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REDOX STATUS DURING PREGNANCY, PERI-PARTURITION AND POST CALVING-FIRST INSEMINATION PERIOD IN FERISIAN COWS

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SUMMARY

A total of fifteen late pregnant Holstein Friesian cows with different parities were selected 3-6 weeks before calving. 231 Jugular Blood samples were collected weekly along 18 weeks for serum separation. Serum Hydrogen peroxide (H_2O_2), Malondialdehyde (MDA) and Total antioxidant capacity (TAC) were measured colorimetrically. Both H_2O_2 and MDA averages were increased gradually and reached the peak($P<0.01$) one week before calving, TAC averages were dropped at the 5^{th} week and increased gradually to peak again at the last week before calving and fluctuated with a peak ($P<0.01$) at the 10^{th} week post- calving. The Parity and cow effect was significant for (H_2O_2& MDA) and TAC averages, $P<0.05$ and $p<0.01$ respectively. Cows having retained placenta were accompanied by high H_2O_2 (323.9± 19.69 µmol/L) and low serum TAC (1790.8 ± 12.32 mmol/L) concentration, ($P<0.01$). Cows not bred after calving showed high ($P<0.01$) H_2O_2 (292.6 ± 11.46 µmol/L) and low TAC averages (1890.1± 17.33 mmol/L) comparing to cows that bred either became pregnant or not.

The study declared that the addition of antioxidant to the ration or the injection of vitamin E may play a role to overcome the redox status of the cows under investigation.

Keywords: Frisian, antioxidant, redox status, calving, insemination period

INTRODUCTION

Oxidation reactions are an essential part of normal metabolism as oxygen is the ultimate electron acceptor in the electron flow system that produces ATP (Davies, 1993). Reactive oxygen species (ROS) play different positive roles *in vivo*, being involved in energy production needed for phagocytosis, cell growth and intercellular signaling regulation. Problems may arise when electron flow and energy production become uncoupled so that oxygen free radicals (ROS) are produced (Nohl *et al.*, 2005). The ROS may be highly damaging , as they can attack biological macromolecules, namely, lipids, proteins and DNA inducing oxidation and cause membrane damage, enzyme inactivation and DNA damage (Halliwell and Gutteridge 1999 and Valko *et al*., 2004).

Antioxidant capacity system consists of two mechanisms: enzymatic and non enzymatic mechanisms those scavenger free radicals. In cattle, characteristics of these mechanisms depend mainly on the nutritional status of antioxidant minerals especially copper, zinc, iron, selenium, silicon and manganese (Kleczkowski *et al.*, 2003). Polyphenols can stimulate antioxidant transcription and detoxification defense systems through antioxidant responsive elements, ARE (Masella *et al*., 2005)

Redox studies in cattle have been sporadic and mainly with mastitis, pneumonia, and retained placenta. Recently, studies have been focused on metabolic diseases that affect dairy cows during the peripartum period. Numerous and rapidly evolving methodologies for evaluating oxidative stress are available to researchers and clinicians, each with their own distinct advantages and disadvantages. Differences in models and methodologies make it difficult to make meaningful comparisons, even for studies that seem quite similar superficially (Pietro Celi, 2010).

Pregnancy was associated with decreased total antioxidant capacity (TAC) and uric acid in the first trimester, which gradually increased during pregnancy, reaching normal values during postpartum period (Toescu *et al.*, 2002). In cattle, the peri-parturient period is especially critical for health and subsequent reproductive performance (Erisir *et al.*, 2006).

The aim of this study was to shed more light on the occurrence of oxidative stress in close up period of Friesian cows and follow it till the post-calving first insemination (PCFI) by the determination of Hydrogen peroxide (H2O2) as free radical indicator and Malondialdehyde (MDA) levels as lipid peroxide indicator and total antioxidant capacity status (TAC).

MATERIALS AND METHODS

Animals:

A total of fifteen late pregnant dry Friesian cows with different parities averaging three - six weeks before calving were selected. Cows closely before calving were fed corn silage(5 kg), rice straw (four kg), hay (two kg) and five kg concentrate (consists of 34% un -decorticated cotton seed cake, 24% wheat bran, 22% yellow

corn, 10% rice bran, 5% line seed cake, 2% molasses, 2% lime stone and 1% common salt). After calving all cows were fed 12 kgs corn silage, four kg rice straw, three kg hay and eight kg concentrate.

Cow had delayed expulsion of fetal membranes > 8 hrs after calving was counted as a retained placental cow and was treated with the protocol applied in the farm as application of systemic and intra uterine antibiotics pessaries, injection of prostaglandin $F_{2\infty}$ and oxytocin before milking on the second day after calving and followed up till complete recovery. All cows examined at day 35 post-calving to evaluate their reproductive status (uterine involution , Type of vaginal discharges if it is present, ovarian activity) and then followed up for heat detection and breeding with maximum 11 weeks postpartum.

