Effect of Pregnancy and Foetal Number on Diameter of Corpus Luteum, Maternal Progesterone Concentration and Oxidant/Antioxidant Balance in Ewes

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Contents
The aim of this study was to determine the changes in diameter of corpus luteum (CL), maternal progesterone (P) concentration, lipid peroxidation and non-enzymatic antioxidant levels along with enzymatic antioxidant activities in pregnant ewes bearing single and twin foetuses. The ewes were selected from healthy animals that were brought to the abattoir for slaughtering. The ewes were divided into three groups: Group 1 (non-pregnant, non-oestrous, n = 30), Group 2 (pregnant bearing a single foetus, n = 30) and Group 3 (pregnant bearing twin foetuses, n = 12) after they were slaughtered. Pregnant ewes were in the first half of the pregnancy. The diameter of CL and P concentration of pregnant ewes bearing a single foetus or twin foetuses were found higher than that found in non-pregnant ewes. Similarly, the P concentration of pregnant ewes bearing twin foetuses was higher than that found in pregnant ewes bearing a single foetus. Malondialdehyde (MDA) level in pregnant ewes bearing twin foetuses was higher than that found in both non-pregnant and pregnant ewes bearing a single foetus. The serum glutathione (GSH) level and glutathione-peroxidase (GSH-Px) activity of pregnant ewes bearing twin foetuses were found lower than that found in non-pregnant ewes. Additionally, the GSH-Px activity of pregnant ewes bearing twin foetuses was found lower than that found in pregnant ewes bearing a single foetus. No significant difference was found between pregnant ewes bearing female and male foetuses with respect to diameter of CL, P concentration and oxidative stress parameters. There were significant positive correlations between foetal number (0, 1, 2) and diameter of CL, P concentration, MDA level, and between P concentration and diameter of CL, MDA level. However, significant negative correlations were found between foetal number (0, 1, 2) and GSH level, GSH-Px activity, and between P concentration and GSH-Px activity. In conclusion, the diameter of CL enlarges, P production increases and oxidant/antioxidant balance impairs because of the gestation stress in ewes during pregnancy.

Introduction
The corpus luteum (CL) develops after the collapse of the follicle at ovulation in cyclic and pregnant mammals and CL of pregnancy is known as the corpus luteum vernum. Sheep CL is necessary to maintain pregnancy until at least day 60 (Al-Gubory et al. 1999). The major secretory product of CL is the progesterone (P). It is the hormone of pregnancy and unequivocally required in all mammals for maternal support of the conceptus (embryo/foetus and associated membranes) survival and development (Spencer and Bazer 2002). P increases throughout the whole length of gestation and reaches its highest level at weeks 19–20 and then declines 2 weeks before parturition in sheep (Ramilla et al. 1994). In cyclic and pregnant sheep, there is a positive correlation between the total volume of luteal tissue and the P concentration (Kaufuss et al. 2003). It has been reported by many authors (Kalkan et al. 1996; Manalu and Sumaryadi 1998; Kaskous et al. 2003; Müller et al. 2003; Kulcsar et al. 2006) that increased foetal number results in increased maternal circulating P concentration in different sheep breeds. However, it has been reported that sex of foetuses does not affect the P concentration during gestation (Mukasa-Mugerwa and Viviani 1992; Kalkan et al. 1996).

