The effect of vitamin E treatment during preovulatory period on reproductive performance of goats following estrous synchronization using intravaginal sponges

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ABSTRACT

The objectives of this study were to investigate whether the use of intravaginal sponge for estrous synchronization of goats causes oxidative stress, and to examine the effect of administering vitamin E during preovulatory period on reproductive performance of estrous synchronized goats. Estrus was synchronized in 36 non-lactating adult does using intravaginal sponges containing 30 mg of fluorogestane acetate (FGA) for 14 days. All females received 500 IU of eCG at the sponge withdrawal. The goats were allocated at random to two groups balanced for breed, age and body weight. Treatment group ($n = 18$) received 200 mg of vitamin E i.m. at the time of sponge removal and again at the time of second artificial insemination. The other 18 goats (control) were administered 1 ml of physiological saline instead of vitamin E on each of these two occasions. All does in estrus was intracervically inseminated at 12 and 24 h after the onset of estrus. Blood samples were collected every 72 h during the experimental period for evaluation of malondialdehyde (MDA) and vitamin E concentrations. Serum MDA level increased and vitamin E concentration decreased during the period of vaginal sponge application. Following the sponge removal, MDA level declined rapidly to below basal level in the treatment group but remained high in the control group.

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Conversely, vitamin E concentration increased in the treatment group after the sponge withdrawal and remained at a low level in the control group. No statistically significant differences ($P > 0.05$) were observed between groups in terms of estrous response, conception rate, gestation length or kidding rate. However, the number of multiple births (70.0% versus 50.0%) and prolificacy rate (2.40 ± 0.37 versus 1.63 ± 0.26 kids per kidding) were significantly higher ($P < 0.05$) for the treatment group than those of the control group. The results indicate that the use of intravaginal sponges for estrous synchronization of goats causes an increase in level of oxidative stress. However, the vitamin E treatment during preovulatory period can prevent the overproduction of reactive oxygen species (ROS), and it may improve the multiple birth rates and the number of kids born in estrous synchronized goats.

1. Introduction

Reactive oxygen species (ROS) are free radicals such as hydroxyl radical ($^\bullet$OH) and superoxide anion ($O_2^\bullet$) or molecules like hydrogen peroxide (H_2O_2). The effect of ROS on the reproductive potential in male is a subject of extensive research worldwide. However, there are limited reports about the possible effects of ROS in the female reproductive system (Agarwal et al., 2005). The production of ROS is a normal physiological event and, there is a delicate balance between ROS and antioxidants in the body. The certain levels of ROS may have a regulatory role for multiple reproductive processes such as oocyte maturation, steroidogenesis and fertilization. However, oxidative stress occurs when the balance is disrupted towards an overabundance of ROS. Lipid peroxidation is one of the most important expressions of oxidative stress induced by ROS. Malondialdehyde (MDA) is an indicator of the lipid peroxidation and, the amount of produced MDA was used as an index of the lipid peroxidation. The oxidative damage has been implicated in the cause of many diseases. Therefore, ROS can play an important role in pathophysiology of infertility and assisted fertility (Agarwal and Allamaneni, 2004).

Estrous synchronization has been successfully used for reproductive management in goats. In the past, estrous synchronization has focused to allow for optimal timing of milk production in dairy goats. However, the expanded popularity of meat goat production leads to increase interest in reliable methods to synchronize estrus in goats (Whitley and Jackson, 2004). The ultimate aims of any estrous synchronization method are to reduce the time used for estrous detection and, to provide an optimum litter size with a high survival to weaning (Kusina et al., 2000). The most widely used procedures for estrous synchronization are 12–14 days of treatment with fluorogestone acetate (FGA) impregnated vaginal sponges and intramuscular injection of equine chorionic gonadotropin (eCG) at the time of sponge removal (Ahmed et al., 1998; Amarantidis et al., 2004). However, the long-term application of this treatment has been associated with a lesser fertility (Larsson et al., 1991; Viñoles et al., 2001). The vaginal sponge can impede drainage of vaginal secretion, with the result that it has foul-smelling discharge on its removal (Motlomelo et al., 2002). In addition, the long-term application of vaginal sponge may cause infection and inflammation with adherence to vaginal mucosa (Larsson et al., 1991). There is a complex interrelationship between inflammation process and an increase in ROS level (Helmersson, 2005; Singh et al., 2005). Therefore, this application may lead to the oxidative stress. There is no report about relationship between the oxidative stress and the application of vaginal sponges.

