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Research Article

# Evaluation of Certain Oxidative Stress Parameters in Heifers that were Administered Short Term PRID

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## Abstract

The purpose of this study was to investigate the effect of short term administration of PRID on the levels of serum progesterone, nitric oxide (NO), malondialdehyde (MDA), total antioxidant capacity (TAC) and total oxidant capacity (TOC) in heifers. This purpose, 200 heifers were synchronized with the Cosynch-72 protocol in which progesterone was administered for 5 days. Blood was taken from the heifers 10 days prior to starting synchronization (day -10), the day the PRID was attached (day 0), the day the PRID was removed (day 5) and on the day of insemination (day 8). Blood taken on day -10 and day 0 were accepted as control values for the study. Serum progesterone, NO, MDA, TAC and TOC were measured on days 5 and 8. The difference in serum NO and MDA levels was found to be statistically significant on day 5 of the study ($P<0.001$). It was determined that the procedure caused a drop in the TAC level, while the TOC level was not affected. In conclusion, it was determined that short-term administration of PRID in heifers caused an increase in serum NO and MDA levels, a decrease in TAC level and had no effect on the TOC level.

***Keywords:*** *Heifer, Nitric oxide, Oxidative Stress, PRID*

# Kısa Süreli PRID Uygulanan Düvelerde Bazı Oksidatif Stres Parametrelerinin Değerlendirilmesi

## Özet

Bu çalışmada, düvelere kısa süreli PRID uygulamasının; serum progesteron, nitrik oksit (NO), malondialdehit (MDA), total antioksidant (TAK) ve oksidant kapasitesi (TOK) düzeyleri üzerine etkisinin araştırılması amaçlanmıştır. Bu amaçla, 200 adet düve 5 gün progesteron uygulanan Cosynch-72 protokolüyle senkronize edildi. Düvelerden senkronizasyona başlamadan 10 gün önce (-10. gün), PRID takılma günü (0. gün), PRID çıkarılma günü (5. gün) ve tohumlama günü (8. gün) kan alındı. Çalışmada -10 ve 0. gün alınan kanlar kontrol olarak değerlendirildi. Beş ve 8. günlerde serum progesteron, NO, MDA, TAK ve TOK değerleri araştırıldı. Çalışmada 5. gün serum NO ve MDA düzeyleri diğer günlerden istatistiksel olarak farklı olduğu tespit edildi ($P<0,001$). Uygulamanın TAK düzeyinde düşmeye neden olduğu, TOK değerini ise etkilemediği belirlendi. Sonuç olarak, düvelere kısa süreli PRID uygulamasının serum NO ve MDA düzeylerinde artışa neden olduğu, TAK düzeyinde düşmeye ve TOK değerini ise etkilemediği belirlendi.

***Anahtar sözcükler:*** *Düve, Nitrik oksit, Oksidatif stres, PRID*

## INTRODUCTION

Oxidative stress is a new and pertinent issue in the field of veterinary medicine. Reactive oxygen metabolites (ROM) are known to be the free radicals of an organism. The body consists of about 1-2% ROM, which play a role in many body activities [1]. These metabolites are found in low concentrations and play a role in functions such as protein phosphorylation, cell maturation, apoptosis, oocyte maturation, steroidogenesis, cell immunity, ovulation, implantation, blastocyst formation, luteolysis, acrosome reactions, fertilization and luteal maintenance during pregnancy [1-4]. However, when ROM is produced in large amounts in an organism, it causes damage to lipids, protein and DNA in the cell, resulting in the loss of cell functions. Disturbance of the balance between ROM and the anti-oxidant systems that eliminate these substances (such as superoxide dismutase and glutathione peroxidase) causes oxidative stress (OS) [1,2,5].

A high level of free radicals reacts with fatty acids of the cell membrane and creates peroxidation products.

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In this process, the fatty acid on the cell membrane loses a hydrogen molecule, which increases the level of malondialdehyde (MDA) in the environment. Measuring the MDA level provides information about lipid peroxidation [6,7]. Peroxynitrites develop after free radicals cause damage to the fatty acids on the cell membrane. Nitric oxide (NO) plays a role in many of the body's physiological functions and is produced by nitric oxide synthesis. NO is mostly produced by macrophage, neutrophil and mast cells [2,8,9].

Studies performed on cattle have reported that OS factors increase when the animals become sick [9-11]. Other studies have shown that the level of OS factors increases in cases of retentio secundinarum, mastitis, subclinical mastitis, udder edema, anestrus, repeat breeders, follicular cysts and metabolic diseases [1,7,12-15].

