significant variation was detected in subsequent sampling stages ($p \geq 0.05$). During the first sampling, a statistically significant elevation in MDA was observed in the ultimately non-pregnant group compared to the groups that experienced singleton and twin pregnancies ($p < 0.05$). As illustrated in Table 1, a statistically significant difference in NO levels was observed among ewes with different numbers of lambs during the initial stage of sampling (mating). However, no significant differences were detected in the subsequent sampling stages ($p \geq 0.05$). During the first sampling (mating), the NO level in the group that ultimately gave rise to twins was notably lower compared to the other groups ($p < 0.05$).

Table 2. Mean ± standard deviation of antioxidant factor concentrations at different sampling stages based on pregnancy status in Gray Shirazi Ewes.

Sampling stage	Pregnancy	SOD (U/ml)	GPX (U/ml)	TAC (mM)	MDA (µM)	NO (µM)
First (Mating)	Non-pregnant	0.16±0.08	424.12±106.97	0.11±0.04	23.57±5.62^{a}	7.68±3.23^{a}
	Singleton	0.18±0.06	434.94±113.65	0.09±0.04	15.35±4.06^{b}	8.76±3.38^{a}
	Twins	0.13±0.02	469.87±124.67	0.11±0.03	5.71±1.05^{b}	3.91±3.07^{b}
Second (Pregnancy)	Non-pregnant	0.28±0.06	218.00±18.59	0.03±0.01^{a}	8.13±0.55	9.95±4.73
	Singleton	0.20±0.07	262.86±13.72	0.08±0.02^{b}	10.08±2.59	13.56±6.17
	Twins	0.17±0.07	207.87±20.03	0.09±0.03^{b}	8.11±0.18	13.78±4.66
Third (Early lactation)	Non-pregnant	0.40±0.11	333.80±19.58	0.02±0.01	9.58±1.01	11.38±3.36
	Singleton	0.39±0.10	491.18±108.36	0.01±0.00	9.64±2.75	8.50±1.33
	Twins	0.31±0.12	467.42±114.67	0.03±0.01	8.95±0.60	10.07±2.18

Different superscript letters within a column indicate significant differences between pregnancy outcome groups within the same sampling stage ($p < 0.05$).

Effect of parity

As indicated in Table 3, a statistically significant difference in SOD levels was observed among ewes with different parities, with this variation being evident only at the onset of lactation ($p<0.05$). Specifically, SOD levels in the case of the four parities, it is lower than in the groups of two and three parities. However, there was no significant difference in GPX, TAC, MDA, and NO levels among ewes with different parities ($p \geq 0.05$).

Table 3. Mean ± standard deviation of antioxidant concentrations at different sampling stages based on the parity number in Gray Shirazi Ewes

Sampling stage	Parity	SOD (U/ml)	GPX (U/ml)	TAC (mM)	MDA (µM)	NO (µM)
First (Mating)	1	0.08±0.02	371.80±31.83	0.09±0.04	21.42±9.19	7.50±1.51
	2	0.16±0.03	382.45±38.62	0.11±0.03	18.87±5.00	8.13±3.25
	3	0.18±0.02	470.14±67.73	0.09±0.02	26.02±7.12	7.48±1.35
	4	0.16±0.07	441.35±42.09	0.12±0.04	14.38±4.82	7.86±2.73
Second (Pregnancy)	1	0.31±0.07	205.50±23.33	0.05±0.02	8.20±0.22	10.77±0.86
	2	0.21±0.08	215.85±14.26	0.04±0.01	10.30±2.93	10.09±2.70
	3	0.23±0.09	258.85±16.29	0.05±0.02	8.73±1.07	13.10±4.21
	4	0.17±0.05	224.00±24.76	0.09±0.03	8.65±1.36	12.29±4.61
Third (Early lactation)	1	0.26±0.06^{ab}	233.00±28.28	0.00±0.00	7.83±0.46	11.38±3.05
	2	0.42±0.13^{a}	497.42±75.78	0.03±0.01	10.87±3.95	13.09±5.76
	3	0.47±0.11^{a}	483.84±32.25	0.01±0.00	9.60±2.61	8.25±2.70
	4	0.27±0.08^{b}	384.63±54.46	0.02±0.00	8.22±1.16	9.25±3.29

Different superscript letters indicate significant differences between parity (or BCS) groups within the same sampling stage ($p< 0.05$).