Antioxidants are necessary to prevent some disorders in female reproduction (Fujii et al., 2005). Vitamin E, chain-breaking antioxidant, is one of the primary components of the antioxidant system. It is particularly important in protecting cells against oxidative damage by induced ROS (Chow, 1991). In addition, vitamin E is also important for oocyte quality and maturation in female reproduction (Tao et al., 2004).

There were two objectives in this study. The first objective was to investigate whether the use of intravaginal sponge for estrous synchronization of goats causes oxidative stress. The second objective

was to examine the effect of administering vitamin E during preovulatory period on reproductive performance of estrous synchronized goats.

2. Materials and methods

2.1. Animals and location

The present study was carried out between 15 October and 15 November, which is the period accepted as natural breeding season for goat in Elazig province of Turkey (located at latitude of 38°40′N, at longitude of 39°14′E and at an altitude of 1067 m). A total of 36 non-lactating adult does of Pure hair (n = 22) and Saanen (n = 14) breeds in good body score condition and weighting between 45 and 55 kg were used in this study. All goats were housed together in farm of Education, Research and Application at the Faculty of Veterinary Medicine, Fırat University in Elazig. They were allowed to graze at pasture of the farm throughout the day, and were kept indoors at night. When the goats were kept indoors, they were given 0.5 kg of concentrate and 1 kg of alfalfa hay per animal per day during the experimental period. The fresh drinking water and mineral salts were available *ad libitum*.

2.2. Treatment schedule

The experimental schedule is summarized in Fig. 1. At the beginning of the study, the females were allocated at random to two treatments balanced for breed, age and body weight. Hormonal treatments for induction of estrus were the same in both groups. Estrus was synchronized in all goats using the intravaginal sponges containing 30 mg FGA (Chrono-gest®, Intervet, Istanbul, Turkey). The intravaginal
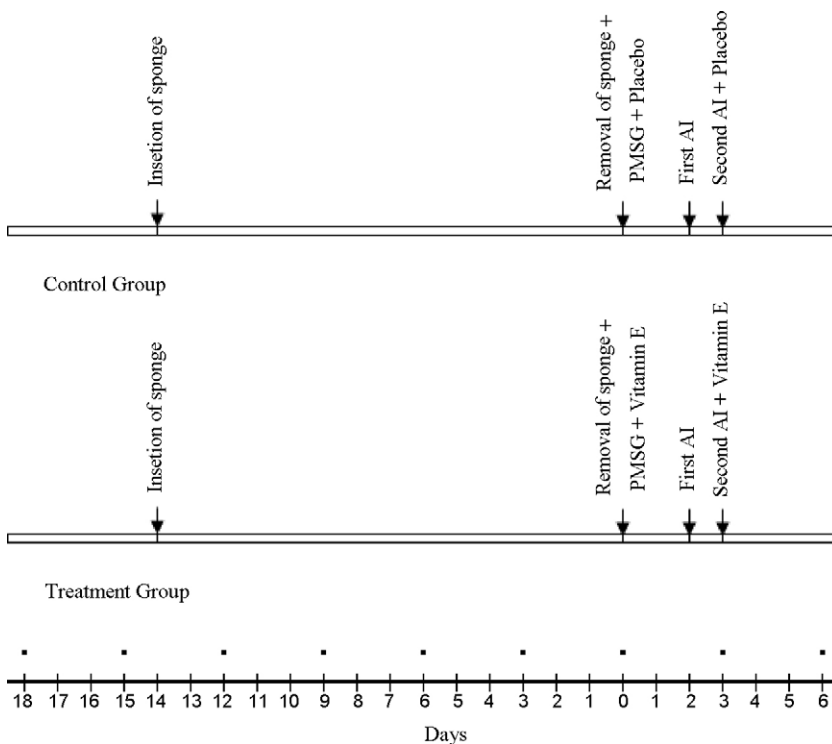

