

# OXIDATIVE ANTIOXIDANT STATUS DURING TRANSITION FROM LATE PREGNANCY TO EARLY LACTATION IN NATIVE AND CROSS BRED COWS IN THE EGYPTIAN OASIS

(With 6 Tables and One Figure)

By

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حالة الأوكسدة ومضادات التأكسد أثناء الانتقال من نهاية الحمل إلى بداية  
الإدرار في الأبقار البلدي والخليط في الواحات المصرية

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تعطي دلالات الإجهاد التأكسدي معلومات متممة عن الاتزان الداخلي للحيوان قياسا بمؤشرات الأيض التقليدية الأخرى. واستهدفت هذه الدراسة تقييم التداخل بين الإجهاد التأكسدي وحالة مضادات التأكسد المصلحية له أثناء الانتقال من نهاية الحمل إلى بداية الإدرار في الأبقار البلدي والخليط وعلاقته بالبيئة المحلية في الواحات المصرية. تم إجراء هذه الدراسة على ٢٢ بقرة عشر منها ١٢ بقرة محلي (بلدي) و ١٠ خليط (بلدي) X فريزيان). تم جمع عينات دم من هذه الأبقار أثناء منتصف العشر (الشهر الرابع أو الخامس) ثم أسبوعيا خلال الأسابيع الثلاثة الأخيرة من العشر وأثناء الولادة ثم أسبوعيا خلال الثلاثة أسابيع الأولى بعد الولادة. وقد أوضحت النتائج أن المتوسط العام لمجموع تركيز البلازما من كل من الألفاتوكوفيرول والبيتاكاروتين وحمض الأسكوربيك بالإضافة إلى السوبر أكسيد ديسميوتيز في خلايا الدم الحمراء (eSOD) كان أقل أثناء الفترة الانتقالية عنه أثناء منتصف الحمل في كلا السلالتين. وكانت نواتج المواد المتفاعلة مع حمض الثيوباريتيوريك (TBARS) كمؤشر لأوكسدة الدهون أعلى أثناء الفترة الانتقالية عنه أثناء منتصف الحمل في الأبقار الخليط فقط ولم تتأثر في الأبقار البلدي. هذا وقد أظهرت النتائج وجود اختلافات في تركيزات كل من الألفاتوكوفيرول والبيتاكاروتين و eSOD خلال الفترة الانتقالية حيث كانت تنجه إلى أقل قيمة لها أثناء الولادة وبالعكس بلغت قيم TBARS ذروتها أثناء الولادة بينما لم تتغير قيم حمض الأسكوربيك خلال الفترة الانتقالية في كلا السلالتين. وقد أوضحت نتائج الارتباط والانحدار الخطي بين TBARS وباقي مضادات التأكسد وجود علاقة عكسية بين TBARS والألفاتوكوفيرول ولكن لم تكن له علاقة بـحمض الأسكوربيك في كلا السلالتين. وكانت العلاقة بين TBARS وكل من البيتاكاروتين و eSOD سلبية في الأبقار البلدي ولم

تتأثر في الأبقار الخليط. ومن نتائج هذه الدراسة نستخلص أن الفترة الانتقالية في الأبقار تتسبب في الإجهاد التأكسدي الواضح من زيادة TBARS المصاحب لاستنفاد مضادات التأكسد، وأن وقت الولادة كان له الأثر الأكبر في هذا الخلل. وقد أظهرت الأبقار البلدي استعداداً أكثر من الأبقار الخليط للتعامل مع الإجهاد التأكسدي خلال تلك الفترة الانتقالية.

## SUMMARY

Markers of oxidative stress and antioxidant capacity could provide complementary information about the homeostasis of the animal than the other conventional metabolic parameters. The aim of this work was to assess the interaction between the oxidative stress and the encountered antioxidant status during transition from late pregnancy to early lactation in native and cross bred cows in relation to the local environment of the Egyptian oasis. The study was carried out on 22 pluriparous pregnant cows, of which 12 native (Balady) and 10 crossbred (Friesian x Balady). Blood samples were collected from the selected cows at the 4-5<sup>th</sup> month of pregnancy, then weekly throughout the last three weeks prepartum, at calving time and weekly through out the first three weeks post-calving. At the peripartum period, the overall mean of the sum of the concentrations of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene, ascorbic acid and the activity of erythrocytic superoxide dismutase (eSOD) were lower than those registered at mid-pregnancy in both breeds. Thiobarbituric acid reactive substances (TBARS) values as a marker of lipid peroxidation were higher at peripartum period than mid-pregnancy only in crossbred cattle, but it did not change in native cattle. Within the peripartum period, variations (F test) of concentrations of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene and the activity of eSOD were significant, which tend to minimize with a conversed corresponding peak of TBARS at calving in both breeds. Levels of ascorbic acid did not change throughout the peripartum period in native and crossbred cows. The correlation and linear regression analysis revealed that TBARS negatively interacted with  $\alpha$ -tocopherol and did not interact with ascorbic acid in both breeds. The relation between TBARS concentration and each of  $\beta$ -carotene concentration and eSOD activity was significantly negative in native cows, but it was non-significant in crossbred cows. In conclusion, the peripartum phase in cows can impose oxidative stress as indicated by the increase of TBARS concentration accompanied by marked depletion in the antioxidants. Stress due to calving has a greater effect on this imbalance. Native cows are well prepared than crossbred cows to deal with the oxidative stress at the peripartum period.

