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## ARAŞTIRMA

F.Ü.Sağ.Bil.Vet.Derg.  
2015; 29 (3): 183 - 186  
http://www.fusabil.org

### Effect of Injection of Vitamin E and Selenium Administered Immediately Before the Ovsynch Synchronization on Conception Rate, Antioxidant Activity and Progesterone Levels in Dairy Cows

The objective of this study was to investigate the effect of a single subcutaneous injection of vitamin E and selenium administered immediately before ovsynch on conception rates, antioxidant and plasma progesterone levels of lactating dairy cows. Twenty four healthy cows were randomly distributed into the experimental group (group E, n=14) or the control group (group C, n=10) and synchronized with the ovsynch protocol. In group E, cows received a single subcutaneous injection of 8 mL Esegal (60 mg/mL vitamin E and 1 mg/mL sodium selenite) immediately before ovsynch. In group C, cows did not receive any injection. Blood samples were taken immediately before application and on day 14 after artificial insemination (AI). Pregnancy detection was performed on day 60 after AI. There were no significant differences in conception rates between the groups. The mean erythrocyte GSH-Px and SOD activities in group E on day 14 after AI were higher than group C and their own activities prior to application. The progesterone levels increased in group E compared to group C on day 14 after AI. In conclusion, a single subcutaneous injection of vitamin E and selenium immediately before ovsynch had no a significant effect on conception rates of lactating dairy cows. However, this application had a beneficial effect on erythrocyte GSH-Px and SOD activities and plasma progesterone levels.

**Key Words:** Conception, glutathione peroxidase, estrus synchronization, superoxide dismutase

#### Ovsynch Senkronizasyonundan Hemen Önce Vitamin E ve Selenyum Enjeksiyonunun Sütçü İneklerde Gebe Kalma Oranı, Antioksidant Aktivite ve Progesteron Düzeyleri Üzerine Etkisi

Bu çalışmanın amacı, ovsynch senkronizasyonundan hemen önce tek doz derialtı uygulanan vitamin E ve selenyumun, laktasyondaki sütçü ineklerin gebe kalma oranları, antioksidan aktiviteleri ve plazma progesteron düzeyleri üzerine etkisini araştırmaktır. Yirmi dört sağlıklı inek rastgele deney (grup E, n=14) ve kontrol (grup C, n=10) grubuna ayrıldı ve ovsynch protokolüyle senkronize edildi. Grup E'de ineklere ovsynch senkronizasyonundan hemen önce tek doz 8 mL Esegal (60 mg/mL vitamin E and 1 mg/mL sodium selenite) derialtı yolla verildi. Grup C'deki ineklere uygulama yapılmadı. Her iki gruptaki ineklerden tohumlamadan hemen önce ve 14 gün sonra kan örnekleri alındı. Gebelik teşhisi tohumlamadan 60 gün sonra yapıldı. Gruplar arasında gebe kalma oranlarında önemli farklılık bulunmadı. Tohumlamadan sonraki 14. günde grup E'de ortalama glutathione peroxidase (GSH-Px) ve superoxide dismutase (SOD) düzeylerinin grup C'dekine göre ve uygulama öncesi kendi düzeylerinden daha yüksek olduğu (P<0.05) tespit edildi. Progesteron düzeyleri tohumlamadan sonraki 14. günde grup C ile karşılaştırıldığında grup E'de arttığı belirlendi. Sonuç olarak, ovsynch'ten hemen önce tek doz derialtı uygulanan vitamin E ve selenyumun gebe kalma oranı üzerinde etkili olmadığı görüldü. Bununla birlikte, uygulamanın eritrosit GSH-Px ve SOD aktiviteleri ve plazma progesteron düzeyleri üzerine olumlu bir etkisi tespit edildi.

**Anahtar Kelimeler:** Konsepsiyon, glutatyon peroksidaz, östrus senkronizasyonu, süperoksid dismutaz

Geliş Tarihi : 09.07.2015  
Kabul Tarihi : 14.08.2015

#### Introduction

Oxidative stress is one of the major causes of the decrease in viability and development of embryo (1). Embryo is highly sensitive to injury to oxidant molecules because of its low antioxidant capacity (2). In the various researchs, studies focused on studying antioxidant strategies to prevent oxidative stress induced damage to gametes and developing embryos (3). It is known that vitamin E (vit E) and selenium (Se) are powerful free-radical scavenger and antioxidants. Vit E prevents the oxidative chain from proliferating prior to the alteration of neighboring molecules by being oxidized into harmless compounds (4, 5). Selenium is important in inhibiting nitric oxide production in cells (6) and is an essential element of several enzyme systems, including glutathione peroxidase (GSH-Px) and deiodinase (7). These enzymes have been shown to be important in embryonic development, through their antioxidant functions and regulatory effects on metabolic rate (8). In addition to, plasma GSH-Px activity is assumed to be indicative of oxidative stress (9). Vit E and Se are capable of improving the