Effect of BCS

As shown in Table 4, the mean values of SOD, TAC, MDA, and NO at varying sampling times, based on BCS, did not differ significantly among the study groups ($p \geq 0.05$). However, the mean GPX in BCS=2.5 and BCS=4 was increased considerably compared to BCS=3.5 in the first sampling stage (mating) ($p<0.05$). Different superscript letters within a column indicate significant differences between sampling stages ($p< 0.05$).

Correlation Analyses

As indicated in Table 5, no statistically significant correlation was identified between the birth weight of lambs and the values of SOD, GPX, TAC, MDA, and NO at any of the sampling stages ($p \geq 0.05$).

Table 4. Mean ± standard deviation of antioxidant concentrations at different sampling stages based on the BCS in Gray Shirazi Ewes

Sampling stage	BCS	SOD (U/ml)	GPX (U/ml)	TAC (mM)	MDA (µM)	NO (µM)
First (Mating)	2.5	0.19±0.05	506.12±150.37^{b}	0.11±0.04	29.76±2.97	10.42±2.65
	3	0.18±0.09	434.64±129.23^{ab}	0.09±0.04	16.57±10.59	7.02±3.20
	3.5	0.15±0.03	387.75±96.97^{a}	0.10±0.05	23.25±7.31	8.57±3.35
	4	0.19±0.04	459.10±194.72^{b}	0.10±0.05	17.65±12.97	6.54±1.17
Second (Pregnancy)	2.5	0.35±0.13	270.00±33.94	0.03±0.04	8.91±1.88^{b}	8.17±1.43
	3	0.15±0.05	205.57±17.75	0.07±0.04	7.99±0.80^{b}	12.94±4.44
	3.5	0.22±0.06	271.66±135.94	0.06±0.06	8.69±1.17^{b}	12.30±5.64
	4	0.22±0.08	212.20±19.13	0.06±0.04	8.50±0.52^{b}	12.84±2.58
Third (Early lactation)	2.5	0.30±0.02	353.50±79.90	0.06±0.01	9.56±2.05	8.44±2.36
	3	0.40±0.12	452.71±101.94	0.01±0.02	10.27±3.21	12.43±3.06
	3.5	0.33±0.11	421.53±183.98	0.01±0.02	8.88±2.10	11.61±4.72
	4	0.38±0.13	408.33±200.45	0.01±0.03	8.97±2.53	6.58±1.32

Table 5. Correlation between antioxidant concentration and lamb weight at different sampling stages in Gray Shirazi Ewes

Sampling Stage	NO		MDA		TAC		GPX		SOD	
	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation
First (Mating)	0.251	0.199	0.152	0.404	0.099	-0.292	0.809	-0.037	0.251	0.199
Second (Pregnancy)	0.585	-0.108	0.410	0.162	0.637	-0.092	0.078	0.375	0.585	-0.108
Third (Early Lactation)	0.098	-0.391	0.141	0.341	0.184	-0.094	0.767	0.065	0.098	-0.391

As shown in Table 6, no statistically significant correlation was observed between ewes' blood serum BCS and SOD, GPX, TAC, and NO levels at various sampling stages ($p \geq 0.05$). Similarly, no statistically significant correlation was observed between MDA values and the BCS of the ewes in the first and third sampling stages ($p \geq 0.05$). However, during the second-stage sampling, as ewes' BCS increased, their serum MDA levels decreased significantly, yielding a correlation coefficient of -0.301 ($p<0.05$). A significant negative correlation was observed between serum MDA concentration and BCS during mid-pregnancy ($r = -0.301$, $p < 0.05$), indicating lower lipid peroxidation in ewes with higher body condition scores.