**Key words:** *Cattle, peripartum, lipid peroxidation, antioxidant Egyptian oasis*

## INTRODUCTION

Oxidative reactions are an essential part of normal metabolism (Dröge, 2002). Problems may arise when electron flow become uncoupled so that oxygen free radicals or the so called reactive oxygen species (ROS) are produced (Nohl, *et al.*, 2005). When level of ROS exceeds the antioxidant capacity of the cell, the intracellular redox homeostasis is altered and oxidative stress ensues (Arts and Hollman, 2005; Berger, 2005; Bernabucci *et al.*, 2005 and Turk *et al.*, 2005).

Oxidative stress is considered to play a pivotal role in the pathogenesis of several degenerative diseases (Valko *et al.*, 2004). In order to cope with an excess of free radicals produced upon oxidative stress, bodies have developed sophisticated mechanisms in order to maintain redox homeostasis (Hundhausen *et al.*, 2005 and Colitti and Stefanon, 2006). These protective mechanisms either scavenge or detoxify ROS, block their production, or sequester transition metals that are the source of free radicals (Masella *et al.*, 2005). In cattle, these mechanisms include enzymatic and nonenzymatic antioxidant defenses produced in the body, namely, endogenous as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and others supplied with the diet, namely, exogenous as Polyphenols (Kleczkowski *et al.*, 2003 & 2004).

Lipids especially polyunsaturated fatty acids are sensitive to oxidation forming a complex series of compounds, leading to the term lipid peroxidation or the thiobarbituric acid reactive substances (TBARS), of which the most abundant is malondialdehyde (Janero, 1990 and Gerard-Monnier *et al.*, 1998). Recent studies showed that the use of oxidative stress markers as a measure of oxidant-antioxidant balance could provide complementary information about the homeostasis of the animal than conventional metabolic parameters alone (Castillo *et al.*, 2006).

The transition peripartum period is a critical phase and particularly important for health of dairy cows (Ingvarsen and Andersen, 2000). Substantial evidence indicates that innate and acquired defense mechanisms are lowest from 3 weeks pre-calving to 3 weeks post-calving (Mallard *et al.*, 1998). The performance of dairy cows at this period is exposed to drastic metabolic stress which alters their

homeostasis and exposes the cows to illness (Miller *et al.*, 1993 and Ronchi *et al.*, 2000). It has been hypothesized that an involvement of oxidative stress during the transition period is the etiology of some diseases and metabolic disorders as udder edema, milk fever, retained placenta, mastitis, and suboptimal reproduction (Lomba, 1996).

Native cattle and their crosses with Friesians are the only breeds utilized for beef and milk production systems in the Egyptian oasis. These breeds are well adapted to high ambient temperature and maximize their efficacy under the effect of harsh conditions (Saleh, 1996). However, metabolic differences have been reported between native and crossbred cows when challenged with the adverse tropical environments (Saleh, 1996).

There is a lack of information concerning the interaction between oxidative and antioxidant status in periparturient crossbred and native dairy cows especially under the Egyptian oasis conditions. The aim of this work was to assess the degree of oxidative stress and the encountered antioxidant status in addition to their interaction during transition from late pregnancy to early lactation in native and cross bred cows in relation to the local environment of the Egyptian oasis.

## **MATERIALS and METHODS**

### **Cows:**

This study was carried out on 22 pluriparous pregnant cows (4-6 years), of which 12 native (Balady) and 10 crossbred (Friesian x Balady). These cows were belonging to small herd scales in the rural areas at El-Kharga oasis. Feeding of these cows was absolutely depending on fresh-cut Barseem Hegazzy (*Medicago sativa*) without additives. These cows were clinically healthy and their previous history revealed no metabolic or reproductive disturbances. The average daily milk yield in the previous milking cycle was 6-9 Kg/day/head for native and 8-15 Kg/day/head for crossbred cows.

### **Sampling:**

Because dairy cows are in a continuous productive reproductive cycle, the cows are either in pregnant or lactating state (Knight, 2001). So that, to select a control period for the judgment on the peripartum phase, the only possibility was to choose animals that were in the declining phase of lactation. We choose a period in which the effect of lactation and pregnancy were minimal and not a cause of major metabolic burdens (Ingvarsen and Andersen, 2000), coinciding with the

4-5<sup>th</sup> month of pregnancy as recently reported by Castillo *et al.* (2005, 2006).

Jugular blood samples were collected in heparinized vacuumed tubes from the selected cows at the 4-5<sup>th</sup> month of pregnancy, then weekly throughout the last three weeks prepartum, at calving time and weekly through out the first three weeks post-calving. The accurate estimation of the day of prepartum sampling was assessed in relation to the calving time.