Free radicals are highly reactive molecules that include reactive oxygen species (ROS) and reactive nitrogen species (RNS). The most prominent ROS are the superoxide anion (O$_2^-$), hydroxyl radical (?OH) and the hydrogen peroxide (H$_2$O$_2$). Nitric oxide (NO), is one of the most significant RNS, is synthesized during the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS, Agarwal et al. 2005). Free radical reactions are essential for host defence mechanisms involving neutrophils, macrophages, and other cells of immune system; however, excessive production of free radicals can lead to tissue injury and cell death and result in antioxidant depletion. Antioxidants are substances or enzymes present in tissues with the capacity to balance or neutralize these free radicals (Chen and Scholl 2005). Placenta is rich in mitochondria and highly vascularised with high metabolic rate. Thus, with increased leakage of electrons from the mitochondrial respiratory chain, there is increased generation of free radicals in normal pregnancy. This physiological response, in the presence of transition metals, potentially contributes to damage of DNA, lipids and proteins by ROS (Casamayor and Viteri 2003; Poston and Raimakers 2004). Many studies have compared biomarkers of oxidative stress in normal pregnant women and non-pregnant control subjects (Zachara et al. 1993; Morris et al. 1998; Mihailovic et al. 2000; Arikan et al. 2001). Results of such studies suggest that erythrocyte or plasma malondialdehyde (MDA), a by-product of lipid peroxidation, and lipid peroxides are generally higher during the second trimester (Mihailovic et al. 2000) as well as late in gestation (Morris et al. 1998; Mihailovic et al. 2000; Arikan et al. 2001). Conversely, the activities of antioxidant enzymes [superoxide dismutase (SOD), glutathione-peroxidase (GSH-Px)] and their related substances [selenium, glutathione (GSH)] are usually decreased (Zachara et al. 1993; Mihailovic et al. 2000; Arikan et al. 2001). Erişir et al. (2009) have reported that although plasma lipid peroxidation level does not show
any significant change, erythrocyte GSH level and GSH-Px activity significantly increase during pregnancy and erythrocyte catalase (CAT) activity significantly decreases after the first month of the pregnancy in Awassi ewes. Öztabak et al. (2005) have reported that while plasma GSH-Px activity was higher and plasma CAT activity was lower in pregnant Chios ewes on days 148 of pregnancy than in the non-pregnant ewes, plasma lipid peroxidation level on days 105 and 148 of pregnancy and plasma GSH-Px and CAT activities on day 105 of pregnancy were not different. It is clearly seen that there is a conflict regarding the changes in oxidative stress parameters during pregnancy in both ewes and women according to literature information mentioned earlier. However, there is no data regarding biomarkers of oxidative stress during normal gestation with multiple foetuses in early pregnancy in sheep. Therefore, this study was conducted to determine the changes in oxidant/antioxidant balance along with diameter of CL and P concentration during first half of the gestation in normal pregnant ewes bearing a single foetus or twin foetuses.

Material and Methods

Animals

This study was conducted in Elazığ province of Turkey located at latitude of 38°40′N during breeding season (between September and December). Seventy-two Akkaraman ewes, approximately 2 years of age, were used in this study. The age of ewes was estimated by examining their teeth and also by taking information from the animal owners. The ewes were selected from healthy animals which were systematically examined by the expert veterinarian before and after slaughter. According to the information obtained from animal owners, the ewes used in this study were generally fed on grass supplemented with alfalfa hay and mated by ram.

The animals were numbered, and blood samples were collected by a sterile syringe and injector from jugular vein before the slaughtering. Blood samples were then dumped into the sterile tubes and brought to the laboratory in appropriate conditions. The samples were centrifuged at 4500 × g for 5 min; sera were removed and stored at −20°C in a deep freezer until assayed.

Blood collection

The animals were numbered, and blood samples were collected by a sterile syringe and injector from jugular vein before the slaughtering. Blood samples were then dumped into the sterile tubes and brought to the laboratory in appropriate conditions. The samples were centrifuged at 4500 × g for 5 min; sera were removed and stored at −20°C in a deep freezer until assayed.

Measurement of diameter of CL

The uterus and ovaries were gently removed from the body after slaughter. Diameter of CL was measured by callipers and measurements were recorded.

Progesterone assay

The serum progesterone concentration was determined by coated-tube radioimmunoassay method using Active® Progesterone RIA DSL – 3900 kit (Diagnostic System Laboratories Inc., Webster, TX, USA) in gamma counter (LKB-Wallac Multigamma, Oy, Turku, Finland) according to the kit manufacturer’s report. The progesterone concentration was expressed as ng/ml. The calibration range and sensitivity of kit were 0.30–60 and 0.12 ng/ml, respectively. The intra-assay and interassay variation coefficient of kit were 4.8% and 9.2%, respectively.