Fig. 1. Schematic representation of the experimental design used to study. (■) The days that blood samples were collected from all females.

sponges were inserted into vagina of each goat for 14 days. At the time of sponge withdrawal (day 0), all does were injected intramuscularly with 500 IU eCG (Chrono-gest®, Intervet, Istanbul, Turkey) for stimulation of estrus and ovulation. Treatment group ($n = 18$) received intramuscularly 200 mg vitamin E (DL-alpha tocopherol acetate, Evigen®, Aksu Farma, Istanbul, Turkey) at the time of sponge removal and again at the second artificial insemination (AI). The other goats ($n = 18$) served as control group and, they were administered 1 ml physiological saline instead of vitamin E on each of these two occasions.

2.2.1. Estrous detection and duration

The time of onset of estrus was detected every 6 h, from 12 to 60 h after the sponges removal by observing the reactions of females to a buck equipped with an apron to avoid penetration. The onset of estrus was considered as the time when the female stood to be mounted. Estrous duration was defined as the time elapsed between the first and last accepted mount within the same estrous period.

2.2.2. Semen collection, processing and insemination

Semen was collected with aid of an artificial vagina at 12-h intervals from three Saanen bucks (3–5 years of age and with a known fertility). The semen samples were protected from temperature shock during collection and examination. Each ejaculate was immediately determined volume and progressive motility (Chemineau et al., 1991). Only ejaculates of more than 0.5 ml and exhibiting a motility score of 4 (scoring 1–5) were used. Sperm concentration was determined using hemocytometer (Bearden et al., 2004). Then, the semen was diluted to a sperm concentration of 400×10^6 motile spermatozoa/ml at 35 °C in tris–fructose–citric acid–yolk (2%) diluent (Leboeuf et al., 2000). The diluted ejaculates were pooled in order to minimize the effect of semen variation and kept at 35 °C in a water bath until insemination. The pooled semen was used within 12 h after the collection. All does in estrus were intracervically inseminated with 0.25 ml of diluted semen containing 100×10^6 motile spermatozoa at 12 and 24 h after the onset of estrus using a speculum with an attached light source and specific insemination pipette.

2.2.3. Pregnancy diagnosis

All does were tested for pregnancy detection on day 25 after insemination using real time ultrasonography (Falco Vet, Pie Data Medical, Maastricht, The Netherlands) with 7.5 MHz linear array rectal transducer. They were confirmed by the occurrence of parturition.

2.2.4. Determination of fertility parameters

The following parameters were recorded in both treatments; estrous response (number of goats in estrus/number of treated goats × 100), estrous duration (the time elapsed from first to last mounting acceptances), interval to estrus (the time elapsed from sponge removal to onset of estrus), conception rate (number of pregnant goats/number of inseminated goats × 100), gestation length (the time elapsed from insemination to kidding), kidding rate (number of goats kidding/number of inseminated goats × 100) and prolificacy rate (number of kids born alive/number of goats kidding). All procedures were conducted in accordance with National Animal Care and Use Committee guidelines.

2.3. Biochemical analyses

Blood samples were collected from jugular vein into sterile tubes every 72 h during the experimental period. This procedure is shown in Fig. 1. The samples were kept for 4 h at room temperature. Then, they were centrifuged at $3000 \times g$ for 15 min. Serum was decanted and stored at −20 °C until assayed for assessment of MDA level and vitamin E concentration.