The erythrocyte hemolysate from the tubes containing whole blood samples was prepared according to the method of Cohn *et al.* (1970). Briefly, immediately after collection, 1 ml of each blood sample was centrifuged at 1500 rpm for 10 min. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic saline (0.89% w/v NaCl), followed by re-centrifugation and removal of the supernatant fluid. The cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate. The rest of the blood was centrifuged at 2000 rpm for 15 min for separation of plasma. The plasma and the lysate of erythrocytes were stored frozen at -20 °C until analyzed.

#### **Biochemical assay:**

Determination of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene and ascorbic acid was carried out colorimetrically according to the standard methods described by Hawks *et al.* (1954), Carr and Price (1926) and Lowery *et al.* (1945) respectively. Erythrocytic superoxide dismutase (eSOD) activity was assayed by the indirect inhibition technique (Misra and Fridovich, 1972) based on the ability of SOD to inhibit the auto-oxidation of L-epinephrine to adrenochrome at alkaline pH. Haemoglobin was assayed in erythrocyte hemolysate according to (Feldman *et al.*, 2000). eSOD was expressed as U/mg haemoglobin. The concentration of lipid peroxide was estimated in the plasma using the method of Placer *et al.* (1966). The method depended on forming a colour complex between the resulting products of lipid peroxidation and thiobarbituric acid (thiobarbituric acid reactive substances, TBARS) at a temperature of 100 °C in an acidic environment. The maximum absorption of this complex occurs at a wavelength of 532 nm. Plasma protein was estimated by Biuret reaction according to Henry *et al.* (1974). The thiobarbituric acid reactive substances (TBARS) were expressed by malondialdehyde (MDA) as nmol MDA/gm protein.

#### **Statistical analysis:**

The results were analyzed by one way analysis of variance (ANOVA) followed by student "t" test for the comparison between breeds. F test followed by pair-wise comparisons (Duncan's new multiple range test) were used for the comparison between periods within the peripartum period of each breed. Linear regression analysis (R<sup>2</sup>) and Spearman correlation (r) were performed on the arranged all-raw data to evaluate the relation between TBARS and antioxidants in each of native and crossbred cows. The computer packaged software program SPSS was used for analysis (SPSS, 2002). Differences were considered significant at P <0.05.

## RESULTS

At mid-pregnancy, no breed differences were noticed in the average plasma concentrations ( $\mu\text{mol/l} \pm \text{SE}$ ) of  $\alpha$ -tocopherol and ascorbic acid between native and crossbred cows, whereas the values of  $\beta$ -carotene were higher (P<0.05) in native ( $7.8 \pm 0.42$ ) than in crossbred cows ( $6.36 \pm 0.40$ ). Activity of eSOD (mean  $\pm$  SE U/mg haemoglobin) at mid-pregnancy did not significantly differ between native and crossbred. On the other hand, plasma concentrations of TBARS at mid-pregnancy (mean  $\pm$  SE nmol MDA/gm protein) were lower (P<0.05) in native ( $17.40 \pm 1.60$ ) than crossbred ( $22.87 \pm 2.00$ ) cows (Tables 1-5 and Figure 1).

At the peripartum period, the overall mean concentrations (the mean of the sum of all periods within the peripartum period) of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene, ascorbic acid and the overall mean activity of eSOD in both breeds were lower than those registered at mid-pregnancy. TBARS values were higher at peripartum period than mid-pregnancy only in crossbred cattle, but it did not change in native cattle (Tables 1-5 and Figure 1).

Within the peripartum period, the variations (F test) of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene, eSOD and TBARS values were significant (P<0.01) in both breeds, but there were non-significant variations in ascorbic acid throughout the peripartum period in native and crossbred cows. Sources of these variations are illustrated in Tables 1-5 and Figure 1. The values of  $\alpha$ -tocopherol and  $\beta$ -carotene decreased substantially during the last 3 weeks of gestation till reached its lowest value at calving (Day 0). After calving, the values of  $\alpha$ -tocopherol were reversed to its earlier precalving state, but  $\beta$ -carotene values remained low throughout the first 3 weeks after calving in both breeds. Despite of the concentrations of ascorbic acid were lower during the peripartum

period than mid-pregnancy, the values did not change significantly throughout the peripartum period in both breeds.

The activity of eSOD was lower during the peripartum period than mid-pregnancy in both breeds. The values of eSOD activity remained constant throughout the last 3 weeks of gestation. At calving the activity of eSOD significantly decreased and continued at this low level during the first week in native and the first two weeks in crossbred cows. At the third week postpartum the eSOD activity restored its previous pre-calving values in both breeds.

The mean plasma concentrations of TBARS as an index of lipid peroxidation did not significantly change throughout the last weeks of gestation and also did not change significantly than the mid-pregnancy values in native and crossbred cows. However, the mean concentrations of TBARS significantly elevated at calving in both breeds. After calving, the mean value of TBARS returned to the pre-calving value during the first week in native breeds, but it declined during the second week after calving in crossbred cows without reaching the pre-calving values.

