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THE RELATIONSHIP BETWEEN OXIDANTS / ANTIOXIDANTS IMBALANCE AND POSTPARTUM FERTILITY IN CATTLE

(With 2 Tables and 2 Figures)

By

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علاقة عدم توازن الأوكسدة و مضادات الأوكسدة بالخصوبة في فترة ما بعد
الولادة في الأبقار

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الهدف من هذه الدراسة هو العلاقة بين عدم توازن الأوكسدة و مضادات الأوكسدة و درجة الخصوبة في أبقار الألبان أثناء فترة ما بعد الولادة. تم في هذه الدراسة اختيار ١٦ حيوان بدء عندها نشاط المبيض و بدء دورة الشبق بعد ٥٦-٦٣ يوم من الولادة وسميت مجموعة (١). و ١٣ حيوان كانت أول دورة للشبق بها بعد الولادة بـ ٧٠-٨٤ يوم وسميت مجموعة (٢). و عشرة حيوانات بدء عندها نشاط المبيض و بدء دورة الشبق بعد أكثر من ٩٠ يوم من الولادة وسميت مجموعة (٣). أخذت عينات دم وريدي من كل الحيوانات وذلك في الأسبوعين الأولين قبل الولادة و بعد الولادة أسبوعيا حتى إعادة دورة الشبق في كل مجموعة. وكذلك تم أخذ عينات دم وريدي من مجموعة من الحيوانات غير عشر. وتم فصل السيرم من كل العينات الدم و حفظت حتى إجراء جميع القياسات. تم قياس دلائل ضغوط الأوكسدة (فوق أكسيد الدهون و أكسيد النيتريك) كما تم قياس مضادات الأوكسدة (نشاط أنزيم السوبر اكسيد ديسميوتيز و فيتامين هـ و السيلينيوم). وكذلك تم قياس بعض الهرمونات مثل البروجسترون و الاستروجين. و أتضح من هذه الدراسة أن دلائل ضغوط الأوكسدة قد ارتفعت معنويا في كل الحيوانات في الأسبوعين الأخيرة قبل الولادة و خاصة في حيوانات المجموعة الثالثة و في الأسبوع الأخير قبل الولادة. بعد الولادة وجد أن مستوي دلائل ضغوط الأوكسدة قد ارتفع معنويا بينما انخفض مستوي مضادات الأوكسدة في المجموعتين ٢ و ٣ و كانت هذه النتائج بصورة أكبر في حيوانات المجموعة (٣). كذلك وجد أن مستوي هرمون الأستروجين قد زاد في حيوانات المجموعة (٣) عن باقي الحيوانات. وكانت هناك علاقة ارتباط سالبية معنوية مع دلائل ضغط الأوكسدة بينما كانت هناك علاقة ارتباط موجبة معنوية مع هرمون البروجسترون. و من ذلك يستنتج أن زيادة مستوي دلائل ضغوط الأوكسدة في حيوانات المجموعتين (٢ و ٣) قد تؤدي إلي عدم عودة نشاط المبيض و تأخر دورة الشبق الطبيعية في هذه الحيوانات و ذلك بسبب موت الجر بيئات و فشلها في النضوج و التبويض.

ولذلك ينصح بإعطاء الحيوانات وخاصة أبقار الألبان أثناء الحمل مضادات الأكسدة (مثل فيتامين هـ و ج والسيلينيوم) والذي يفرز بكميات كبيرة في اللبن طبيعياً مما يؤدي إلى ضغط الأكسدة وزيادة دلائلها.

SUMMARY

The objective of this study was to determine the relationship of postpartum oxidative stress and the ovarian anestrus in the dairy cows. Sixteen cows showed normal ovarian cyclicity (normal estrous cycle) detected after 56-63 days postpartum and kept as G1. Thirteen cows showed ovarian cycle after 70-84 days postpartum, G2 and other ten animals showed marked delayed in the ovarian cyclicity more than 90 days, G3. Venous blood samples were taken weekly from all animals in the last two weeks of pregnancy and in postpartum period as well as, sera of blood samples were kept for measuring all parameters. Another blood samples from ten non-pregnant cows in diestrus were taken. The oxidative stress (lipid peroxides and nitric oxide), antioxidants (superoxide dismutase, vitamin E and selenium), as well as estradiol-17 β and progesterone were measured in all animals. In prepartum, lipid peroxides as well as nitric oxide were increased in the last week of pregnancy. In postpartum the preoxidative indices were elevated in the animals of G3 while antioxidants were decreased significantly in G3 and to some extent in G2. Estradiol-17 β hormone levels were increased significantly in animals of G3 and it was negatively significant correlated with LPO and NO. While progesterone levels were positively significant correlated with antioxidants in the animals of G3. The oxidative stress in these animals may be led to follicular damage and failure of maturation and ovulation. It was concluded from this study that excess free radical production may be play a role in the prolongation of the postpartum anestrus in dairy cows. These may be due to exhaustion of antioxidant system in milk production. Therefore, it was advise to supplement the dam with antioxidants during the late stage of pregnancy and in the postpartum period to stimulate the normal ovarian activity.