2.3.1. Vitamin E concentration

Vitamin E concentration (α-tocopherol) in the serum samples was determined by a modification of the method described by Desai (1984). Briefly, the serum samples were saponified by the addition of 0.3 ml of 60% (w/v in water) KOH and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70 °C for 30 min. After the samples were cooled on the dry ice, 2 ml of water and 1 ml of *n*-hexane were added and mixed with the samples and then, they rested to allow phase separation for 10 min.

Table 1
Estrus responses (mean ± S.E.M.) in goats synchronized for estrus using the intravaginal sponges and administered vitamin E (treatment group) or placebo (control group).

Group	n	Estrus response (%)	Estrus onset (h)	Estrus duration (h)
Treatment	18	100 (18/18)	31.0 ± 1.83	30.3 ± 1.64
Control	18	94.4 (17/18)	33.5 ± 1.71	27.9 ± 1.62

Calibration was performed using standard solutions of α-tocopherol in methanol. The absorbance of supernatant was measured at 330 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). Vitamin E concentration was expressed as mg/ml protein.

2.3.2. MDA level

MDA level was measured according to concentration of thiobarbituric acid reactive species (Okawa et al., 1979). Briefly, one volume of test sample and two volume of stock reagent (15%, w/v trichloroacetic acid in 0.25N HCl and 0.375%, w/v thiobarbituric acid in 0.25N HCl) were mixed in a centrifuge tube. The solution was heated for 15 min in boiling water. After cooling, the precipitate was removed by centrifugation at 2500 rpm for 10 min and then, the absorbance of the supernatant was measured at 532 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). MDA level was expressed as nmol/ml.

2.4. Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was performed using all tests for statistical significance at 95% confidence interval. The differences in MDA level, vitamin E concentration, the average interval (h) from sponge removal to onset of estrus, estrous duration, gestation length and prolificacy rate were compared between control and treatment groups using the Student's *t*-test. The proportions of does in estrus, conception rate and kidding rate were analyzed using Pearson Chi-square test. The SPSS/PC program (Version 10.0; SPSS, Chicago, IL) was used for all analyses.

3. Results

The proportion of goats in estrus, the average time elapsed from removal of sponges to onset of estrus and the estrous duration following estrous synchronization are shown in Table 1. The overall proportion of does exhibiting clinical signs of estrus between 12 and 60 h after the sponge removal was 97.2% (35/36). While all does receiving vitamin E came into estrus, one doe from control group did not show any overt signs of estrus during the observation period. There was no significant difference ($P > 0.05$) in estrous response between the two treatments.

The mean estrous duration was 30.3 ± 1.64 h for treated and 27.9 ± 1.62 h for control goats. The average time elapsed from sponges removal to onset of estrus were 31.0 ± 1.83 and 33.5 ± 1.71 h for the treatment and control groups, respectively. No significant difference was observed between groups regarding the interval to estrus and estrous duration.

The conception rate, kidding rate, gestation length, multiple birth rates and prolificacy rate are shown in Table 2. The overall conception rate was 62.9% (22/35). The gestation length was similar between groups and averaged 148.4 ± 0.5 day. The kidding rate was 55.6% (10/18) and 47.1% (8/17) for the treatment and control groups, respectively. The prolificacy rate was 2.40 ± 0.37 (24/10) and 1.63 ± 0.26 (13/8) for the treatment and control group, respectively. No statistically significant differences were observed between groups in terms of conception rate and kidding rate. However, the number of multiple births (70.0% versus 50.0%) and prolificacy rate were higher ($P < 0.05$) for the treatment group than those of control group.

The mean MDA level and vitamin E concentration for the treatment and control group throughout the experimental period are shown in Figs. 2 and 3, respectively. MDA level and vitamin E concentration in the treatment group were similar to the control group before the insertion of vaginal sponges. MDA

Table 2
Reproductive responses (mean ± S.E.M.) in goats synchronized for estrus using the intravaginal sponges and administered vitamin E (treatment group) or placebo (control group).