Blood sampling:

Jugular Blood samples were collected weekly and let for clotting. Serum was separated by centrifugation at 4000 rpm for 10minutes and then frozen and preserved at -40 °c till the chemical analysis was done.

Chemical analysis:

- Hydrogen peroxide was measured colorimetrically at 510 nm using Biodignostic kits, Cairo, Egypt, Cat. No. HP 25(Aedi H. 1984).
- Malondialdehyde (MDA) was measured colorimetrically at 534 nm using Biodignostic kits, Cairo, Egypt, Cat. No. MDA 25 29 (Satoh K.1978 and ohkawa *et al.*, 1979).
- Total antioxidant were measured colorimetrically using Biodignostic kits, Cairo, Egypt, Cat. No.TA 25 13 at 505nm (Koracevic *et al.*, 2001).

All the colorimetric assays were quantified using spectrophotometer

Statistical analysis:

It was carried out using SPSS version 17. Results were expressed as mean± SEM. Analysis of variance (one way ANOVA) followed by Duncan' test were used to determine whether there were significant differences among the groups. Differences were considered significant when P values were less than 0.05.

RESULTS AND DISCUSSION

Weekly averages of fifteen blood serum samples along 18 weeks revealed that, both H_2O_2 and MDA averages had a clear trend, it is increased gradually and reached the peak (P<0.01) one week before calving and then declined again but not reached the bottom level again (Figures 1&2,).

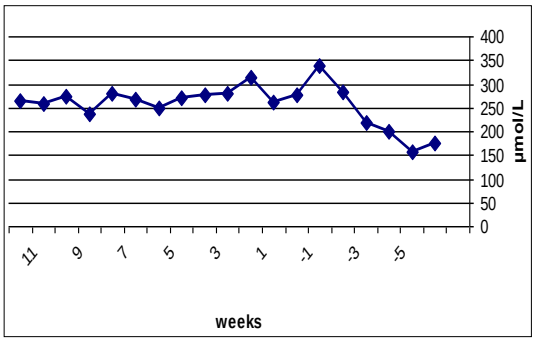

Fig. 1. Pre and post calving means of H_2O_2 in Frisian cows

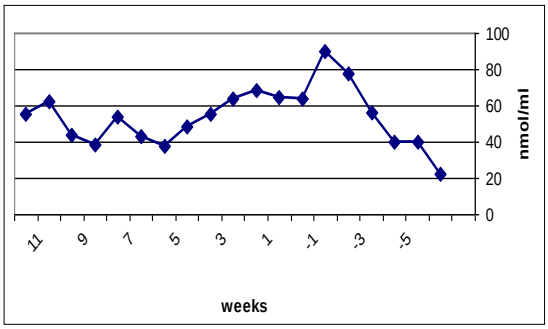

Fig. 2. Pre and post calving means of MDA in Frisian cows

On the other hand, TAC averages had a clear trend pre-calving, it is dropped at the 5th week and increased gradually to the peak again on the last week before calving and then declined and fluctuated with a peak (P<0.01) at the 10th week post- calving (Figure 3). Castillo *et al.* (2005) noticed no trend for plasma MDA level in close cows 10 weeks before calving till two weeks after calving. The TAC averages had a trend pre-calving. Castillo *et al.* (2005) noticed the same TAC level trend, it was peaking (P<0.05) one week after calving and then declined. Kandofer *et al.* (2010) added that TAC differs (P<0.05) with time pre and postpartum. The maximum level of peroxidation (MDA level) was observed in the first week post partum (pp) comparing with its level in late lactation (Adela *et al.*, 2006). The oxidative stress was increased in cows after parturition under hot climatic condition (Tanaka *et al.*, 2011), while, Bernabucci *et al.* (2002) concluded that heat stress effect on oxidative status in transition cows doesn't clear and cows with pre-calving high body condition score (BCS) and get higher BCS losses are more sensitive to oxidative stress (Bernabucci *et al.*, 2005) with high ß-hydroxy butyric acid (BHBA) and non-esterified fatty acids (NEFA). On contrast, Gaal *et al.* (2oo6) reported that no significant differences were found between pre-calving, calving and post-calving means of both MDA and TAC.