Lipid peroxidation (MDA) assay

Lipid peroxidation (as MDA) level in serum samples was measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966). The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of MDA equivalents generated by acid catalysed hydrolysis of 1,1,3,3 tetramethoxypropane. The values of MDA were expressed as nmol/ml.

Glutathione (GSH), glutathione-peroxidase (GSH-Px) and catalase (CAT) assay

The GSH level in serum was measured at 412 nm using the method described by Sedlak and Lindsay (1968). The samples were precipitated with 50% trichloroacetic acid and then centrifuged at 1000 × g for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris-EDTA buffer (0.2 M; pH 8.9) and 0.1 ml of 0.01 M 5,5-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min and then read at 412 nm on the spectrophotometer. The GSH level was expressed as nmol/ml. Glutathione-peroxidase activity in serum samples was measured at 37°C and 412 nm according to Lawrence and Burk (1976). The GSH-Px activity was expressed as U/g prot. The method described by Goth (1991) was used for the determination of CAT activity in serum. The yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank using a spectrophotometer. The CAT activity was expressed as kU/l.

Protein determination

The protein content in serum was measured by method of Lowry et al. (1951) using bovine serum albumin as the standard.

Plasma vitamins A and E analyses

Vitamins A (retinol) and E (alpha-tocopherol) were determined in serum samples by a modification of
Pregnant bearing a single foetus

Pregnant bearing a single foetus

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pregnant (p < 0.05). **Different from non-pregnant (p < 0.001)

(values are presented as means ± SEM). *Different from non-
pregnant bearing a single foetus and pregnant bearing twin foetuses

Fig. 1. Diameters of corpus luteum in ewes with non-pregnant,
congestant and pregnant bearing twin foetuses were found higher
than in non-pregnant ewes (p < 0.001). Similarly, the P
concentration in pregnant ewes was higher than in pregnant ewes bearing a single foetus (p < 0.01).

The lipid peroxidation, non-enzymatic antioxidant levels and enzymatic antioxidant activities in ewes that
non-pregnant, pregnant bearing a single foetus and twin foetuses are presented in Table 1. There was no signif-
icant difference in MDA levels between non-pregnant and pregnant ewes bearing a single foetus. However,
MDA level in pregnant ewes bearing twin foetuses was higher (p < 0.05) than that found in both non-pregnant
ewes and pregnant ewes bearing a single foetus. The
GSH level and GSH-Px activity in pregnant ewes bearing twin foetuses were found lower (p < 0.05) than in
non-pregnant ewes. Additionally, the GSH-Px activity in pregnant ewes bearing twin foetuses was found lower (p < 0.05) than in pregnant ewes bearing a single foetus. With respect to serum vitamins A and E levels,
and CAT activity, no significant differences were deter-
mined between the groups.

The mean diameters of CL, P concentrations and
oxidative stress parameters in groups including both
types of pregnant ewes bearing female or male foetuses
are shown in Table 2. No significant difference was

Table 1. The serum lipid peroxidation, non-enzymatic antioxidant levels and enzymatic antioxidant activities in ewes with non-pregnant, pregnant bearing a single foetus and pregnant bearing twin foetuses (values are presented as means ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-pregnant (n = 30)</th>
<th>Pregnant bearing a single foetus (n = 30)</th>
<th>Pregnant bearing twin foetuses (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.83 ± 0.09*</td>
<td>1.91 ± 0.08*</td>
<td>2.80 ± 0.17*</td>
</tr>
<tr>
<td>GSH (nmol/ml)</td>
<td>0.34 ± 0.01*</td>
<td>0.33 ± 0.02*</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>Vit A (μg/dl)</td>
<td>17.01 ± 1.80</td>
<td>19.24 ± 3.52</td>
<td>24.46 ± 5.76</td>
</tr>
<tr>
<td>Vit E (mg/dl)</td>
<td>0.40 ± 0.06</td>
<td>0.28 ± 0.04</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>GSH-Px (U/g prot.)</td>
<td>3.95 ± 0.13*</td>
<td>3.83 ± 0.22*</td>
<td>2.37 ± 0.25*</td>
</tr>
<tr>
<td>CAT (kU/l)</td>
<td>16.45 ± 1.48</td>
<td>15.26 ± 2.24</td>
<td>20.90 ± 5.51</td>
</tr>
</tbody>
</table>