Spearman correlation and linear regression analysis between the TBARS and the antioxidant levels in native and crossbred peripartum cows are presented in Table 6. The results revealed that TBARS negatively interacted with  $\alpha$ -tocopherol ( $P < 0.01$  in native and  $P < 0.001$  in crossbred cows) and did not interact with ascorbic acid. The relation between TBARS values and each of  $\beta$ -carotene and eSOD was significantly negative ( $P < 0.001$  for each) in native cows, but it was non-significant in crossbred cows

**Table 1:** Plasma concentrations of  $\alpha$ -tocopherol (mean  $\pm$  SE  $\mu$ mol/l) in crossbred and native peripartum cows.

	Native	Crossbred	P
Mid-pregnancy	11.42 $\pm$ 0.47 <sup>a</sup>	10.11 $\pm$ 0.49 <sup>a</sup>	0.069
3 weeks before calving	10.06 $\pm$ 0.45 <sup>b</sup>	08.58 $\pm$ 0.44 <sup>b</sup>	0.029
2 weeks before calving	09.59 $\pm$ 0.50 <sup>b</sup>	07.54 $\pm$ 0.45 <sup>b</sup>	0.007
1 week before calving	09.77 $\pm$ 0.45 <sup>b</sup>	06.86 $\pm$ 0.48 <sup>bc</sup>	<0.001
Calving day	07.58 $\pm$ 0.44 <sup>c</sup>	05.21 $\pm$ 0.34 <sup>c</sup>	<0.001
1 week after calving	09.70 $\pm$ 0.40 <sup>b</sup>	05.71 $\pm$ 0.30 <sup>c</sup>	<0.001
2 weeks after calving	09.52 $\pm$ 0.40 <sup>b</sup>	07.56 $\pm$ 0.53 <sup>b</sup>	0.009
3 weeks after calving	10.41 $\pm$ 0.38 <sup>ab</sup>	08.37 $\pm$ 0.41 <sup>b</sup>	0.002
Mean of peripartum	09.52 $\pm$ 0.25 <sup>b</sup>	07.12 $\pm$ 0.33 <sup>b</sup>	<0.001
F test of peripartum	4.343***	8.880***	-

Means in the same column with unlike superscript letters are significantly different ( $P < 0.05$ ); \*\*\* Significant F test at  $P < 0.001$ .

**Table 2:** Plasma concentrations of  $\beta$ -carotene (mean $\pm$  SE  $\mu$ mol/l) in crossbred and native peripartum cows.

	Native	Crossbred	P
Mid-pregnancy	7.80 $\pm$ 0.42 <sup>a</sup>	6.36 $\pm$ 0.40 <sup>a</sup>	0.022
3 weeks before calving	5.93 $\pm$ 0.27 <sup>b</sup>	4.84 $\pm$ 0.26 <sup>b</sup>	0.009
2 weeks before calving	4.91 $\pm$ 0.28 <sup>b</sup>	4.28 $\pm$ 0.19 <sup>b</sup>	0.084
1 week before calving	4.71 $\pm$ 0.25 <sup>bc</sup>	3.82 $\pm$ 0.26 <sup>c</sup>	0.025
Calving day	3.83 $\pm$ 0.28 <sup>c</sup>	2.86 $\pm$ 0.19 <sup>d</sup>	0.009
1 week after calving	3.50 $\pm$ 0.23 <sup>c</sup>	3.23 $\pm$ 0.19 <sup>cd</sup>	0.376
2 weeks after calving	4.10 $\pm$ 0.25 <sup>c</sup>	2.88 $\pm$ 0.26 <sup>d</sup>	0.003
3 weeks after calving	4.38 $\pm$ 0.23 <sup>bc</sup>	3.25 $\pm$ 0.23 <sup>cd</sup>	0.002
Mean of peripartum	4.48 $\pm$ 0.22 <sup>bc</sup>	3.59 $\pm$ 0.22 <sup>cd</sup>	0.010
F test of peripartum	9.766***	10.649***	-

Means in the same column with unlike superscript letters are significantly different (P<0.05); \*\*\* Significant F test at P<0.001.

**Table 3:** Plasma concentrations of ascorbic acid (mean $\pm$  SE  $\mu$ mol/l) in crossbred and native peripartum cows

	Native	Crossbred	P
Mid-pregnancy	23.81 $\pm$ 1.53 <sup>a</sup>	21.28 $\pm$ 1.11 <sup>a</sup>	0.197
3 weeks before calving	18.23 $\pm$ 1.21 <sup>b</sup>	16.79 $\pm$ 0.83 <sup>b</sup>	0.339
2 weeks before calving	18.66 $\pm$ 1.06 <sup>b</sup>	15.43 $\pm$ 1.23 <sup>b</sup>	0.062
1 week before calving	16.87 $\pm$ 1.29 <sup>b</sup>	15.82 $\pm$ 1.29 <sup>b</sup>	0.572
Calving day	15.81 $\pm$ 1.25 <sup>b</sup>	13.86 $\pm$ 0.99 <sup>b</sup>	0.236
1 week after calving	16.14 $\pm$ 1.02 <sup>b</sup>	13.08 $\pm$ 0.87 <sup>b</sup>	0.034
2 weeks after calving	16.87 $\pm$ 1.10 <sup>b</sup>	13.47 $\pm$ 0.94 <sup>b</sup>	0.029
3 weeks after calving	17.83 $\pm$ 1.12 <sup>b</sup>	15.56 $\pm$ 0.89 <sup>b</sup>	0.129
Mean of peripartum	17.20 $\pm$ 0.94 <sup>b</sup>	14.86 $\pm$ 0.78 <sup>b</sup>	0.069
F test of peripartum	0.857 <sup>NS</sup>	1.851 <sup>NS</sup>	-

Means in the same column with unlike superscript letters are significantly different (P<0.05); NS: Non-significant F test at P<0.05.