Table 6. Correlation between antioxidant concentration and BCS at different sampling stages in Gray Shirazi Ewes

Sampling Stage	NO		MDA		TAC		GPX		SOD	
	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation	Sig. (2-tailed)	Correlation
First (Mating)	0.596	-0.069	0.544	0.113	0.510	-0.101	0.483	0.082	0.559	0.074
Second (Pregnancy)	0.460	0.114	0.042	-0.301	0.142	-0.243	0.972	-0.006	0.978	-0.005
Third (Early Lactation)	0.744	-0.056	0.598	0.088	0.606	-0.068	0.143	-0.260	0.171	-0.248

Discussion

Adequate antioxidant status, both before and during pregnancy, is a critical condition for optimal placental and fetal growth and function, reduced fetal mortality, improved lambing outcomes, and neonatal well-being (13). In twin pregnancies, the production of oxidants is elevated relative to singleton pregnancies. This is due to the increased oxygen requirements and more severe lipid peroxidation (14). During pregnancy, oxidative stress increases due to the placenta's elevated oxygen demand, which is supported by a high mitochondrial density (15). According to Santarosa et al. (2021), SOD levels were higher in the postpartum period than in the preceding periods. Their study examined the relationship between the highest SOD activity and the timing of sample collection in two distinct groups: pregnant singletons and pregnant twins. The findings indicated that the highest SOD activity was observed in samples collected during the delivery process and within 24 hours postpartum (16). Furthermore, Gaal et al. (2006) reported that SOD activity in red blood cells increased significantly at parturition in dairy cows compared with the prepartum period (17). These three findings are consistent with results of our study. According to Mutinati et al. (2013), these results can be explained by the activation of additional antioxidant mechanisms in response to increased oxidant production during this period (18). This phenomenon is associated with processes such as tissue regeneration, uterine rotation, and cervical dilation. As pregnancy progresses, challenges related to antioxidant status become more apparent, especially in twin pregnancies, which are most evident in the last third of pregnancy (16).

A comparison of ewes at different sampling stages across three groups —non-pregnant, singleton, and twin — revealed no statistically significant differences in SOD levels ($p \geq 0.05$). According to Santarosa et al. (2021), the activity of SOD was elevated in the singleton group than in the twin group at the time of artificial insemination, on days 30 and 140 of pregnancy, and 24 and 48 hours after lambing. However, on day 90 of pregnancy, SOD activity was higher in the twin group (16). This difference may be due to the fact that, in the present study, sampling was conducted only during mid-pregnancy.

According to Santarosa et al. (2021), GPX levels increased markedly in the postpartum period compared with the preceding periods (16). Furthermore, the study by Salinas-Rios et al. (2017) revealed that the highest GPX levels were observed in the second month of pregnancy and the fifth day of lactation, while the lowest levels occurred in the first and third months of pregnancy (19). This increase is likely attributable to the prevention of lipid peroxidation. In the present study, a comparison of ewes at different sampling stages across three groups (non-pregnant, singleton, and twin) revealed no significant difference in GPX levels at any of the sampling stages ($p \geq 0.05$). However, Gur et al. (2011), in a study by conducted on slaughter ewes, ewes with two fetuses had lower GPX levels than ewes with one fetus or non-pregnant ewes (14). In addition, Erisir et al. (2009) conducted a study on 12 Awassi ewes aged 4-5 years. Blood samples were obtained from the ewes before estrus synchronization and during pregnancy on the 25th day of each month. The findings of the study indicated that GPX activity levels increased during pregnancy (20). This difference in results may be due to variations in ewe breed and age, as well as the time period and method of blood sampling.

In the present study, TAC values were significantly lower during early lactation compared to mating and pregnancy, suggesting possible consumption of antioxidant reserves at the onset of lactation. These results are consistent with those reported by Soriano et al. (2015), who examined 30 ewes and their lambs. In their study, the

authors observed an increase in maternal TAC levels on days 1 and 5 postpartum, with a sustained elevation persisting until day 10 postpartum (21). However, a comparison of lambs with their mothers on day 10 after birth revealed that there was a discrepancy in TAC levels between the ewes and lambs, with lower levels in the lambs. As stated by Nawito et al. (2016), the levels of TAC in pregnant goats and ewes were found to be lower in comparison to those observed in non-pregnant goats and ewes (22). In the present study, no statistically significant differences were observed in the TAC levels among ewes with different numbers of lambing ($p \geq 0.05$). In the study by Salinas-Rios et al. (2017), the TAC did not demonstrate any significant differences in ewes with different numbers of lambings (19).