GSH, glutathione; GSH-Px, glutathione-peroxidase; MDA, malondialdehyde.
The differences between the values bearing different superscript letters (a and b) in the same line are statistically significant (p < 0.05).

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Table 2. Diameters of corpus luteum (CL), levels of progesterone, serum lipid peroxidation and non-enzymatic antioxidants and activities of enzymatic antioxidant in pregnant ewes bearing female or male foetus (values are presented as means ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female (n = 15)</th>
<th>Male (n = 15)</th>
<th>p-value</th>
<th>Female (n = 6)</th>
<th>Male (n = 7)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of CL (cm)</td>
<td>0.82 ± 0.17</td>
<td>0.80 ± 0.15</td>
<td>0.829</td>
<td>0.86 ± 0.04</td>
<td>0.88 ± 0.03</td>
<td>0.711</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>5.29 ± 0.23</td>
<td>5.11 ± 0.26</td>
<td>0.588</td>
<td>9.28 ± 0.81</td>
<td>9.62 ± 0.75</td>
<td>0.745</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.82 ± 0.11</td>
<td>2.00 ± 0.15</td>
<td>0.367</td>
<td>2.79 ± 0.23</td>
<td>2.80 ± 0.24</td>
<td>0.980</td>
</tr>
<tr>
<td>GSH (nmol/ml)</td>
<td>0.32 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>0.710</td>
<td>0.20 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.400</td>
</tr>
<tr>
<td>Vit A (µg/dl)</td>
<td>18.41 ± 2.93</td>
<td>20.07 ± 6.77</td>
<td>0.813</td>
<td>27.49 ± 2.91</td>
<td>21.52 ± 0.77</td>
<td>0.331</td>
</tr>
<tr>
<td>Vit E (mg/dl)</td>
<td>0.22 ± 0.04</td>
<td>0.34 ± 0.07</td>
<td>0.620</td>
<td>0.21 ± 0.04</td>
<td>0.31 ± 0.07</td>
<td>0.630</td>
</tr>
<tr>
<td>GSH-Px (U/g prot.)</td>
<td>3.74 ± 0.28</td>
<td>3.92 ± 0.36</td>
<td>0.746</td>
<td>2.50 ± 0.18</td>
<td>2.24 ± 0.19</td>
<td>0.372</td>
</tr>
<tr>
<td>CAT (kU/l)</td>
<td>16.02 ± 2.96</td>
<td>14.56 ± 3.47</td>
<td>0.674</td>
<td>16.98 ± 3.10</td>
<td>24.82 ± 7.97</td>
<td>0.448</td>
</tr>
</tbody>
</table>

GSH, glutathione; GSH-Px, glutathione-peroxidase; MDA, malondialdehyde.

found between pregnant ewes bearing female foetus and male foetus with respect to all these studied parameters. There were significant positive correlations between foetal number (0, 1, 2) and diameter of CL (r = 0.528, p = 0.000), P concentration (r = 0.903, p = 0.000), MDA level (r = 0.358, p = 0.003). However, significant negative correlations were found between foetal number (0, 1, 2) and GSH level (r = -0.307, p = 0.012), GSH-Px activity (r = -0.308, p = 0.012). Additionally, there were significant positive correlations between P concentration and diameter of CL (r = 0.421, p = 0.000), MDA level (r = 0.294, p = 0.016). However, a significant negative correlation was determined between P concentration and GSH-Px activity (r = -0.311, p = 0.011).