**Table 4:** Activity of eSOD (mean $\pm$  SE U/mg haemoglobin) in crossbred and native peripartum cows.

	Native	Crossbred	P
Mid-pregnancy	4.30 $\pm$ 0.39 <sup>a</sup>	3.75 $\pm$ 0.31 <sup>a</sup>	0.279
3 weeks before calving	3.55 $\pm$ 0.33 <sup>b</sup>	2.78 $\pm$ 0.18 <sup>b</sup>	0.060
2 weeks before calving	3.83 $\pm$ 0.32 <sup>b</sup>	2.31 $\pm$ 0.17 <sup>b</sup>	<0.001
1 week before calving	3.32 $\pm$ 0.30 <sup>b</sup>	2.53 $\pm$ 0.27 <sup>b</sup>	0.065
Calving day	2.43 $\pm$ 0.17 <sup>c</sup>	1.93 $\pm$ 0.16 <sup>c</sup>	0.049
1 week after calving	2.53 $\pm$ 0.15 <sup>c</sup>	2.02 $\pm$ 0.20 <sup>c</sup>	0.055
2 weeks after calving	3.18 $\pm$ 0.21 <sup>b</sup>	1.84 $\pm$ 0.13 <sup>c</sup>	<0.001
3 weeks after calving	3.28 $\pm$ 0.23 <sup>b</sup>	2.57 $\pm$ 0.27 <sup>b</sup>	0.063
Mean of peripartum	3.16 $\pm$ 0.19 <sup>b</sup>	2.28 $\pm$ 0.19 <sup>b</sup>	0.004
F test of peripartum	4.057**	3.121**	-

Means in the same column with unlike superscript letters are significantly different (P<0.05); \*\* Significant F test at P<0.01.