Many different synchronization protocols are used to control reproduction in cattle. Synchronization methods include the use of progesterone-releasing intravaginal devices (PRID or CIDR), ear implants and feed additives [16-18]. It is known that the removal of progesterone-releasing intravaginal devices irritates the tissue, causing vaginitis, which in turn causes the animal to undergo stress [19-21]. For example, Aksu et al.[22] conducted a study on cows in which the use of CIDR in the synchronization protocol resulted in higher MDA levels than in those cows for which the devices were not used. Other studies conducted on cattle found that TAC and TOC levels were higher during the period of the cycle which had high progesterone levels than they were during the estrus period [3,23]. It has also been reported that estrogen and progesterone can affect OS parameters in the body [2].

The purpose of this study was to determine the effect of short-term administration of PRID on the levels of serum progesterone, NO, MDA, TAC and TOC in heifers.

## MATERIAL and METHODS

This study was conducted after obtaining approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ HADYEK - Submission: 2014/04-Decision no: 040). The study material consisted of 200 clinically healthy Holstein heifers age 14-16 months and weighing 350-420 kg.

The heifers were synchronized with the method described by Colazo and Ambrose [24], using the Cosynch-72 protocol with short-term progesterone (5 days) administered via PRID. For all the heifers, blood was taken from the *Vena coccygea* into evacuated 8.5 ml gel tubes without an anticoagulant (BD Vakutainer®, Tıpkimsan, Turkey) 10 days prior to the synchronization, on the day the PRID was attached (day 0), on the day the PRID was removed (day 5) and on the day of artificial insemination (day 8). The samples were centrifuged at 4.000 RPM for 10 min, after which they were stored at -18°C until the analysis was performed.

Serum nitric oxide concentration was measured according to the method reported by Miranda et al.[25] and serum MDA levels were measured according to the method reported by Yoshioka et al.[26].

A colorimetric assay (PowerWave XS, Biotek, Instruments, USA) was performed on serum total antioxidant and oxidant capacity using a commercial kit (Rel Assay Diagnostic®, Turkey).

Progesterone levels in blood taken from the heifers was measured with an Enzyme-Linked Immunosorbent Assay (ELISA) reader (Epoch®, Biotek, USA) using ELISA commercial kits (EIA 1561®, DRG International, Germany).

Statistical evaluation of the results was performed using SPSS® (SPSS 20, IL, USA). One-Way ANOVA was used to evaluate statistical differences between the groups. The results were analyzed as X±SE. $P<0.05$ was considered to be statistically significant.

## RESULTS

On the day of the synchronization protocol in which the PRID was removed (day 5), it was noted that vaginitis had developed in all heifers and when the device was removed, there was a purulent discharge from between the vulva lips.

Analysis showed that the serum NO level varied depending on the time of administration *(Fig. 1)*. Although no statistically significant difference was found between samples taken 10 days prior to administering synchronization and on day 0, a significant difference was found between all of the days in which the procedure was administered ($P<0.001$).

No statistically significant difference was found in the MDA levels between the control samples (days -10 and 0) and the serum samples taken during the days in which synchronization was administered. A statistically significant difference was found between all days in which the procedure was administered (*Fig. 2*; $P<0.001$).

Analysis of the changes in total antioxidant capacity during the administration of the procedure revealed that the level dropped up until day 5 after the PRID was administered and increased from day 5 through day 8, with a statistically significant difference between these levels and the other days (*Fig. 3*; $P<0.001$). No statistically significant difference was found between the groups after measuring total oxidant capacity (*Fig. 4*; $P>0.05$).

Measurements of serum progesterone levels performed during the synchronization were found to be similar to those conducted on days -10 and 0 ($P>0.05$), although

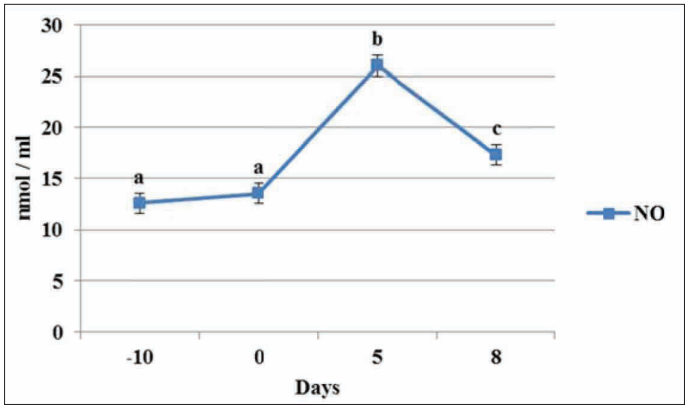
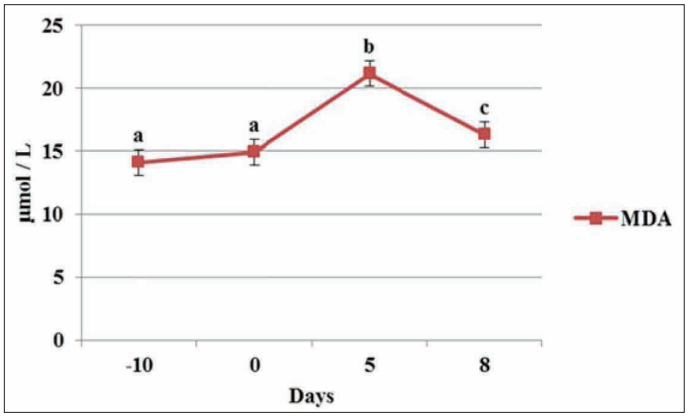