**Discussion**

The primary function of the CL in pregnancy is the production of P. The development of the CL begins immediately after ovulation. After an ovum is extruded at ovulation, the follicle collapses; this is followed by haemorrhage into the follicular cavity within a matter of hours. Over the next 2–4 days, the wall is penetrated with thin-walled blood channels that pass centrally towards the lumen with fresh blood. This neovascularisation is essential for the delivery of cholesterol, a principal substrate for P synthesis within the wall of the CL. As the CL matures, blood is gradually resorbed from within the cavity, and the overall structure enlarges. If conception is not achieved, the CL regresses. If pregnancy occurs, the CL continues to enlarge (Hafez and Hafez 2000). Although the sheep CL is necessary to maintain pregnancy at least during the first 60 days, it remains functional throughout the pregnancy. In addition, CL together with placenta secretes more P than required for pregnancy maintenance after day 60 (Al-Gubory et al. 1999). The presence of high levels of luteal tissue P until day 142 of pregnancy in sheep indicates that luteal cells have a sustained and high steroidogenic capacity (O’Shea and McCoy 1988). Many authors have reported that maternal plasma P concentration of different pregnant ewe breeds such as Menz (Mukasa-Mugerwa and Viviani 1992), Javanese thin-tail (Manalu and Sumaryadi 1998) and Awassi (Kaskous et al. 2003) is higher than in non-pregnant ewes. It has been reported that pregnant Awassi (Kalkan et al. 1996; Kaskous et al. 2003), German blackheaded mutton × long-wool Merino crossbred (Müller et al. 2003) and prolific Merino (Kulscar et al. 2006) ewes bearing multiple foetuses have higher P concentration than that found in ewes bearing a single foetus until the last week of pregnancy. Additionally, Mukasa-Mugerwa and Viviani (1992) and Kalkan et al. (1996) have reported that maternal P concentration is not affected by the sex of the foetuses. In this study, it was found that diameter of CL and maternal serum P concentration of pregnant ewes were higher than in non-pregnant ewes, and ewes bearing a single foetus had lower P concentration than that found in ewes bearing twin foetuses. In addition, there was no significant difference between ewes bearing female and male foetuses with respect to diameter of CL and P concentration in this study. Our findings are in agreement with those previous studies. Kaufluss et al. (2003) have reported that corpora lutea of sheep formed after multiple ovulations produce more P than CL formed after one ovulation and also reported that there is a positive correlation between total volume of CL and P concentration in cyclic and pregnant sheep. In addition, Manalu et al. (1996) have alleged that increased number of corpora lutea contributes more P secretion in pregnant sheep and goats bearing more than one foetus. In this study, a significant positive correlation was found between foetal number (0, 1, 2) and diameter of CL, maternal P concentration, and between diameter of CL and P. The enlargement in diameter of CL in pregnant ewes versus non-pregnant ewes may be explained by increase in luteal cell volume to provide more P production to maintain pregnancy at least during early pregnancy. The increase in P concentration in ewes bearing twin foetuses versus one foetus is mainly because of the increased number of corpora lutea in this study.

The use of oxygen during normal metabolism in living cells can cause normal production of ROS. In a healthy body, ROS and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress occurs. Reactive oxygen species are a double-edged sword: they serve as key signal molecules in physiological processes but also have role in pathological processes involving the female reproductive tract. Reactive oxygen species affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy (Agarwal et al. 2005). Normal pregnancy is characterized by a high energy demand for many physiological functions.