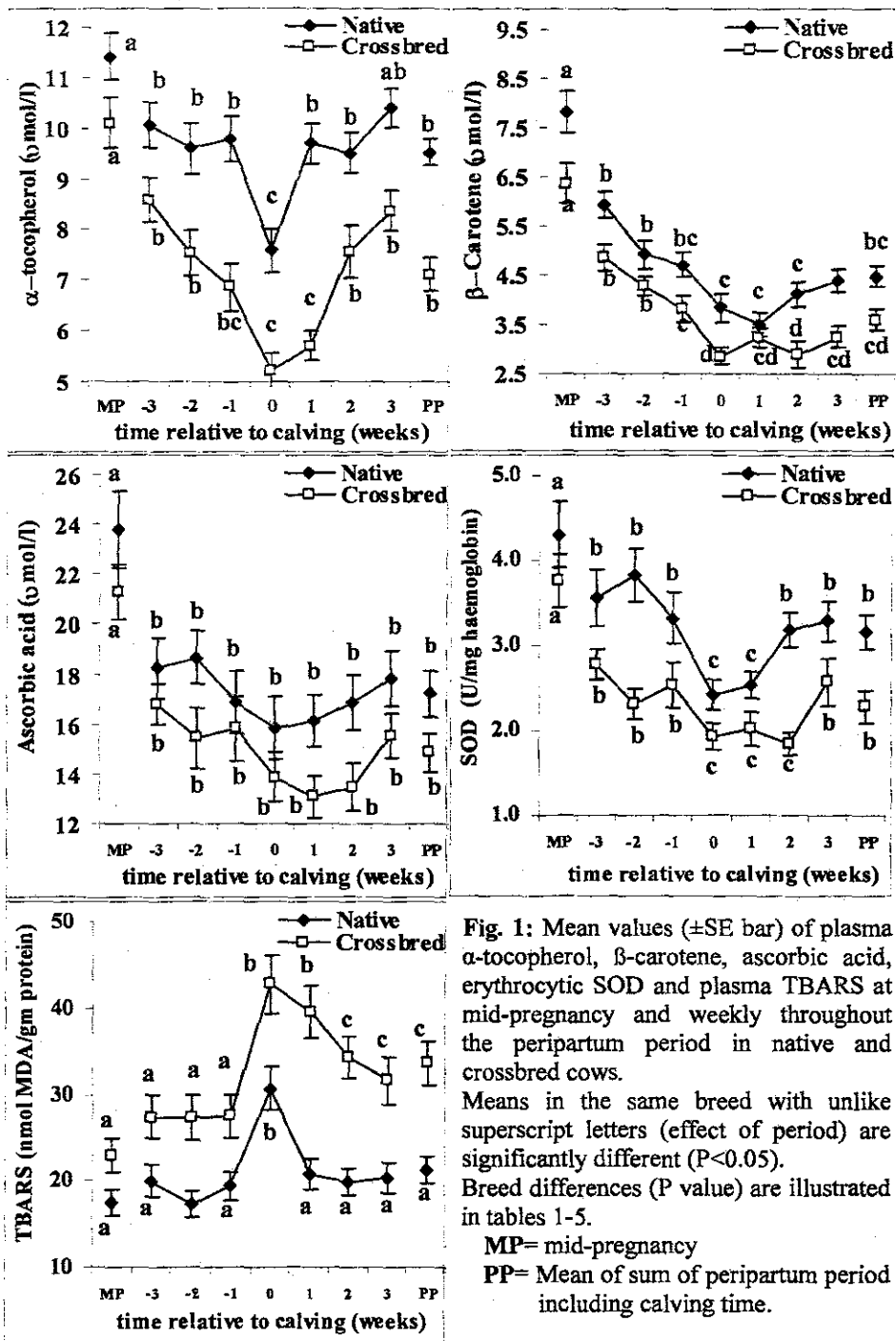


Fig. 1: Mean values ( $\pm$ SE bar) of plasma  $\alpha$ -tocopherol,  $\beta$ -carotene, ascorbic acid, erythrocytic SOD and plasma TBARS at mid-pregnancy and weekly throughout the peripartum period in native and crossbred cows.

Means in the same breed with unlike superscript letters (effect of period) are significantly different ( $P < 0.05$ ).

Breed differences (P value) are illustrated in tables 1-5.

MP= mid-pregnancy

PP= Mean of sum of peripartum period including calving time.

**Table 5:** Plasma concentrations of TBARS (mean± SE nmol MDA/gm protein) in crossbred and native peripartum cows.

	Native	Crossbred	P
Mid-pregnancy	17.40±1.60 <sup>a</sup>	22.87±2.00 <sup>a</sup>	0.046
3 weeks before calving	19.94±1.90 <sup>a</sup>	26.33±2.54 <sup>a</sup>	0.033
2 weeks before calving	17.20±1.51 <sup>a</sup>	26.11±2.64 <sup>a</sup>	<0.001
1 week before calving	19.38±1.63 <sup>a</sup>	26.24±2.43 <sup>a</sup>	0.006
Calving day	30.64±2.60 <sup>b</sup>	42.68±3.47 <sup>b</sup>	0.013
1 week after calving	20.68±1.73 <sup>a</sup>	39.49±3.07 <sup>b</sup>	<0.001
2 weeks after calving	19.85±1.58 <sup>a</sup>	34.22±2.34 <sup>c</sup>	<0.001
3 weeks after calving	20.26±1.83 <sup>a</sup>	31.58±2.67 <sup>c</sup>	0.003
Mean of peripartum	21.14±1.54 <sup>a</sup>	33.59±2.50 <sup>c</sup>	<0.001
F test of peripartum	5.452***	4.161**	

Means in the same column with unlike superscript letters are significantly different (P<0.05); \*\*, \*\*\* Significant F test at P<0.01 and P<0.001 respectively.

**Table 6:** Spearman correlation (r) and linear regression analysis (R<sup>2</sup>) between TBARS and antioxidants in native and crossbred peripartum cows.

	α-tocopherol		β-carotene		Ascorbic acid		eSOD	
	N	CB	N	CB	N	CB	N	CB
R	-0.391	-0.558	-0.333	-0.124	-0.041	-0.135	-0.335	-0.087
R <sup>2</sup>	0.153	0.311	0.111	0.016	0.002	0.018	0.112	0.008
P- value	0.002	<0.001	<0.001	0.634	0.694	0.089	<0.001	0.807

## DISCUSSION

In the last few years, oxidative stress markers have involved in the mechanisms of metabolic disorders, especially important in dairy cows, in which peripartum period imposes great demands on the body's homeostatic mechanisms (Bernabucci *et al.*, 2002 & 2005; Chawl and Kaur, 2004; Castillo *et al.*, 2006 and Gaál *et al.*, 2006).

The mean values of α-tocopherol and β-carotene at mid-pregnancy in both breeds were higher than the values reported previously for temperate breeds by Brzezinska-Slebozinska (1994), Michal *et al.* (1994), Weiss *et al.* (1994), Trout *et al.* (1998), and LeBlanc *et al.* (2002, 2004). The values obtained by these authors ranged from 1.4 to 4.1 µg/ml (3.25-9.5 µmol/l) for α-tocopherol and from 1.1 to 3.5 µg/ml (2.0-6.5 µmol/l) for β-carotene. Cows used in the current study were depending on freshly harvested forages in comparison with manufactured diets prepared for feeding of temperate breeds in the previously mentioned studies. The higher concentration of α-tocopherol and β-carotene in fresh grass (Gatellier *et al.*, 2004) confer an improved overall antioxidant and redox status (Descalzo *et al.*, 2007)

when compared to a grain-finishing diet. On the other hand, tropical breeds, which are adapted to high ambient temperature, have greater concentrations of metabolic hormones and metabolites than temperate breeds (Obeidat *et al.*, 2002).

