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Relation Between Oxidant/Antioxidant Status and Postpartum Anestrous Conditions

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***Abstract*— The aim of the present study was to detect the relation between oxidant/antioxidant status and postpartum anestrous (PPA) conditions in dairy cows.The postpartum period is a very critical time that influenced mainly in cattle reproduction. A little information is obtainable in literature concerning antioxidant defense mechanisms during anestrus.The purpose of the following study is detection of the relation between oxidant/antioxidant status and postpartum anestrous (PPA) condition in dairy cows. Seventy five postpartum anestrous (PPA) and twenty five normal cyclic Holstein Friesian pluriparous dairy cows were selected on the basis of their reproductive history gained from farm records. Depend on the rectal findings and ultrasonography in addition to progesterone profile; the studied animals were classified into three groups (each 25 animals) as inactive ovaries group, persistent corpus luteum group and silent heat group. Blood samples from anestrous and normal cyclic animals were gathered at day 0, day 10, day 21. These samples were utilized for detection of MDA, Vitamin C, Nitric Oxide and Total antioxidant capacity. Results of the present study revealed that MDA and Nitric Oxide were be significantly ($p<0.05$) higher in the groups of PPA than the normal cyclic group. Vitamin C (Ascorbic acid) levels were seen to be significantly ($p<0.05$) lower in normal cyclic animals in comparison to inactive ovaries group and persistent C.L group ,while there is no significant difference with the silent heat group .**

No statistically significant difference was detected in the total antioxidant capacity between the group of silent heat and the normal cyclic group, while the groups of persistent C.L and inactive ovaries were found to have statistically significant difference ($p<0.05$) with the normal cyclic group. It is concluded that supplementing diets with optimal levels of micronutrients with antioxidant capabilities is a good advice to farmers to avoid post-partum anestrum. Moreover, early approaches to conflict the progression of stress and to promote the antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands appears to be Pertinent.

***Index Terms*— oxidant/antioxidant, anestrous Conditions, nitric oxide, total antioxidant capcity.**

I. INTRODUCTION

The reproductive efficiency in dairy cattle is affected by the postpartum ovarian activity. So, it is preferable that such activity must be resumed as early soon as possible after parturition .The duration of postpartum anestrus has enormous effect on reproductive performance [1], [2]. It has been recommended that the occurrence of anestrus happen in high rate in high-yielding dairy cows [3], [4].

In the past, a lot of research has been carried out in the field of reproductive endocrinology to recognize specific problems of reproduction and takeover procedures to improve reproductive efficiency. However, little information is obtainable in literature concerning antioxidant defense mechanisms during anestrus[5] .Studies accomplished on cattle have displayed that oxidative stress (OS) factors increase when the animals suffered from anestrus, repeat breeders, follicular cysts and metabolic diseases [6],[7].

Stress responses in heat, pregnancy and milk production result in formation of reactive oxygen and nitrogen species (ROS and RNS). These ROS and RNS comprise hydroxyl radicals, super oxide ion, hydrogen peroxide, nitric oxide radicals and are included in free radical chain reaction influencing lipid peroxidation plus apoptosis, and fertility [8]. The biological outcome of these ROS and RNS mediated free radical chain reaction result in infertility by influencing folliculogenesis, steroidogenesis and retained placenta [9], [10].

Antioxidant defense system decrease the free radical damage by get rid these ROS and blocking the free radical chain reaction to preserve the animals healthy. This defense system made out of two segments enzymatic and non-enzymatic. The enzymatic segment constituted of catalase ,superoxide dismutase (SOD) , and glutathione peroxidase (GPX) while the non-enzymatic segments contains reduced glutathione (R-GSH), vitamin C, vitamin E, β- carotene, and various macro and micro elements. Consequently, hypothesizing that the pro-oxidant and antioxidant balance emphasize the animal reproductive efficiency[11].The physiological events, during the period of postpartum in dairy cows, concerning the role done by these antioxidants and their levels relative to the ovarian changes are lacking [1].

The purpose of our investigation was to identify the connection between oxidant/antioxidant status and postpartum anestrous (PPA) condition in dairy cows.

II.MATERIAL AND METHODS

A. Animal management:

The present study was carried out at the Farm of dairy cows of Qena governorate for milk production and completed in labs of faculty of veterinary medicine in Aswan University. Seventy five postpartum anestrous (PPA) and twenty five normal cyclic Holstein Friesian pluriparous dairy cows were selected on the basis of their reproductive history gained from farm records.

According to the herd records, the cows that had shown anestrous for more than 120 days were selected in postpartum anestrous group (PPA) whilst cows coming in estrous before

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65 days of postpartum for more than three successive lactations involving present lactation were selected in normal cyclic group (control group).

Current status of reproductive organs of all cows in the study was also examined and confirmed by rectal examination, ultrasonography and progesterone assay.