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and an increased oxygen requirement by different organs, including the feto-placental unit. During gestation and embryogenesis, the organism of the mother and embryo is the site of physiological high rates of ROS production, primarily involved in cell signalling and control of the foetal development; replication, differentiation, and maturation of the foetal cells and organs. However, excessive ROS production can be involved in harmful effects and can lead to breakdown of vitamins and mineral trace-elements associated with enzymes specialized in the defence against ROS and can end in peroxidation of cell components and induction of pathologies and gestation failure (Aurousseau et al. 2006). It has been reported (Catalona et al. 1999; Homko et al. 2001) that physiological increases in insulin resistance during gestation result in an increase in circulating lipids [e.g. triglycerides, free fatty acids, total cholesterol and low density lipoprotein (LDL) levels]. Increased plasma LDL is associated with increased lipid hydroperoxides in normal pregnancy (Toescu et al. 2004). In sheep and cows, the blastocyst loses its zona pellucida 7 and 10 days before implantation, respectively, whereas this phenomenon occurs at the moment of implantation in women. Therefore, an extended period of high rates of cell to cell contacts and triggering of NADPH oxidases and ROS fluxes is likely to occur in the ruminants (Aurousseau et al. 2006).

Moreover, NO radical production by peripheral bovine lymphocytes has been reported to be steadily increased by a factor of 3 between the non-pregnant state and seventh day after conception and by a further factor of 1.5 between day 7 and days 90–120 of gestation (Dixit and Parvizi 2001). Evidences based on the circulating biomarkers indicate that there is an increase in oxidative stress during normal pregnancy in women (Chen and Scholl 2005). Erisir et al. (2009) have reported that plasma MDA level was 2.99 ± 0.52 nmol/ml in non-pregnant healthy Awassi sheep. Simsek et al. (2006) have reported that serum MDA level was 2.83 ± 0.42 nmol/ml in non-pregnant healthy Akkaraman ewes. Similarly, plasma MDA level was found as 1.43 ± 0.48 nmol/ml in non-pregnant healthy Anatolian goats by Kizil et al. (2007). The serum MDA levels (1.83 ± 0.09 nmol/ml) reported here show similarity with these results. It has been reported that plasma lipid peroxidation level on days 105 and 148 of pregnancy in Chios ewes is not different from non-pregnant control ewes (Oztabak et al. 2005). Similarly, Erisir et al. (2009) have reported that no significant change is seen in plasma MDA level during early and late pregnancy in Awassi ewes compared with non-pregnant ewes. Although the MDA levels in pregnant ewes bearing twin foetuses were found higher than in both non-pregnant and pregnant with a single foetus, there were no significant differences between non-pregnant and pregnant with a single foetus in this study. In addition, a significant positive correlation was found between foetal number (0, 1, 2) and MDA level, and P concentration and MDA level. Our findings are in agreement with previous studies in ewes and women. The increased MDA level in normal pregnant ewes bearing twin foetuses may be attributed to an increased oxygen requirement and increased circulating lipids.

