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RESEARCH ARTICLE

Dynamics of Oxidants, Antioxidants and Hormones During Different Phases of Pregnancy in Hairy Goats

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Abstract

The aim of the present study was to observe the variation in oxidant (MDA), antioxidants (SOD, GSH, GSH-Px) and hormones (P4 and E2) levels in pregnant hairy goats during breeding season. In the this study, twenty hairy goats were synchronized by using sponges containing progesterone (fluorogestone acetate). The animals showing oestrus were inseminated twice, first at 18th -24th h and second at 36th - 48th h of oestrus. On 35th and 42nd days after insemination, pregnancy diagnosis examination was performed with transrectal ultrasonography. The blood samples were collected from pregnant goats at 0, 11, 24 , 57, 100, 134, 141 days of gestation and immediately after the parturation. Serum samples collected at estrus and different stages of gestation were analyzed for MDA, SOD, GSH-Px, GSH, P4 and E2 concentrations using standard protocols. The results showed that MDA level did not change in pregnant goats. The SOD, GSH and GSH-Px levels also shown a similar pattern throughout pregnancy period. The peak ($P<0.05$) level of progesterone was recorded between 11th to 134th days of gestation. At time of estrus (day 0) and late gestation (day 134 and 141), the concentrations estradiol reached at maximal level ($P<0.05$) in hairy goats. In conclusion, the oxidants and antoixidants do not change with respect to dynamics of progesterone or estradiol level during gestation period in black hairy goats.

Keywords: *Antioxidants, Oxidants, Pregnancy, Hormones, Hairy Goat*

Kıl Keçilerinde Gebeliğin Farklı Aşamalarında Oksidanların, Antioksidanların ve Hormonların Dinamikleri

Öz

Bu çalışmanın amacı, üreme sezonundaki gebe kıl keçilerinde oksidan (MDA), antioksidan (SOD, GSH, GSH-Px) ve hormon (P4 ve E2) seviyelerindeki farklılıkları gözlemlemekti. Bu çalışmada 20 adet kıl keçisi progesteron içeren (fluorogeston asetat) vaginal süngerler kullanılarak senkronize edildi. Östrüs gösteren hayvanlar ilk olarak östrüsün 18-24. saatlerinde daha sonra 36-48. saatlerde olmak üzere iki defa tohumlandı. Tohumlama sonrası 35-42. günlerde transrektal ultrasonografi ile gebelik muayenesi yapıldı. Gebe keçilerden gebeliğin 0, 11, 24, 57, 100, 134, 141. günlerinde ve doğumdan hemen sonra kan örnekleri alındı. Gebeliğin farklı dönemlerinde toplanan serum örnekleri standart protokoller kullanılarak MDA, SOD, GSH-Px, GSH, P4 ve E2 konsantrasyonları için analiz edildi. Sonuçlar, MDA seviyesinin gebe keçilerde değişmediğini gösterdi. SOD, GSH ve GSH-Px seviyeleri de gebelik süresince benzer kaldı. Gebeliğin 11 ile 134. günleri arasında progesteron en yüksek seviyedeydi ($P<0.05$). Gebeliğin 0, 134 ve 141. günlerinde estradiol konsantrasyonu en yüksek seviyeye ulaştı. Sonuç olarak oksidanlar ve antioksidanlar, progesteron ve estradiol seviyelerine kıyasla gebelik süresince herhangi bir değişiklik göstermedi.

Anahtar sözcükler: *Antioksidanlar, Oksidanlar, Gebelik, Hormonlar, Kıl Keçisi*

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Introduction

During the pregnancy phase, body of the dam and conceptus demands the surplus supply of oxygen. The high demand of oxygen promotes the reactive oxygen species (ROS) production level during implantation to parturition [1]. The normal ROS level is required for the process of steroidogenesis, embryonic development, maternal recognition and implantation during pregnancy. Any imbalance in ROS level leads to cellular disintegration in the dam and conceptus that could result in embryonic mortality, placental degeneration, and pregnancy failure [2]. The pregnancy associated oxidative stress is balanced by regulation of exogenous and endogenous antioxidants level [3] and pre-conception increase in antioxidants favors the establishment of pregnancy [4,5]. Previously, the mechanism of ROS generation and antioxidant response during gestation have been documented in bovine [6] and ovine [7] but little information is available in pregnant goats [8] with respect to antioxidant response and oxidative stress to hormonal changes. Enzymatic scavenging mechanism i.e. glutathione peroxidase, catalase (CAT) and superoxide dismutase (SOD) against oxidative damage prevent dam and fetus from oxidative stress during the pregnancy. Alteration in enzymatic antioxidants occurres due to multiple fetuses, change of environment, low body condition scores and provision of low quality forage during pregnancy [9]. A change in oxidants and antioxidants is also associated with the status of the animal such as pregnancy, parturation and lactation [10].