The studied animals were classified into three groups (each of them 25 animals) as inactive ovaries group, persistent corpus luteum group and silent heat group.

The animals were preserved as per the standard feeding and managementpractices followed at the farms and were fed for body maintenance and according to the level of milk production, for this reason the green and dry fodder was supplemented with concentrate mixture which having mineral mixture. Cows were raised in open and closed system.

B. Rectal examination, blood sampling and progesterone assay:

Each animal of the selected anestrus cows was subjected for two successive rectal palpations and examined ultrasonographically at ten days interval to follow up the reproductive status.

Blood samples from anestrous and normal cyclic animals were collected by tail vein puncture at day 0 (day of estrus in cyclic group), day 10, day 21,into heparinized tubes then centrifuged to separate plasma and kept at -20°C until the analysis was accomplished..

Progesterone assay:Serum progesterone (P4) levels were measured by an enzyme linked immunoassay (ELISA) using progesterone kit (Human, PROG ELISA, GmBh, Germany).

C. Detection of blood plasma oxidant/ antioxidant levels

Determination of Malondialdehyde (MDA) levels were resolved by the technique detailed by [12]. Estimation of vitamin C (Ascorbic corrosive) in plasma was finished by strategy for [13]. Nitric oxide (NO) levels were recognized by technique revealed by [14] while Total antioxidant capacity (TAC) was resolved by [15].

D. Statistical analysis

Results of our study are displayed as means ± SEM for each group. Groups were tested for differences by the utilizing of the ANOVA test using version 20 of IBM SPSS software computer program.

III. RESULTS

The mean Plasma MDA, vitamin C (Ascorbic acid), Nitric oxide and Total antioxidant capacity levels were compared between PPA and normal cyclic animals (Table I).

Analysis showed that MDA levels appeared to be significantly ($p<0.05$)greater in the three groups of postpartum anestrous animals in comparison to the normal cyclic group as shown in Figure 1.

Vitamin C (Ascorbic acid) levels were seen to be significantly ($p<0.05$) lower in normal cyclic animals in comparison to inactive ovaries group and persistent C.L group ,while no presence of significant difference with the silent heat group as illustrated in Figure 2.

Nitric oxide scavenging activity was found to be significantly ($p<0.05$) elevated in the three groups of PPA than the normal cyclic group as shown in Figure 3.

No significant difference from statically point of view of the total antioxidant capacity was detected between the group of silent heat and the normal cyclic group, while the group of persistent C.L and inactive ovaries were found to have statistically significant difference ($p<0.05$) with the normal cyclic groupas in Figure 4.

Table I.Comparison of mean (±SE) values of plasma MDA, plasma Vitamin Plasma nitrous oxide scavenging activity and Total antioxidant capacity between PPA and normal cyclic animals.

Tests	Inactive ovaries	Persistent C.L	Silent heat	Normal cyclic cows
MDA	9.82 ±0.49*	8.10 ±0.49*	7.37 ±0.49*	3.10 ±0.49
Vitamin C	9.49 ±0.50*	8.09 ±0.50*	4.03 ±0.50	2.99 ±0.50
NO	42.21 ±.57*	39.55 ±.57*	41.02 ±.57*	14.69 ±.57
TAC	0.611 ±0.03*	0.310 ±0.03*	0.798 ±0.03	0.908 ±0.03
P value	P <0.05	P <0.05	P <0.05	P <0.05

- Values are (mean ± SE).
- Means carrying superscripts are significantly different at (p-value<0.05).

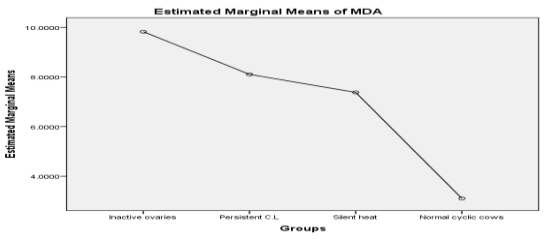

Fig.1 Estimated marginal means of MDA.

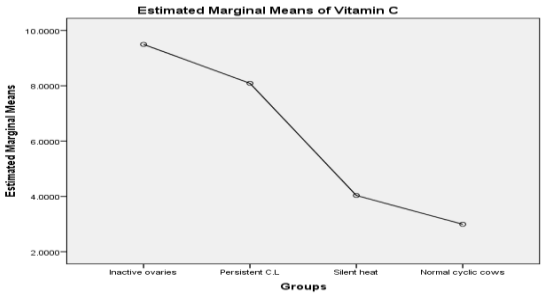

Fig. 2 Estimated marginal means of Vitamin C.

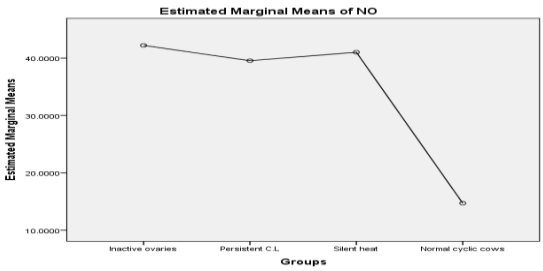

Fig. 3 Estimated marginal means of Nitric oxide.