Variable	Group	
	Control	Treatment
Number of does inseminated	17	18
Pregnancy rate (%)	58.8 (10/17)	66.7 (12/18)
Kidding rate (%)	47.1 (8/17)	55.6 (10/18)
The number of kids	13	24
Singleton	4 (4)	3 (3)
Twin	3 (6)	2 (4)
Triplet	1 (3)	3 (9)
Quadruplet	–	2 (8)
Multiple birth rates (%)	50.0[a] (4/8)	70.0[b] (7/10)
Gestation length (day)	149.1 ± 0.69	147.8 ± 0.71
Prolificacy	1.63 ± 0.26[a]	2.40 ± 0.37[b]

The values within a same line with different superscripts (a, b) are significantly different ($P < 0.05$).
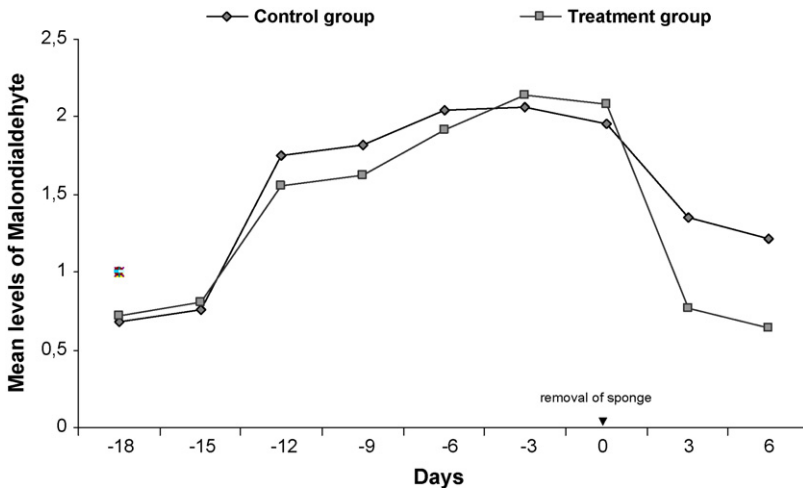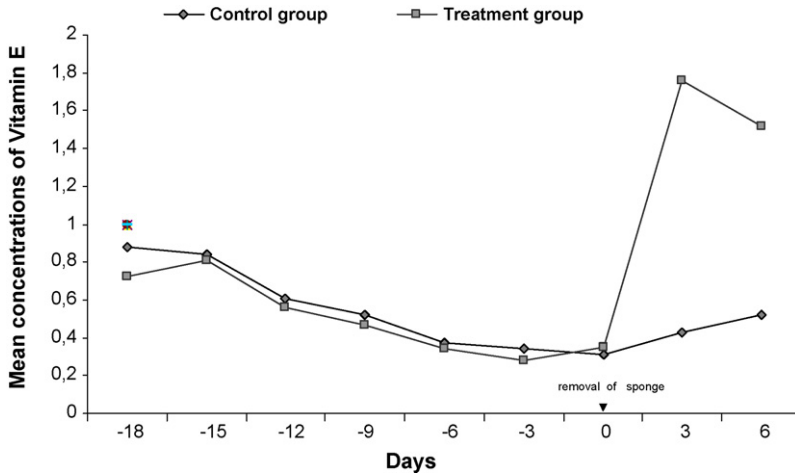

Fig. 2. Mean levels of malondialdehyte (MDA; nmol/ml) in goats synchronized for estrus using intravaginal sponges and administered vitamin E (treatment group) or placebo (control group).

level began to increase sharply within 1–4 days following the insertion of sponges. These high levels were maintained both groups until the sponge withdrawal, and it was arrived to highest level at the day of sponge withdrawal. Following the sponge removal, MDA level declined rapidly to below basal level in the treatment group but remained high in the control group.