Cotyledonary placenta is present in the goats and performs similar functions for optimal fetal growth and pregnancy maintenance alike to other species [11,12]. In contrast, the corpus luteum is considered the sole organ for pregnancy maintenance in caprine compared to bovine or ovine where the shift of corpus luteum (CL) to placenta for progesterone production occurs in mid of gestation [13]. Under such conditions, mechanisms underlying oxidants (MDA)-antioxidants (SOD, GSH, GSH-Px) variability in repsonse to progesetrone or estradiol level in pregnant goats need to be elucidated. Therefore, the present study was aimed to evaluate the malondialdehyde (MDA) concentrations, antioxidant enzyme activities superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and total glutathione (GSH) and progesterone (P_4) and estradiol (E_2) during the different phases of pregnancy in hairy goats.

Material and Methods

Ethical Approval

Prior to the execution of study, an approval was obtained from Animal Welfare and Ethical Committee Van Yüzüncü Yıl University (VAN YUHADYEK), Van, Turkey (Decision No: 2015/07-B).

Animals

In the present study, about twenty (n=20) 2-5 years old multiparous black hairy goats with no history of reproductive problems were selected. The research was conducted in Small Ruminants Research Center, Van Yüzüncü Yıl University, Van, Turkey. The goats were reared under semi-intensive conditions where each goat was given approximately 0.50 kg/head of concentrate daily in addition to grazing. The black hairy goat breed is present in Eastern Anatolia Region and this breed exhibits seasonal breeding pattern in this region. For optimum conditions and to mimimize the variability in experiments, the peak breeding season (September to November, 2019) was chosen to conduct the study.

Study Design, Estrus Synchronization and Pregnancy Diagnosis

Initially, the goats were synchronized using progesterone sponges (Chronogest-CR, fluorogestone acetate FGA, 20 mg Intervet®, Istanbul, Turkey) by placing intravaginally for a period of 11 days. Later, the cloprostenol (Estrumate 50 mg, Intervet®, Istanbul, Turkey) and equine chorionic gonadotropin (Chronogest/PMSG, 400 IU, Intervet®, Istanbul, Turkey) were adminstered intramuscularly 48 h before the removal of sponges. Estrus response detection was started after the removal of sponges till possible window of estrus display using a teaser buck at twelve hours intervals. The estrus goats were inseminated twice (First at 18-24 h and second after 36-48 h of estrus) using frozen-thawed semen (75×10^6 sperm/0.50 mL) through transcervical intrauterine insemination technique [14]. The pregnancy diagnosis was performed at day 35th and 42nd after insemination through transrectal ultrasonography (7.5 MHz Linear Probe, Honda HS 1500).

Blood Sampling

The blood samples were collected through jugular vein puncture at 0, 11th, and 24th days after insemination from all experimental goats (n=20). Upon pregnancy confirmation, the blood samples were collected from pregnant goats (n=10) on 57th, 100th, 134th and 141st days of gestation and immediately after the parturation. The blood samples were centrifuged at 3000 rpm for 10 min for serum separation. Then serum samples were stored at -20°C and later assayed for oxidants (MDA), antioxidant (SOD, GSH-Px, Total GSH,) and hormone (P_4 and E_2) titer.

MDA, SOD, GSH and GSH-Px Analyses

Serum MDA, GSH, SOD, GSH-Px were measured by commercially available ELISA kits (LOT # OK18104O, Rel Assay Diagnostics, Clinical Chemistry Solutions, Gaziantep, Turkey) as described earlier [15]. These kits were based on the spectrophotometric measurement and presence of the oxidants present in serum samples shown the colored compund. The detection principle was based

on the colored compund orgination by conversion of ferrous (Fe^{+2}) ions into ferric (Fe^{+3}) ions as a chromogen in an acidic environment. Hydrogen peroxide was used as a calibrator index. The MDA, GSH, SOD, GSH-Px levels were measured by ELISA spectrophotometric device (TECAN Firm, Sunrise model). The results were expressed as µmol H_2O_2 Eq/L, MDA levels as µmol/L, SOD activity as U/mL, GSH-Px activity as nmol/min/ml, and total GSH levels as µm.