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\* International Mesopotamia Agriculture Congress, 22-25 September 2014, Diyarbakır/TURKEY.

developmental capacity of bovine embryos in vitro (10, 11). Vit E and Se may suppress these oxidants. Several studies have shown that administration of Se, vit E or vit E and Se in combination resulted in higher pregnancy rates (12-14). However, other studies have shown little or no beneficial effect of administration of vit E and/or Se (15-17). Oxidative stress and maternal low blood progesterone levels during early embryonic development are a major cause of decreased pregnancy rate in dairy cows. Vierk et al. (18) suggested that luteolysis of the corpus luteum is due to accumulation of oxidative species. Circulating progesterone concentrations are critical for maintenance of pregnancy. It has been suggested that some of early pregnancy losses in livestock may be due to insufficient circulating progesterone concentrations (19). Hence, use of appropriate antioxidants can help to improve conception rate by preventing accumulation of oxidative species during early embryonic development in dairy cows. However, whether vit E and Se possess similar benefits on the conception rate in cows has yet to be determined.

The objective of this study was to investigate the effect of a single subcutaneous injection of vit E and Se administered just before ovsynch on erythrocyte GSH-Px and superoxide dismutase (SOD) activities and plasma progesterone levels and pregnancy rates of lactating dairy cows.

## Materials and Methods

The study was carried out in a conventional dairy farm located in Elazığ (Eastern Turkey). A total of 24 healthy multiparous Holstein cows, aged 4-7 years, and with mean body condition score of  $2.74 \pm 0.17$  (on scale 1 to 5) (20) were used in this study in the period of February–April. The cows were in 55–70 days postpartum. They were all examined by rectal palpation and vaginoscopic examination to evaluate the normality of their reproductive tract. They had not any disorders in their reproductive tracts. The cows were fed a total mixed ration (TMR) of corn silage and grains that was balanced according to nutritional requirements based on milk production, and housed in a tie-stall confinement facility. Ovsynch program was performed with 2 injections of GnRH (10 µg Busereline acetate, Receptal®, Intervet Istanbul, Turkey), 7 d before and 48 h after an injection of PGF2 alfa (25 mg, Dinoprost tromethamine, Dinolytic®, Etkin, Istanbul, Turkey). The cows were divided randomly into two groups. The experimental

group (group E) (n= 14) was administered a single subcutaneous injection of 8 mL Esegal per animal (Esegal inj.: 60 mg vitamin E and 1 mg sodium selenite in 1 mL, Galenka) just before the first GnRH injection and the control group (group C) was not received vit E and Se (n = 10). Cows were artificially inseminated 16 h after the second injection of Gonadotrophin releasing hormone (GnRH). Pregnancy was determined by palpation per rectum at 60 d after AI.

Blood samples were taken from jugular vein into tubes containing EDTA immediately before treatment and on d 14 after AI for cows per group, to monitor erythrocyte GSH-Px, SOD activities and plasma progesterone levels.

Erythrocyte GSH-Px enzyme activity was determined using the method previously described in Paglia and Valentine, (21) and was expressed as Units/gram of Hemoglobin (U/g Hb).

Erythrocyte SOD activity was measured at 560 nm and was expressed as U/gHb (22).

Plasma progesterone level was quantified by a commercial solid-phase RIA kits (Coat-a-count; Diagnostic Products Corp., Los Angeles, CA, USA) (23). The assay sensitivity was 0.05 ng/mL. The intra- and inter- assay coefficients of variation were 7.6 and 7.9% respectively.

The pregnancy rate was compared between groups by Fisher's exact test. Progesterone levels, GSH-Px and SOD activities means between groups were tested using student T test, whereas pre and post-treatment means of GSH-Px and SOD activities were compared using paired t test. Mean values are presented as mean±standard deviation (SD). Differences were considered as significant at  $P < 0.05$ .

## Results

The conception rate for first insemination was 42.9% (6 to 14) in group E, and 30.0% (3 to 10) in group C. Conception rate was proportionately higher in cows of treatment group, compared to cows in control group. However, this increase was not statistically significant at the 5% level ( $P > 0.05$ ). The mean GSH-Px and SOD activities of group E were similar to group C immediately before vit E and Se treatment (Table I).