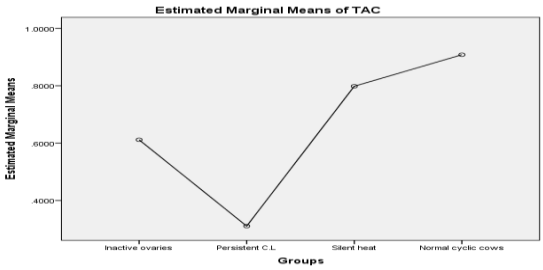

Fig. 4 Estimated marginal means of Total antioxidant capacity.

IV. DISCUSSION

In the present study lipid peroxidation (plasma MDA production) was significantly ($p<0.005$) higher in the groups of postpartum anestrous cows than normal cyclic group. The findings of our study are in corroboration with [16], [17] who reported that (plasma MDA production) was significantly higher in early lactating cows than advanced pregnant cows.

From the veterinary studies of view, OS factors increase when certain diseases happen. In the same time, oxidative stress in cows is a participatory factor to increase disease susceptibility [18], as metabolic demands accompanied by late pregnancy, parturition and initiation of lactation would be predictable to increase the production of reactive oxygen species (ROS), resulting oxidative stress. During advanced pregnancy and early lactation increased demand of micronutrients do not commonly full fill the resulting shortages occurring due to natural protective substances or excrescent exposure to stimulators of "reactive oxygen metabolites" (ROM), and this might lead to increased lipid peroxidation and decreased level of antioxidant enzymes.

In the present study Vitamin C (Ascorbic acid) levels were recognized to be significantly ($p<0.05$) lower in normal cyclic animals in comparison to inactive ovaries group and persistent C.L group ,while there is no statically significant difference with the silent heat group.

This result doesn't agree with that of [19], [20]who found that there is non-significant variation in vitamin C level in between postpartum anestrous animals and normal cyclic animals.

The role of Vitamin C as antioxidants is that it helps oocyte survival in calcium-free media and prolonged inhibition of oocyte maturation by CAMPbut did not prohibit oocyte maturation in the absence of CAMP[21]. Low levels of Vit C have an antioxidant effect but higher levels were able to exert pro-oxidative effects on the cell. This may explain their low levels in the normal cyclic animals and its high level in anestrous cows. In the same time deficiency of Vit C make ovarian atrophy, extensive follicular atresia. While, Supplementation of this antioxidant inhibits follicular apoptosis[22]. So the balanced presence of reactive oxygen species and antioxidants has a positive effect on oocyte maturation, fertilization and embryo development in vitro [23].This indicates that animal showing PPA is under oxidative stress resulting in decreased fertility.

Concerning nitric oxide, the obtained results indicate a significantly ($p<0.05$) higher in the three groups of PPA than the normal cyclic group.

This result is in agreement with that concluded by [24] who said that serum NO levels in diseased animals were greater than those of healthy animals. NO has been assured to work directly at the ovarian level, where it is emitted by the vasculature and neurons, plus various cell types, including granulosa, theca, and luteal cells. Nitric oxide production is adjusted by several hormones as estradiol 17β, luteinizing hormone, follicle-stimulating hormone, and human chorionic gonadotropin [25]. This may explain the high level of NO in anestrous animals as the hormonal disturbance of these cases of animals lead to this disturbance in the levels of NO. Furthermore, [26] concluded that a low level of nitric oxide is important for ovarian activity and implantation and on the other hand 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.

Furthermore, [26] concluded that a low level of nitric oxide is important for ovarian activity and implantation .

The obtained results of Total antioxidant capacity (TAC) in the groups of persistent C.L and inactive ovaries have statistically significant difference ($p<0.05$) with the normal cyclic group. Its values in the persistent C.L group were lower than that of cyclic group. While there is no significant difference between the silent heat group and normal cyclic one. This result is in concord with that of [27] who reported that there is an increase in the TAC during period of estrus in the study conducted to measure TAC and TOC levels at various points of the reproductive cycle in cows. On the other hand, it was detected that TAC levels were lower during the times when the progesterone level was high.

V.CONCLUSION

Stress resulted from pregnancy and lactation affect the reproductive performance in PPA animals which could be inherently more sensitive to these stressors than those cyclically normal ones. The performance of High yielding dairy cattle could become optimal for a specific degree by providing diets with optimal levels of micronutrients accompanied by antioxidant capabilities. As, oxidative stress keeps on being a big trouble during postpartum period, early approaches to conflict the progression of stress and to promote the antioxidant defense mechanisms during times of increased metabolic request gives an impression of being firmly related.

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