Progesterone (P_4) and Estradiol (E_2) Hormone Analyses

Serum E_2 and P_4 concentrations were measured by automated Elecsys Immunoanalyser method (Roche Diagnostics, Mannheim, Germany). The estrogen concentration was expressed in pg/ml following the method described by Souza et al.[16], while progesterone concentration was expressed in ng/ml as reported previously [17].

Statistical Analyses

The data was analysed using SPSS (IBM SPSS for Windows, Ver.23) statistical software. The samples were analysed by selecting the values 0.80 and Type 1 Error 0.05. Normality distribution was calculated by using Shapiro-Wilk (n<50) test. In case of data unequallity, the non-parametric tests were applied to observe the difference among the variables. The descriptive statistics were used to express the data (average±Standard Deviation). The Friedman test was used to express the repeated measures for different variables at different days of pregnancy. Additinally, the Wilcoxon test was used for the analysis of dependant variables at different gestational days. Spearman correlation coefficient test was applied to determine the relationship between the measurements. A level of P<0.05 was used to denote the significiance difference between the variables.

Results

The results of serum lipid peroxidation, enzymatic antioxidant activities and hormones at estrus time (0), gestation (day 11, 24, 57, 100, 134 and 141) and immediately after parturation were presented in *Table 1*. The level of MDA, SOD, GSH-Px and total GSH did not change at estrus (day 0), gestation (day 11, 24, 57, 100, 134 and 141) and after parturation. The progesterone was at peak (P<0.05) level at day 11 with a decreasing pattern at day 24, day 57; however, it again increased (P<0.05) on day 134. The least (P<0.05) level of progesterone was observed at the time of AI and after parturition. In contrast, the estradiol was reached to maximum level on day 0, 134th and 141st of gestaion in goats.

The correlations presented in *Table 2* indicated that there was no positive or negative correlations between hormones (P_4 or E_2) and oxidant (MDA) or antioxidants (SOD, GSH-Px and total GSH activity).

Discussion

In the present study, we investigated the variation of oxidants and antioxidants levels in association to progesterone and estradiol levels at estrus or during different phases of

Table 1. *The results of serum lipid peroxidation, enzymatic antioxidant activities hormone at the time of estrus/AI (day 0), blastogenesis (days 11), embryogenesis (days 24), fetal period (days 57, 100, 134 and 141) and after parturition in pregnant goats (values are presented as means ± SD)*

Variables	Day 0 (Estrus/AI)	Day 11 (B)	Day 24 (E)	FP				After Parturition
				Day 57	Day 100	Day 134	Day 141	
MDA (µmol/L)	1.57±0.10	1.57±0.09	1.56±0.08	1.53±0.06	1.59±0.04	1.59±0.11	1.56±0.15	1.59±0.08
SOD (U/mL)	1.93±0.57	2.19±0.49	2.17±0.58	2.43±0.36	2.08±0.43	2.01±0.62	1.65±0.54	2.36±0.91
GSH-Px (nmol/min/mL)	176.0±100.0	288.4±131.1	215.4±373.7	161.9±60.7	225.4±135.2	165.2±97.7	237.6±146.6	184.3±71.3
Total GSH (µm)	3.30±1.96	5.46±2.48	4.07±1.45	2.98±1.22	4.35±2.58	3.69±2.79	4.48±2.87	3.46±1.41
P_4 (ng/mL)	0.32±0.35^{d}	20.37±7.54^{a}	8.01±2.84^{c}	9.72±3.19^{c}	10.82±3.76^{bc}	15.50±3.40^{ab}	7.48±2.91^{c}	0.30±0.08^{d}
E_2 (pg/mL)	35.20±16.3^{bc}	13.20±2.04^{d}	12.40±1.35^{d}	16.00±2.55^{d}	23.56±2.60^{cd}	50.33±12.69^{ab}	67.89±24.43^{a}	39.60±15.58^{bc}

AI: *artificial insemination;* ***B:*** *blastogenesis;* ***E:*** *embryogenesis;* ***FP:*** *fetal period;* ***MDA:*** *malondialdehyde;* ***SOD:*** *superoxide dismutase;* ***GSH-Px:*** *glutathione-peroxidase;* ***GSH:*** *glutathione;* ***P_4:*** *progesterone;* ***E_2:*** *estradiol; Different superscripts (a, b, c, d) in same raw indicate the statistical difference (P<0.05)*

Table 2. *Correlation analysis between hormones, oxidants and antioxidants in pregnant goats*

Variables	MDA	P_4	E_2
MDA (µmol/L)		-0.02	0.05
SOD (U/mL)	0.21	-0.02	-0.2
GSH-Px (nmol/min/mL)	-0.01	0.06	-0.22
Total GSH (µm)	-0.02	0.09	-0.2

P<0.05; r: Spearmen correlation coefficient

pregnancy in goats. The MDA level and blood antioxidant status (SOD, GSH, GSH-Px) in the pregnant goats did not change at any phase of pregnancy. The data shows that no oxidative stress occurs in goats during any phase of pregnancy and oxidants-antioxidants homeostasis is maintained in response to hormonal change.