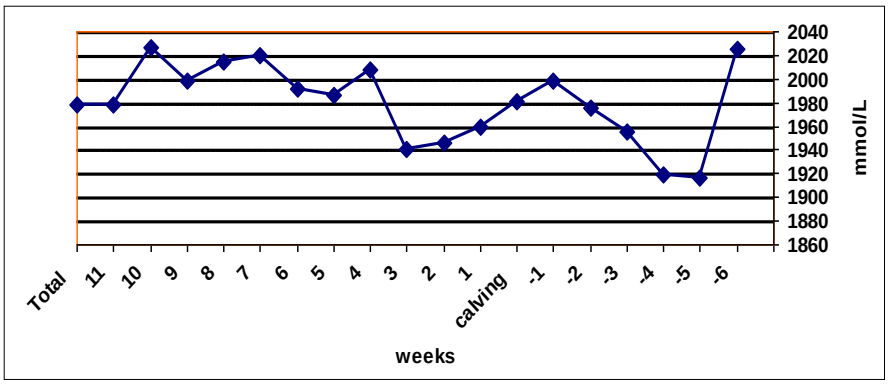

Fig. 3. pre and post calving means of TAC in Frisian cows

Table 1. Serum H_2O_2, MDA and TAC among cows pre and post-calving

Cow number	Sample N	H_2O_2 (µmol/L)		MDA (nmol/ml)		TAC (mmol/L)	
		Average	SEM	Average	SEM	Average	SEM
4053	14	247.2[b]	35.73	45.7[c**]	6.43	2202.0[b]	15.51
4074	14	289.6[a]	26.95	60.1[b]	6.38	1836.4[c]	12.66
4083	18	241.6[b]	29.06	54.5[b]	8.50	1871.7[c]	20.36
4132	14	242.6[b]	26.73	53.2[b]	6.94	2210.7[a**]	16.51
4200	15	123.0[c**]	18.98	51.6[b]	5.44	2220.2[a**]	15.53
4238	17	268.2[a]	25.35	53.1[b]	8.88	1844.3[c]	16.10
4341	14	166.9[c**]	29.00	59.1[b]	9.96	2087.0[b]	18.41
4422	12	291.7[a]	24.44	48.5[c**]	5.09	1859.0[c]	11.38
4437	15	337.8[a]	44.66	72.7[a**]	6.31	2076.5[b]	19.92
4461	15	349.2[a]	35.74	73.4[a**]	7.29	1778.7[d**]	21.78
4463	18	292.2[a]	38.68	51.1[b]	7.73	2144.0[b]	11.61
4467	18	326.0[a]	33.20	55.9[b]	6.40	1802.9[d**]	11.54
4476	17	257.8[b]	25.18	57.1[b]	7.14	1858.7[c]	12.59
4483	18	248.1[b]	16.58	48.8[b]	8.05	1857.7[c]	11.25
4508	12	289.2[a]	32.31	50.4[b]	8.26		
Total	**231**	**265.2**	**8.53**	**55.7**	**1.94**	**1978.7**	**12.52**

Averages marked with a, b, c & d differ at p<0.05 within the same column
**means differ at P<0.01 within the same column.

Table 2. Serum concentration of H_2O_2, MDA and TAC pre and post-calving in cows in relation to Parity

Parity	Sample N	H2O2 (µmol/L)		MDA (nmol/ml)		TAC (mmol/L)	
		Mean	SE	Mean	SE	Mean	SE
1st lactation	113	297.9[b]	11.39	57.3	2.67	1922.6[b]	16.57
2nd Lactation	26	224.5[a]	22.62	54.2	5.84	1986.7[a]	25.69
3rd Lactation	92	232.8[a]	11.26	51.8	2.83	2030.7[a]	20.62
Total	231	264.6	7.87	54.7	1.84	1977.9	12.48

Averages marked with a & b in the same column are different at P<0.05

Table (1) shows that individual cow effect was significantly clear for H_2O_2 , MDA and TAC averages where parity has a significant effect on serum H_2O_2 mean (297.9±11.39) µmol/L which was high (P<0.05) in the first lactation cows while TAC concentration was low (1922.6±16.57) mmol/L comparing to the 2nd and >2 lactations. The serum MDA level was decreased insignificantly with the increase of the lactation number as shown in Table (2).

As shown in Table (3), cows had got retained placenta were accompanied by significant high H_2O_2 (323.9± 19.69 µmol/L) and low serum TAC (1790.8 ± 12.32 mmol/L) concentration (P<0.01) as shown in table-4, while, MDA were insignificantly high in ROP cows (60.2 ± 4.31 nmol/ml) than normal cows (54.6 ± 2.17 nmol/ml). Erisir *et al.* (2006) and Yildiz *et al.* (2011) agreed that serum or plasma MDA concentration in ROP cows did not significantly altered from normal one while, the erythrocyte MDA markedly increased in Dystocia cow compared with eutocia cow (Yildiz *et al*., 2011). On contrast, Kandofer *et al.* (2010) found that blood means of oxidants and antioxidants did not differ among animals with or without retained placenta. The mean MDA concentration in the acute puerperal metritis was significantly higher in the diseased cows than in controls. Hanafi *et al.* (2008) noticed that MDA was significantly

high (P<0.001) in buffalo cows exhibited endometeritis while, TAC was low. These results demonstrate the occurrence of an oxidative stress in cows with acute puerperal metritis which is exacerbated throughout antioxidant overutilization (Kizil *et al.*, 2010 and Heidarpour *et al.*, 2012). After successful treatment for clinical endometritis, serum MDA concentrations have also significantly decreased and TAS values were increased when treatment was successful in subclinically and clinically affected cows 7 days after (Heidarpour *et al.*, 2012).