Vitamin E concentration decreased gradually in both treatments during the time elapsed from insertion of sponges to removal of sponges. It increased in the treatment group after the sponge withdrawal. Conversely, it remained at low level in the control group during this period. A significant difference ($P < 0.05$) was observed at the time of second insemination between groups regarding vitamin E concentration.

4. Discussion

The use of progestagen–eCG-based estrous synchronization protocols can be effectively synchronized in goat, in especially, during breeding season (Romano, 2004). In this study, it was observed that this protocol was quite effective on induction of estrus. The mean estrous response was 97.2%. Estrus

Fig. 3. Mean concentrations of vitamin E (mg/ml protein) in goats synchronized for estrus using intravaginal sponges and administered vitamin E (treatment group) or placebo (control group).

was observed in 100% of does (n = 18) following the sponges removal in the treatment group. Similarly, all of the does except one doe in the control group were detected in estrus during the observation period. These results are comparable to the findings of Freitas et al. (1997) and Lehloenya et al. (2005).

An important requirement for successful estrous synchronization is uniformity of the time elapsed from the end of treatment to the onset of estrus. The overall average interval elapsed from sponge removal to onset of estrus was 32.2 ± 1.26 in this study (Table 1). The interval obtained in this trial was comparable to the findings of Romano (2004) and Lehloenya et al. (2005). However, this interval is longer than the 25.0 ± 1.56 h reported by Pierson et al. (2001), but shorten than the 52.3 ± 14.3 and 49.7 ± 15.7 h reported by Ahmed et al. (1998) and Fonseca et al. (2005), respectively. It may be affected many exogenous factors such as nutrition (Mani et al., 1992), use of eCG (Regueiro et al., 1999), presence of male after sponge removal (Romano, 1998). The estrous duration was similar between treatment and control groups, and the overall estrous duration was 29.1 ± 1.15 h in this study. This is in agreement with the findings of Motlomelo et al. (2002) and Fonseca et al. (2005). However, the present result was found to be much shorter when compared to those reported by Ahmed et al. (1998) and by Romano (1996). These differences might be due to breed (Lehloenya et al., 2005), present of male (Romano, 1998) and use of eCG (Ahmed et al., 1998).

It was observed that the vitamin E treatment did not showed any significant advantage with respect to the estrous response, the average elapsed time from sponge removal to onset of estrus and the estrous duration in this study. This may be explained that almost all of the goats had normal ovarian cyclic activity in breeding season.

The mean gestation length was 148.4 ± 0.5 days, and it was similar to those reported by Amarantidis et al. (2004) and Kusina et al. (2000) in different breeds of synchronized goats under different environmental conditions. The average overall conception rate and kidding rate in this trial is 62.9% and 51.4%, respectively, and there were no differences in pregnancy rate and kidding rate between treatment and control groups. These results were similar to the findings of Jackson et al. (2006) and by Motlomelo et al. (2002). However, these rates were lower when compared to those reported by Freitas et al. (1997) and by Greyling and van der Nest (2000). In goats, while some researchers have observed that the progestagen treatment decreased pregnancy and kidding rates, particularly in cycling animals (Jackson et al., 2006; Baril et al., 1993), others have found no differences (Greyling and van der Nest, 2000; Kusina et al., 2000). In the other hand, it is reported that there is positive relationship between optimum insemination time and high conception rate. The ovulations generally occur between 30 and 36 h after the onset of estrus in goats, and the females are artificially inseminated at 12 and 24 h after the estrous detection (Jainudeen et al., 2000). It was observed that the overall pregnancy rate was lower

than expected in this study although the does was inseminated at 12 and 24 h after the onset of estrus. The reason of this low conception rate could be due to the use of cervical insemination technique. The fertility may be markedly lower for cervical insemination than for intrauterine insemination (Gordon, 1997).