Plasma ascorbic acid concentration at mid-pregnancy lies within the range of 2.59 to 4.98 mg/l (14.7 to 28.3  $\mu\text{mol/l}$ ) reported for dairy cows by Verma *et al.* (1993), Hidioglou *et al.* (1995), Santos *et al.* (2001), Weiss (2001) and Weiss *et al.* (2004). Ascorbic acid in foods is quickly degraded in the rumen systems with a half-life of 3.5 hours (Macleod *et al.*, 1999a). So that, vitamin C required for ruminants is not absolutely dependent on the ingested ascorbate but it depend on the production of ascorbic acid in the liver according to the endogenous homeostasis and the body need (NRC, 2001).

Values of eSOD activity in cattle varied according to the methods used. It registered 140.5-215.3 U/ml PCV (Bernabucci *et al.*, 2002) and 802-1415 U/g RBC proteins (Gaál *et al.*, 2006). Wide ranges were also previously reported for plasma TBARS levels which ranged between 1.5 to 2 nmol/ml (Bernabucci *et al.*, 2002) and 34.57 to 68.99  $\mu\text{mol/l}$  (Castillo *et al.*, 2005). In this concern however, our results were comparable with those reported for normal cattle by Imre *et al.* (2001), Castillo *et al.* (2006) and Rezaei and Dalir-Naghadeh (2006).

At mid-pregnancy, there were no breed differences in the average plasma concentrations of plasma  $\alpha$ -tocopherol, ascorbic acid or the activity of eSOD, while the values of  $\beta$ -carotene were higher in native than crossbred cows. On the other hand, plasma concentration of TBARS at mid-pregnancy was lower in native than crossbred cows. It is known that the body mass index and milk production of temperate cattle breeds and their crosses are higher than that of native breeds (Payne and Wilson, 1999). Bernabucci *et al.* (2005) and Castillo *et al.* (2006) found that cows with high body mass index and high milk output were more sensitive to oxidative stress. This can result from the excess accumulation of ROS, depletion of antioxidant defenses, or combination of both (O'Boyle *et al.*, 2006). The excessive macronutrients intake, the higher metabolic rate and the increased secretion of pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor- $\alpha$ ) by adipose tissue in individuals with high body mass index (Dandona *et al.*, 2004) might played a role in the higher TBARS in crossbred than native cows.

The reduction of plasma  $\alpha$ -tocopherol and  $\beta$ -carotene around parturition in dairy cows was previously reported (Goff *et al.*, 2002; Chawl and Kaur, 2004 and LeBlanc *et al.*, 2004). It has been suggested

that the decrease of  $\alpha$ -tocopherol and  $\beta$ -carotene is one of the mechanisms of the decrease in peripartum immune system efficiency (Mallard *et al.*, 1998 and O'Boyle *et al.*, 2006). Plasma concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene in the current work were lower during the peripartum period than mid-pregnancy. They decreased substantially during the last 3 weeks of gestation till reached their lowest value at calving. After calving, the decrease in  $\alpha$ -tocopherol status was reversed to its earlier value, but  $\beta$ -carotene values remained low. Periparturient cows undergo intense mammary growth and marked production of colostrum and milk (Weiss *et al.*, 1997). Since colostrum is rich in fat-soluble vitamins A and E, the circulatory levels of these vitamins decrease at the time of parturition (Michal *et al.*, 1994; Hayangmi *et al.*, 1999 and Baldi *et al.*, 2004). Due to the decreased dry matter intake, the peripartum cows have much lower concentrations of plasma lipids (Murondoti *et al.*, 2004 and Baldi, 2005). Circulating  $\alpha$ -tocopherol and  $\beta$ -carotene are associated with the lipid fraction in plasma (Singh *et al.*, 2005). The lower concentrations of circulating lipids could reduce the transport capacity of fat soluble vitamins (Arts and Hollman, 2005). On the other hand, the consumption of these vitamins as protective antioxidants against the initiated peripartum oxidative stress as recently reported by LeBlanc *et al.* (2004) might be a potent convincing interpretation for the decrease of these vitamins.

Ascorbic acid is a water soluble antioxidant that scavenges the aqueous ROS by very rapid electron transfer that inhibits lipid peroxidation (Hathcock *et al.*, 2005). In spite of absence of variations in ascorbate concentrations between pre-calving, calving and early lactation in the present work, its concentrations decreased at the peripartum period than the values recorded at mid-pregnancy. Santos *et al.* (2001) and Padilla *et al.* (2005) found that plasma ascorbate concentrations are not correlated metabolic profile or metabolic disorders in lactating and dry cows. However, our results agree with Macleod *et al.* (1999b) who found that peripartum cows had a decreased synthesis of ascorbic acid in the liver resulting from high demands for glucose by the mammary gland. The consumption of ascorbate as a protective antioxidant (Weiss *et al.*, 2004) against the initiated oxidative stress at peripartum period in cattle may be also a logic explanation of the decrease of ascorbic acid at this period.