Cows that did not exhibit estrus signs and not bred after calving showed high (P<0.01) H_2O_2 (292.6±11.46) µmol/L and low TAC averages (1890.1±17.33) mmol/L comparing to cows came in heat and bred either became pregnant or not as shown in table-4. In agreement with this, Ali *et al.* (2014) noticed that MDA levels of repeat breeder and anestrous cows was high (P<0.01) than those came in heat. Excess oxidation, however, causes oxidative stress, resulting in the dysfunction of reproductive processes and antioxidant that reduce the levels of ROS maintain the quality of gametes and support reproduction (Fujii *et al.*, 2005).

Lastly, we concluded that fine addition of antioxidant as copper, zinc and selenium to the ration or by injection of vitamin E may play a role to overcome the oxidant stress and might help in improving the post-calving reproductive status of the animal.

Table 3. Serum concentration of H_2O_2, MDA and TAC pre and post-calving in cows in relation to the incidence of retained placenta (ROP)

ROP	Sample N	H_2O_2 (µmol/L)		MDA (nmol/ml)		TAO (mmol/L)	
		Mean	SE	Mean	SE	Mean	SE
nil	186	250.9	9.19	54.6	2.17	2012.7**	12.97
ROP	45	323.9**	19.69	60.2	4.31	1790.8	12.32
Total	**231**	**265.2**	**8.53**	**55.7**	**1.94**	**1978.7**	**12.52**

** The average means in the same column significantly differ at P<0.01

Table 4. Serum concentration of H_2O_2, MDA and TAC pre and post-calving in cows in relation to post partum breeding

Post calving status	Sample N	H_2O_2 (µmol/L)		MDA (nmol/ml)		TAO (mmol/L)	
		Mean	SE	Mean	SE	Mean	SE
Not Bred	100	292.6[a]	11.46	55.2	2.86	1890.1[b]	17.33
Repeat bred	105	246.4	11.76	54.5	2.67	2037.5[a]	17.85
Bred & get pregnant	26	224.5[b]	22.62	54.2	5.84	1986.7 [a]	25.69
Total	231	264.6	7.87	54.7	1.84	1977.9	12.48

a & b differ at P<0.01 within the same column.

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حالة الأكسدة اثناء فترة الحمل المتأخر والولادة وما بعد الولادة حتى الشياع فى الأبقار الفريزيان

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خمس عشرة بقرة جافة عشار اختيرت عشوائيا ما بين الاسبوع الثالث و السادس قبل الولادة. ٢٣١ عينة دم جمعت اسبوعيا على مدى ١٨ اسبوع لفصل السيرم لقياس تركيز فوق اكسيد الهيدروجين (H_2O_2) و مالوندای الديهايد(MDA) واجمالى مضادات الاكسدة (TAC) مستخدما مقياس مطياف الضوء. فقد أظهرت النتائج أن كلا من متوسط H_2O_2 و MDA اوضحا اتجاها حيث ازدادا بشكل معنوى قبل الولادة ليصلا القمة عند الاسبوع الاول قبل الولادة($P>0.05$) وفى المقابل انخفضت مضادات الاكسدة عند الاسبوع الخامس قبل الولادة لتأخذ فى الارتفاع ولتصل اول قمة قبل الولادة بأسبوع ثم تتموج القيم لتصل الى اعلى قمة عند الأسبوع العاشر بعد الولادة($P>0.01$). وكذلك فالاختلافات الفردية و عدد الولادات أوضحت اختلافا معنويا حيث ازدادت كل من H_2O_2 عند ($P>0.01$) و MDAعند ($P>0.05$) وانخفاض TAC عند ($P>0.01$) فى الابقار ذات الولادة الأولى عن مثيلاتها ذات الولادات المتعددة. وكذلك ازداد H_2O_2 بشكل معنوى فى الابقار التى لم يظهر عليها شياع مع انخفاض فى تركيز مضادات الاكسدة بشكل معنوى($P>0.01$).

ومن هنا نستخلص ان الابقار وخاصة التى تلد للمرة الاولى يزداد بها مواد الاكسدة بشكل ملحوظ وانه يجب مراعاة اضافة او حقن مضادات الاكسدة فى الفترة ما قبل الولادة بأسبوعين والتى ربما تساعد على تجاوز مثل هذه الانضغاطات.