**Fig 1.** Serum NO levels according to the days of synchronization (P<0.001)

**Şekil 1.** Senkronizasyon günlerine göre serum NO düzeyleri (P<0.001)

**Fig 2.** Serum MDA levels according to the days of synchronization (P<0.001)

**Şekil 2.** Senkronizasyon günlerine göre serum MDA düzeyleri (P<0.001)



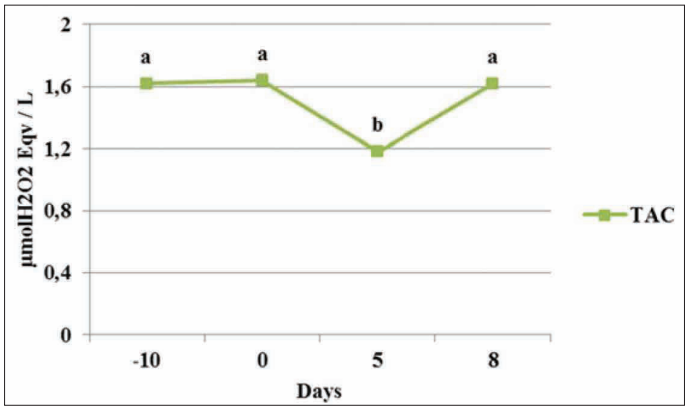
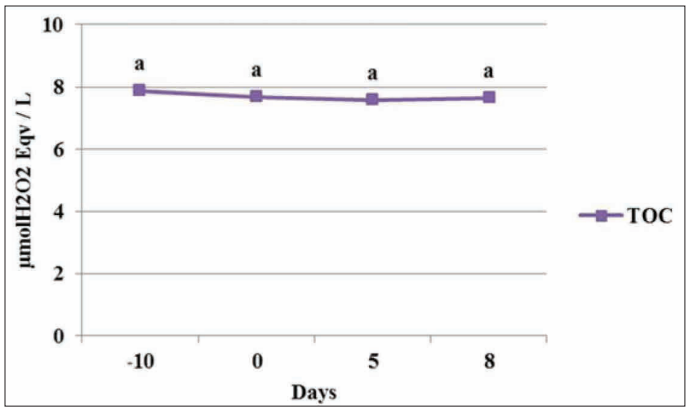




**Fig 3.** Serum TAC levels according to the days of synchronization (P<0.001)

**Şekil 3.** Senkronizasyon günlerine göre serum TAK düzeyleri (P<0.001)

**Fig 4.** Serum TOC levels according to the days of synchronization (P>0.05)

**Şekil 4.** Senkronizasyon günlerine göre serum TOK düzeyleri (P>0.05)




the highest level was found to be on day 5 and the lowest level was on day 8 *(Fig. 5)*. Serum NO, MDA, TAC, TOC and progesterone levels have been shown in *Table 1* according to the days of synchronization.

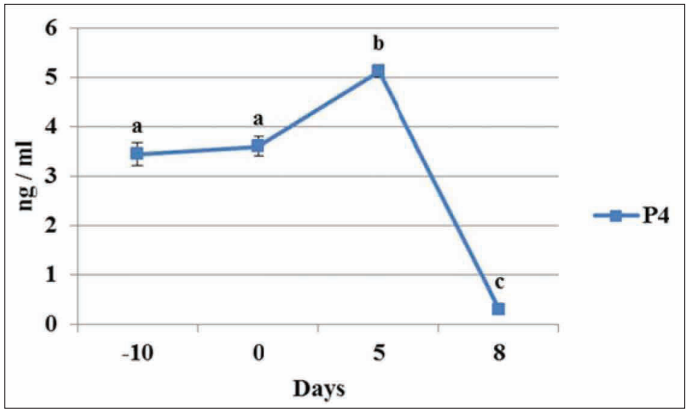



**Fig 5.** Serum P4 (progesterone) levels according to the days of synchronization (P<0.001)

**Şekil 5.** Senkronizasyon günlerine göre serum P4 (progesteron) değerleri (P<0.001)

***Table 1.*** *Changes in NO, MDA, TAC, TOC and progesterone according to days*
***Tablo 1.*** *Günlere göre NO, MDA, TAK, TOK ve progesteron değişimleri*