The level of MDA or CAT, SOD and GSH prior to breeding or mating time also affect the future pregnancy. Although, the ROS is a prerequisite for oocyte maturation, fertilization, embryogenesis, implantation and steriodogenesis from CL or plancenta but enormous rise in ROS during synchronization through application of CIDR or vaginal sponges could adversely affect the pregnancy [4]. In contrast, we did not notice any change in MDA or antioxidants levels comapring to nonpregnant or any other phase of pregnancy in the present study, though, the goats were also synchronized using progesterone sponges by placing intravaginally as reported earlier [4,18].

Increasing trend of MDA with decreasing level of antioxidants in blood profile is an indicator of oxidative stress. The current results showed neither MDA nor antioxidants levels change in pregnant goats; although, previous studies in different species describe that pregnancy induces oxidative stress and variation in oxidant-antioxidants is observed [1,2,6]. It is also noted that fluctutaions in hormonal changes during the pregnancy did not influence the oxidative and antioxidative parameters. Keeping in view of previous literature, generally rise of oxidative stress is notable during the twinning and multiple pregnancies compared to singleton [19]. In addition, the rise in MDA during pregnancy is an indication of stress that occurred due to climate change, low plane of nutrtion or poor body condition [5]. In the present study, unaltered antioxidative homeostasis during the pregnancy might be due to high level of progesterone at each phase of pregnancy and high level of estradiol at estrus or parturition. Because the progesterone and estradiol have the antioxidative properties [20] and hormonal fluctution during pregnancy could lead to imbalance between oxidative and antioxidants level as seen in multiple pregnancies [21]. In addition, similar level of oxidant-antioxidants during pregnancy in goats in the current study might be linked to similar level of progesterone production solely by CL compared to other species where shift from CL to placenta occur. In current study, we did not notice any oxidative stress in those goats carrying twins because two goats were carrying twins and rest of them having singleton. In contrast to current study, Jimoh et al.[22] observed a decline in antioxidant enzyme activity from 2nd to the 3rd trimester and assumed that this decline might be due to depletion of the antioxidants in response to higher physiological demand of immunity at neonates birth.

The goat breeds present in subtropical or in tropics shows the seasonality in breeding pattern and majority of the population exhibit estrus activity and concieved during breeding season with small portion of goats in estrus during non-breeding season. In addition, the extreme climatic conditions during non-breeding season could be major influential factor on cyclicity, variation in serum metabolites, hormones and oxidative/antioxidative marker in sheep and goats [23]. The present study was conducted during the breeding season when small ruminants are present under comfort zone with no significant change in serum metabolites and oxidative markers [24]. The current data showed no variations in oxidative and antioxidative variables throughout the pregnancy in goats that might be linked to the season or husbandry practices. Previously, Teama [25] reported that hot climatic conditions of Egypt influenced the hormonal, serum metabolites, oxidative and antioxidative variables in cyclic goats compared to mild climate. Similarly, Rathwa et al.[24] reported that non-breeding season affect most of the biochemical, hormonal and oxidative/antioxidative markers in sheep in Indian conditions. The changes in stress hormones during non-breeding season lead to change of different body metabolites that could reinforce the incidence of oxidative stress even in nonpregnant goats in Damascus goats [26] in climatic conditions of Southern Turkey. Based on the data, it is indicated that the seasonal comparison concerning to pregnancy and oxidative stress needs to be further elucidated. It is also speculated that breed of species, feeding regimen during pregnancy, and husbandry practices might be contribuotry factors for onset of oxidative stress in goats.

In conclusion, no oxidative stress occurs at estrus or during pregnancy in black hairy goats carrying single fetus during the breeding season. However, the oxidative stress markers across the different breeding seasons and managemental systems could be investigated to explore the oxidative stress with ameliorative strategies in different tropical and subtropical goat breeds.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

This work was carried out in collaboration between all authors. Designed the experimental procedures and conducted the research work NÇ, FE, MB. Interpretation and editing of results NÇ, FE, ZN, LM. All authors read and approved the final manuscript.

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