In this study, there was no difference in MDA level between treatment and control groups before the insertion of vaginal sponges. MDA level began to increase within 1–4 days following the insertion of vaginal sponges. This increment continued both groups until the sponge removal, and it was arrived to highest level at the day of sponge withdrawal. In normal conditions, the production of ROS is a normal physiological event in various organs including female reproductive tract and, the certain levels of ROS are necessary for several biological processes in the body (Agarwal et al., 2005). However, some physical, chemical or biological stressors can cause the overproduction of ROS. The increase in formation of ROS can be defined as oxidative stress. The application of vaginal sponge often causes infection and inflammation with adherence to vaginal mucosa (Larsson et al., 1991). The rich discharge with unpleasant odor was observed following the sponge withdrawal in this study. There is a complex interrelationship between oxidative stress and inflammation (Helmersson, 2005; Singh et al., 2005). Therefore, the reason of the increase in MDA level may be the vaginal inflammation by the application of vaginal sponge.

Vitamin E concentration decreased gradually both groups throughout the application of vaginal sponge in this study. While vitamin E concentration increased in the treatment group after the sponge removal, it remains to low levels in the control group. In the other hand, it was observed that while MDA level in the treatment group declined rapidly to below basal level after the vitamin E treatment, it remained high level in the control group. Vitamin E is an essential vitamin for mammalian reproduction. It functions not only as cellular antioxidant, but also as modulators of many intracellular or extracellular biochemical processes. Therefore, vitamin E is important in restoring and maintaining the oxidant–antioxidant balance in the body. It is the primary free radical scavenger and, it protects cells from damage of ROS (Chow, 1991).

Jozwik et al. (1999) reported that ROS have been localized in the follicular fluid before ovulation. The preovulatory follicle has a potent antioxidant defense. Therefore, the level of ROS was lower in the follicular fluid to compare with the serum level. However, the increase in plasma level of ROS may affect the level of ROS in the preovulatory follicle fluid. The follicular fluid has a crucial role in determining the quality of oocyte. This in turn impacts fertilization rate and embryo quality. Agarwal et al. (2005) suggested that the oxidative stress influences oocyte quality and fertilization rate. The oocyte quality is a very important factor for a successful fertilization. Poor quality oocytes may be associated with increase in generation of ROS in the follicular fluid. The oxidative stress can damage oocytes in developing follicles. The fertilized morphologically poor-grade oocytes may result in fertilization failure or poor embryo quality. Das et al. (2006) suggested that the high level of ROS in the follicular fluid may inhibit embryo formation or fertilization. Similarly, Seino et al. (2002) reported that the increase in level of ROS in the follicular fluid lowered fertilization rates and subsequently led to a decrease in quality of embryos. Therefore, there is a negative correlation between fertilization rate and level of ROS.

Vitamin E is also present in the preovulatory follicle. The plasma vitamin E concentration may affect its follicular concentration, and it was found to be significantly higher in the follicular fluid than in the plasma. Therefore, the increase in vitamin E concentration causes to reduce level of ROS in the follicular fluid (Agarwal et al., 2005). Vitamin E is important for oocyte quality and maturation, implantation and fetal growth in female reproduction. It protects denuded oocytes from degeneration and facilitates meiotic maturation of cumulus-free oocytes, especially from MI to MII (Tao et al., 2004). Therefore, the vitamin E treatment may increase fertilization rate of oocytes. In addition, vitamin E decreases the portion of follicles undergoing follicular atresia by counteracting potential generation of oxidative stress in oocytes. In this study, the vitamin E treatment during preovulatory period increased multiple birth rates and the mean number of kids born per doe in the treatment group in comparison to those of the control group. This effect may be associated with increase in fertilization rate of viable oocytes. Vitamin E has a significant role in maturation and development of ovum, and the insufficiency of oocyte development and maturation may cause higher the number of fetal resorption during early embryonic period.

5. Conclusion

The results indicate that the use of intravaginal sponges for estrous synchronization of goats causes an increase in the level of oxidative stress. However, the vitamin E treatment during preovulatory period can prevent the overproduction of ROS, and it may improve the multiple birth rates and the number of kids born in estrous synchronized goats.

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