The foetus and placenta are protected by antioxidant defence system to avoid any lethal effect of the high rates of ROS production. Glutathione production and metabolism are of the utmost importance to prevent pathologies of gestation, both for the radical scavenging properties of GSH and for its role in the control of redox status inside cells. Superoxide dismutase, CAT and GSH-Px are a set of enzymes specialized in defence against ROS (Agarwal et al. 2005; Aurousseau et al. 2006). Whole blood or erythrocytes are frequently used to measure the activities of these intracellular enzymes. However, plasma and/or serum are also used to measure enzyme activity (Oztabak et al. 2005; Simsek et al. 2006; Kizil et al. 2007) because activity values in these samples reflect those found in erythrocytes (Naziroğlu and Kökçam 2000). In normal pregnancy, it seems plausible that the antioxidant defence system may be able to compensate through induction and increased activity of antioxidant enzymes as well as non-enzymatic free radical protection and scavenging (e.g. by protein thiols, and vitamins A, E and C, Morris et al. 1998; Chen and Scholl 2005). It has been reported that maternal SOD and GSH-Px activity either increases, declines, or shows no change during gestation in women (Carone et al. 1993; Zachara et al. 1993; Loverro et al. 1996; Chen et al. 2003). Zachara et al. (1993) and Mihailovic et al. (2000) have reported that maternal GSH-Px activity gradually declines with gestation, whereas Chen et al. (2003) have reported that GSH-Px activity increases by 15% between week 16 and third trimester. In addition, some researchers have reported that SOD, GSH-Px and their substrates (e.g. selenium, GSH) usually decreases during gestation in women (Zachara et al. 1993; Mihailovic et al. 2000; Ankan et al. 2001). In a study made in sheep throughout the gestation, Al-Gubory et al. (2004) have reported that enzymatic activity of SOD, GSH-Px and glutathione-S-transferase in CL increases significantly from day 15 to day 40 of pregnancy and thereafter remains constant until day 128. Kizil et al. (2007) have reported that plasma GSH level, GSH-Px and CAT activities were 0.244 ± 0.028 nmol/ml, 1.01 ± 0.15 U/g prot. and 28.88 ± 14.02 kU/l, respectively, in non-pregnant healthy goats. Oztabak et al. (2005) have reported that plasma GSH-Px and CAT activities were 15.91 ± 7.23 U/l and 2.06 ± 1.08 kU/l respectively, in non-pregnant healthy ewes. Similarly, Koza et al. (2007) have reported that serum CAT activity in non-pregnant healthy Akkaraman lambs was 14.16 ± 0.99 kU/l. Our findings regarding serum GSH level (0.34 ± 0.01 nmol/ml) and CAT activity (16.45 ± 1.48 kU/l) are normal values for non-pregnant ewes, and these values show similarity with the aforementioned reports. Erisir et al. (2009) have reported that erythrocyte GSH level and GSH-Px activity significantly increase during pregnancy, and erythrocyte CAT activity significantly decreases after the first month of the pregnancy in Awassi ewes. Oztabak et al. (2005) have reported that while plasma GSH-Px activity was higher and plasma CAT activity was lower in pregnant Chios ewes on day 148 of pregnancy than in the non-pregnant ewes, plasma GSH-Px and CAT activities on day 105 of pregnancy were not different. In this study,
the serum GSH level and GSH-Px activity in pregnant ewes bearing twin foetuses were significantly lower than in non-pregnant ewes. Additionally, the GSH-Px activity in pregnant ewes bearing twin foetuses was significantly lower than in pregnant ewes bearing a single foetus. However, there was no significant difference in GSH level and GSH-Px activity between the non-pregnant and pregnant ewes with a single foetus. The reason of the discrepancy between our findings and previous reports may be because of breed differences or to the different type of samples used to measure the enzymatic activities (serum, plasma or erythrocytes). With respect to serum vitamins A and E levels, and CAT activity, no significant differences were found between the groups. Significant negative correlations were found between the foetal number (0, 1, 2) and both, GSH levels and the GSH-Px activity. The reason of the decreased antioxidants in pregnant ewes bearing twin foetuses in the first half of the pregnancy observed in this study may depend on two factors; first, it is possible that there is an insufficient increase in antioxidant defences to offset the increase in oxidative stress and lipid peroxidation in pregnant ewes bearing multiple foetuses. This may explain the reduced circulating levels of antioxidants. Second, maternal circulating lipid levels and plasma volume can influence the values of biomarkers as well. For example, increased maternal lipids potentially induce lipid peroxidation; expanded plasma volume may lower the concentration of antioxidant enzymes (Chen and Scholl 2005).

In conclusion, the results of this study show that normal gestation with single and twin foetuses causes enlargement in diameter of CL and increases maternal P concentration. Although pregnancy with a single foetus does not affect the oxidant/antioxidant balance, pregnancy with multiple foetuses causes oxidative stress by increasing serum lipid peroxidation levels and decreasing serum GSH level and GSH-Px activity. Therefore, powerful antioxidants may be administered to pregnant ewes bearing more than one foetus to protect both mother and foetuses against detrimental effects of oxidative stress.

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Conflict of interest
None of the authors have any conflict of interest to declare.

Author contributions
G Türk and E Demirci planned the study. G Türk, S Gür, M Sönmez, Ş Özer and EH Aksu collected the samples from abattoir. A Yüce made the analyses of lipid peroxidation and antioxidants in blood samples. G Türk also analysed the progesterone concentrations and wrote the first draft of the manuscript.

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Pregnancy Affects Maternal Oxidative Stress


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