Imre *et al.* (2001) and Dröge (2002) reported that the oxidation or auto-oxidation of hemoglobin (Hb-Fe<sup>2+</sup> into Hb-Fe<sup>3+</sup>) in the erythrocytes results in the continuous formation of superoxide anion

( $\bullet\text{O}_2^-$ ). The eSOD initiates the antioxidant process, transforming  $\bullet\text{O}_2^-$  into hydrogen peroxide which is neutralized by catalase (Petersen and Enghild, 2005). In the current work, the peripartum eSOD activity was lower than that at mid-pregnancy in both breeds, which was more pronounced at calving. The reduction of erythrocyte antioxidant enzymes in the transition cow was previously reported (Bernabucci *et al.*, 2002). Our results however, differed than those reported by Bernabucci *et al.* (2005) who found that the activity of eSOD increased around calving. In fact, SOD activity is modulated by mineral content of the diet such as Cu, Zn and Mn (Okado-Matsumoto and Fridovich, 2001). It is well established that Cu and Zn deficiency induces decrease in the activity of Cu-Zn-SOD in Cu deficient animals and man (Ho, 2004 and Uriu-Adams and Keen, 2005). Predictably, the activity of Cu-Zn-SOD is sensitive to tissue Cu as this enzyme requires Cu as a catalytic cofactor (Uriu-Adams *et al.*, 2005). Moreover, Cu deficiency affects components of the oxidant defense system, and increases ROS and oxidative damage to lipid (Johnson and Thomas, 1999; Hawk *et al.*, 2003 and Uriu-Adams *et al.*, 2005). In the Egyptian oasis, livestock including cattle are suffering from Cu and Zn deficiency due to the shortage of these elements in the soil and in turn the food allowed for these animals (Saleh, 1996 and Yousef, 2006). In addition, zinc and copper concentrations are reduced in the periparturient cows (Muehlenbein *et al.*, 2001). On the other hand Miller *et al.* (1994) hypothesized that the increase of metabolic rate after calving might be responsible for the elevation of respiratory electron transfer and ROS production. Under these circumstances, the reduced eSOD activity at calving and shortly postpartum due to shortage of copper supply and its consumption as scavenger of free radicals in cows in the current study is acceptable.

Plasma TBARS were hypothesized to represent a composite number of lipid oxidative end products, including malondialdehyde (Gerard-Monnier *et al.*, 1998). Therefore, TBARS measurement is indicative of lipid peroxidation and is considered as a good general indicator of oxidative stress (Trevisan *et al.*, 2001). The overall mean values TBARS were higher at peripartum period than mid-pregnancy in crossbred cattle, but it did not change in native cattle. At calving, the mean values of TBARS elevated in both breeds then restored their earlier values directly after calving in native breed but still elevated in crossbred cattle. Castillo *et al.* (2005) and Mudron and Konvicna (2006) suggested that the increased lipid peroxidation around parturition provides an accurate reflection of the internal metabolic status of the

animal but with wide individual variations. The higher plasma TBARS levels observed at calving in the current study suggested that: (i) at calving the body presents high levels of free radicals which cause lipid peroxidation; (ii) this effect is related to the intensity of the metabolic changes, under endocrine regulation, that occur at this period (Castillo *et al.*, 2006), (iii) a short-term redox imbalance occurred in the cow at calving time (Gaál *et al.*, 2006) and (iv) the activity of lipid peroxidation is maintained only in crossbred cows, which might result from the relatively higher metabolic and endocrine activity of these cows after calving than native breeds (Obeidat *et al.*, 2002 and O'Boyle *et al.*, 2006).

The possibility that the oxidative marker (TBARS) may determine the redox status in peripartum cows in the current study is supported by various correlations depending on the antioxidants condition and breed of cows. Spearman correlation and linear regression analysis between TBARS and antioxidant levels revealed that TBARS negatively interacted with  $\alpha$ -tocopherol and did not interact with ascorbic acid in both breeds. The relation between TBARS values and each of  $\beta$ -carotene and eSOD was significantly negative in native cows, but it was non-significant in crossbred cows. Previous reports had informed that under normal homeostatic conditions, lipid peroxidation increases as antioxidant protection decreases and vice versa (Halliwell & Chirico, 1993 and Castillo *et al.*, 2003). So that, the significant correlations between TBARS and other antioxidants in native cows might have indicated that there was more peripartal stability of redox reaction in this breed. Conversely, the absence of interactions in crossbred cows had indicated a relatively more imbalance between production of ROS, their safe disposal and the initiated lipid peroxidation during the peripartum period. In temperate breeds, Trevisan *et al.* (2001) found loss of redox homeostasis in the peripartum period. In such conditions, ROS are produced faster than they can be safely neutralized by antioxidant mechanisms with induction of lipid peroxidation (Castillo *et al.*, 2005 & 2006). These conditions can contribute and/or lead to the onset of peripartum disorders in high producing dairy cows (LeBlanc *et al.*, 2004 and Kleczkowski *et al.*, 2006).

In conclusion, the peripartum phase in cows can impose oxidative stress as indicated by the increase of TBARS accompanied by marked depletion in the antioxidant status. Stress due to calving has a

greater impact on this imbalance. Native cows are well prepared than crossbred cows to deal with the oxidative stress at the peripartum period.

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