**Table 1.** The mean levels and standard deviations (±SD) of GSH-Px, SOD and Progesterone for group E and group C

Parameters	Time	Group C (n=10)	Group E (n=14)	P values
GSH-Px (U/gHg)	BA	25.50 ± 0.54	25.86 ± 0.44	0.874
	AA	26.30 ± 0.56	40.10 ± 0.96	0.032
	P values	0.756	0.028	
SOD (U/gHg)	BA	582.67 ± 26.74	614.14 ± 23.40	0.456
	AA	656.34 ± 21.62	852.25 ± 29.34	0.044
	P values	0.546	0.036	
Progesterone (g/mL)	AA	3.83 ± 0.06	4.02 ± 0.04	0.048

BA: Before application, AA: After application

On day 14 after AI, the GSH-Px and SOD activities were higher in group E ( $P<0.05$ ) than group C. On day 14 after AI, GSH-Px and SOD activities in group C were higher than the initial activities but this increase was not considerable ( $P>0.05$ ). Whereas, the differences for GSH-Px and SOD activities between immediately before application and on day 14 after AI in the in group E were statistically significant ( $P<0.05$ ). The progesterone levels increased significantly ( $P<0.05$ ) in group E when compared with group C on day 14 after AI.

## Discussion

The effects of Se and vit E supplementation on conception rates in cattle have been examined in feeding trials, but the effect of Se and vit E on conception rate remains controversial. Many studies have reported to demonstrate a significant difference in conception rates between treatment groups following Se or Se plus vit E supplementation (13, 24), but this finding was not consistent (25, 26). In the present study, although conception rate for group E were observed to be proportionately higher than group C, this was not considerable ( $P>0.05$ ). A similar lack of effect on conception rates was also found by Paula-Lopes et al. (26), Aréchiga et al. (27) and Awadeh et al. (28). However, this is in contrast with the results of Castro et al. (13) and Zanella et al. (14). The variation in results may all contribute to different study designs and methods, conditions and management in farms, change of diet, breeding of animals, stress factors. Several possible reasons may explain why vit E and Se injection in this study did not improve conception rate in cows. First, the period in which cows injected vit E and Se may not be suitable to prevent effects of oxidative stress during early embryonic development. Also, more complex antioxidant therapies may be necessary to achieve beneficial effects. Finally, antioxidants did not totally prevent effects of reactive oxygen species on embryonic survival.

On day 14 after AI, the mean GSH-Px activity in treated group were higher ( $P<0.05$ ) when compared to control group. The same variations were observed in mean GSH-Px activity of group E on day 14 after AI in

comparison with the initial level, and these changes were considerable ( $P<0.05$ ). The mean GSH-Px and SOD activities in group C on day 14 after AI was higher than the initial level, but this increase was not considerable ( $P>0.05$ ). Thus, this result indicated that vit E and Se injection can responsible for an increase in antioxidant activity. Similar to our results, a significant increase in GSH-Px activity due to Se supplementation was reported in the erythrocytes of cows (29, 30). GSH-Px has been shown to be important in embryonic development, through their antioxidant functions and regulatory effects on metabolic rate (8). In the present study, however, pregnancy rates were not affected by increased GSH-Px and SOD activities.

In the present study, vit E and Se injection raised plasma progesterone levels. Some antioxidant systems actively work in the corpus luteum during pregnancy (31). It is known that vit E and Se is also an antioxidant, and could play a role in the antioxidant system in the corpus luteum (32). It is possible that Se degrades  $H_2O_2$  or peroxides in the corpus luteum as a component of GSH-Px (33) or phospholipid hydroperoxide glutathione peroxidase (34). This would support its hormone production activity. Kamada and Hodate (32) have reported the positive effects of Se on plasma progesterone concentration in the progesterone production of luteal cells.

In conclusion, a single subcutaneous injection of vitamin E and selenium immediately before ovsynch may not have a significant effect on conception rates of lactating dairy cows. However, this application had a beneficial effect on antioxidant activity and plasma progesterone levels. Vit E and Se could induce increased progesterone secretion by the corpus luteum which is necessary for successful pregnancy by playing a role in the antioxidant system in the corpus luteum. Treatment with vitamin E and Se proportionally increased conception rates of cows in this study. Lack of difference in conception rates in this study was likely due to the use of limited numbers of animals. Therefore, the results highlight the need for additional studies to investigate the beneficial effects of vitamin E and Se on conception rates.

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