| Days | NO (nmol/ml) | MDA (µmol/l) | TAC (mmol Trolox Eqv/L) | TOC (µmol$H_2O_2$ Eqv/L) | Progesterone (ng/ml) |
|---|---|---|---|---|---|
| -10 | 12.59± 020$^a$ | 14.10±0.21$^a$ | 1.62±0.01$^a$ | 7.89±0.08 | 3.44±0.23$^a$ |
| 0 | 13.52±0.23$^a$ | 14.93±0.25$^a$ | 1.64±0.01$^a$ | 7.68±0.10 | 3.60±0.19$^a$ |
| 5 | 26.02±0.33$^b$ | 21.16±0.28$^b$ | 1.18±0.02$^b$ | 7.59±0.11 | 5.12±0.13$^b$ |
| 8 | 17.30±0.25$^c$ | 16.31±0.27$^c$ | 1.62±0.01$^a$ | 7.65±0.09 | 0.30±0.02$^c$ |
| P | P<0.001 | P<0.001 | P<0.001 | P=0.172 | P<0.001 |

# DISCUSSION

A number of different progesterone-based synchronization protocols have been developed for cattle. Some studies have reported that progesterone-release intravaginal devices frequently cause vaginitis and cause the animal stress [20,21]. The present study also studied the degree to which progesterone releasing devices affect OS factors in animals.

Studies in the field of veterinary medicine have shown that OS factors increase when certain diseases occur [1]. In a study conducted by Ergönül and Kontaş Aşkar [8], the authors had the goal of identifying MDA levels in cattle with Anaplasmosis and in healthy cattle (control group). At the conclusion of the study, they found that MDA levels averaged 15.23 µmol/L in the control group. They reported that this level was much higher in diseased animals. In our study, we found that MDA levels were 14.10 and 14.93 µmol/L in the control samples (days -10 and 0). The results of our study were similar to that of the aforementioned study. Aksu et al.[22] conducted a study in which they administered CIDR to a group of cows for the purpose of synchronization. They found that there was a change in serum MDA levels on the day the CIDR was removed and on the day of insemination. They identified a statistically significant difference in MDA levels between the first blood sample and the second sample. The authors reported that the MDA level was higher on the day the CIDR was removed than on the day of insemination and that the progesterone-release intravaginal device caused stress, resulting in an increase in the MDA levels. Similarly, our study also found that MDA levels were at the highest on the day the PRID was removed but lower on the day of insemination. MDA level is thought to increased due to the stress that occurs after PRID application.

A number of studies have been conducted to measure NO levels in order to analyze oxidative stress. A study conducted by Bozukluhan et al.[9] found that serum NO levels in healthy animals (control group) were lower than those of diseased animals. In our study, the PRID was administered to healthy animals and because the device caused stress to the animal, we found that NO levels rose after the procedure. Agarwal et al.[2] reported that a low level of nitric oxide is important for ovarian activity and implantation. Furthermore, they reported that a high level of NO has a harmful effect on motile sperm, that it can have a toxic effect on the embryo and that it can prevent implantation. In our study, we found that the NO level increased when the PRID was administered and that it decreased notably from day 5 to day 8 of the protocol. Nitric oxide rising by PRID application, it was determined that fell to normal levels at the artificial insemination day. Declining in the value of nitric oxide will not adversely affect fertility parameters and in synchronization protocols concluded that PRID application can be used in a safe manner.

Aydilek et al.[3] conducted a study in which they attempted to measure TAC and TOC levels at various points of the reproductive cycle in cows. The study identified an increase in both factors during the estrus period. On the other hand, it was determined that TAC and TOC levels were lower during the times when the progesterone level

was high. In our study, we found that the TAC level was at its lowest at the end of the administration of progesterone (the day the PRID was removed) (P<0.001), while there was no change in the TOC level (P>0.05). In the present study, it is believed that after the PRID application which forms stress causes a decrease on the levels of TAC. Talukder et al.[23] reported that some OS parameters decreased together with progesterone from the time the CIDR was removed until ovulation. In our study, we also observed a drop in the level of progesterone, MDA and NO after the PRID was removed.

In conclusion, it was determined that short-term administration of PRID in heifers caused an increase in serum progesterone, NO and MDA levels, a decrease in the TAC level and had no effect on the TOC level.

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