In the present study, the MDA level was significantly higher at the initial sampling stage compared with other sampling stages ($p < 0.05$). According to Salinas-Rios et al. (2017), MDA levels remained relatively constant during pregnancy and lactation, except on day 143 of pregnancy, three days before parturition, when a decrease was observed (19). A study by Nawito et al. (2016) revealed that, in goats and ewes, MDA levels were significantly higher in pregnant animals than in non-pregnant animals (22). It is well established that parturient ewes experience increased energy demands, particularly during the postpartum period. This increased energy requirement can be attributed to the enhanced milk production that characterizes this phase. Consequently, there is a concomitant increase in the oxidation rate. Given these physiological changes, it is not surprising that antioxidant levels also increase. In the present study, MDA levels at the initial stage of sampling exhibited a significant difference among ewes with different numbers of lambs. However, no significant differences were observed at other sampling stages ($p \geq 0.05$). In the initial phase of the sampling stage, a statistically significant increase in MDA levels was observed among non-pregnant subjects. This increase was notably more pronounced than in the groups with singleton and twin pregnancies ($p<0.05$). Gur et al. (2011) found that the mean MDA levels in pregnant twin ewes were higher than those in pregnant and non-pregnant singleton ewes. However, no significant difference in MDA concentration was observed between pregnant and non-pregnant singleton ewes. In addition, a positive and significant correlation was observed between the number of embryos (0, 1, 2) and CL diameter, phosphorus concentration, and MDA level. A similar correlation was also identified between phosphorus concentration and CL diameter and MDA level (14). The study design, which involved the examination of healthy ewes brought to the slaughterhouse, underscores the importance of considering the impact of variables such as age, breed, and dietary regimen on the observed outcomes. The sample size consisted of 30 non-pregnant ewes, 30 singleton-pregnant ewes, and 12 twin-pregnant ewes. All of these differences could explain discrepancies between the results of his study and the present study.

During pregnancy, there is an increased need for NO to support vascular function in placental and fetal tissues (23). King RG et al. (1995) and Shaamash et al. (2000) reported that NO inhibits uterine contractions (23, 24). The onset of parturition is accompanied by an increase in uterine contractions, which, in turn, reduces the need for NO (25). In the present study, no significant differences in NO levels were found across sampling stages ($p \geq 0.05$). A number of studies have yielded contradictory results regarding the NO levels in pregnant subjects. According to Hata et al. (1999), NO levels declined during pregnancy (26). Conversely, Brown et al. (1995) and Smarason et al. (1997) reported no alterations in NO levels during this period (27, 28). As demonstrated by Yüksel et al. (2013), maternal blood NO levels increased significantly during pregnancy. Furthermore, maternal blood exhibited higher NO levels compared to cord blood in the third trimester of pregnancy (29). These findings are consistent with the results of studies by Takauchi et al. (1998), King RG et al. (1995), Shaamash et al. (2000), and Choi et al. (2002).

Conclusion

The findings of the present study in Gray Shirazi ewes suggest that the levels of antioxidants examined undergo changes and fluctuations influenced by different stages of pregnancy (mating, mid-pregnancy, and early lactation). These fluctuations may be related to the varying metabolic demands of the ewe and the developing fetus at each stage, as well as to interactions among the antioxidants themselves. Additionally, serum antioxidant levels, including NO, TAC, and MDA, appear to be associated with the number of litters. Overall, measuring the level of antioxidants in ewes at different stages, considering their multiple gestation and examining their relationship with reproductive and nutritional conditions, can yield valuable and practical insights.

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Authors' Contributions

Sina Samaei: Data Curation and Visualization, **Meysam Makki:** Writing-Original Draft, Writing- Review and Editing, Methodology and Conceptualization, **Seyedeh Misagh Jalali:** Supervision and Validation, **Alidad Boostani:** Resources

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

All animal protocols were approved by and performed in accordance with the institutional animal ethics committee of Shahid Chamran University of Ahvaz (protocol number: SCU.VC1404.39117). Also, all protocols complied with ARRIVE guidelines, and all procedures strictly followed Animal Scientific Procedures Act (1986).

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable

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