Key words: *Antioxidants, Oxidative stress, Vitamin E, Selenium, Prepartum, Postpartum, SOD, Nitric Oxide, Lipid Peroxidation.*

INTRODUCTION

Cattle production may be divided into two sectors, dairy and beef production. The main objective in the dairy herd is to produce milk as

economically as possible. Reproduction is a vital factor in determining the efficiency of animal production (Ball and Peters, 2004). Reproduction efficiency can be described as a measure of the ability of a cow to become pregnant and produce viable off-spring. Infertility or sub-fertility are varying degrees of aberration from typical levels of reproductive performance. Reproductive performance of cows affects the efficiency of milk production in the herd because of its influence on the calving to service interval, calving pattern, length of lactation and culling rate (Roche, *et al.*, 1992).

Parturition is followed by a period of ovarian inactivity and sexual quiescence before reproductive cycle recommence. Nutrition plays an important role on the beginning of the reproductive cycles (Borghese, *et al.*, 1997). The postpartum anestrus period is the time after parturition that is necessary for recuperation and reorganization of the brain and reproductive organs. Normally the postpartum interval is 60-70 days in dairy cows.

The antioxidants vitamins (e.g.vitamin E) is important for improve the fertility of the dairy cows (Allison and Laven, 2000). In late pregnancy, the production of free radicals was increased (Toescu, *et al.*, 2002). These elevation in free and increasing in oxidative damage may involved in the of parturition, but the over increase in the oxidative process after parturition may be led to placental retention and/or postpartum infertility. Selenium (Se) and vitamin E are considerable antioxidant agents. Se is an essential co-factor for the enzyme glutathione peroxide which is vital to prevent the production of free radicals (Basini, *et al.*, 1996) as well as, Se catalyses the reduction of peroxides to less harmful hydroxyl acids in the cytoplasm. Moreover, vitamin E is localized in the cell membranes as a biological antioxidant. If this protective function of vitamin E and Se fails, the increased quantities of free radical and may lead to the damage of biological membranes and cell death.

The reactive oxygen species have been implicated as major initiators of tissue damage and can up-regulate enzyme activity, signal transcription, and gene expression may be apoptotic genes (Palmer and Paulson, 1977, Sen and Packer 1996). Antioxidant defenses made up of intracellular and extracellular components work together to obtain an optimal redox balance. Reactive oxygen species play a number of significant diverse roles in female reproductive biology including uterine environment, oocyte maturation and ovulation, corpus luteum function and regression (Riley and Behrman, 1991 and Megahed *et al.*, 2002).

Oxyradicals in the ovarian tissues may affect the growth of the follicles and cell-permeant antioxidants inhibit spontaneous resumption of meiosis, which may implicate a role of oxygen radicals in oocyte maturation (Behrman *et al.*, 2001).

In the present work we will consider the relationship between oxidants/antioxidant imbalance and postpartum hormonal condition in the cattle as well as their possible interactions with fertility.

MATERIALS and METHODS

1. Animals and their managements.

Forty-five pregnant and ten non-pregnant (in luteal phase of estrous cycle) dairy cows were used in this study. The age of the animals ranged from 4-7 years and their parity was 2-4. They were housed in Assiut village under the same ordinary or the same feeding and management conditions. In addition, the animals were given a free access to drinking water. The animals were selected at the last two weeks prepartum. Following parturition, the cows were allowed for suckling. Calves were removed about 3 days after birth. Milking was carried twice daily. The animals were observed twice daily for estrus. Thirty-nine out of 45 cows shows estrus after observation. According to observable heat, the animals were divided into 3 groups. The 1st group (G1, n=16), which considered an animals shows the first estrus after 56-63 days postpartum. Second group (G2, n=13), where the animals shows the first estrus after 70-84 days postpartum as well as the animals shows the first estrus after more than 90 days postpartum were considered as 3rd group (G3, n=10). The animals calved in the period between March and May as well as the annual average value of air temperature and relative humidity were 39.3°C and 69.2% respectively. All animal were inseminated naturally at the first detectable estrus after calving and observed again for return heat then examined rectally 45-60 days after insemination for pregnancy. The animals, which returned again to heat were inseminated again.

2. Blood sampling.

Blood samples were taken weekly (prepartum and postpartum) by via jugular venipuncture and collected in a clean sterile centrifuged tubes to separate serum for all measurements investigated. The time of collection of all samples was subsequently standardized from the time of parturition taken as day 0. The blood samples of the non-pregnant animals were taken in diestrus phase. Serum was pipetted into glass vials and stored at -20°C until assay for Biochemical and hormones.

3. Biochemical analysis.

Lipid peroxidation (LPO) was measured as thiobarbituric acid reactive substances (TBARS). The product of the reaction between malondildehyde and thiobarbituric acid was measured as describes by Buege and Aust (1977), with using 1, 1, 3, 3- tetramethoxypropane as standard. Nitric oxide (NO) levels were measured as nitrate concentration after reduction of nitrate to nitrite with Griees reagent. The reaction was measured using sodium nitrite as standard according to Ding *et al.* (1988). Superoxide dismutase (SOD) activity was estimated according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to Misra and Fridovich (1972). Vitamin E was determined according to Aebischer *et al.* (1999) by using a Hewlett Packard 1090 automated HPLC equipped with diode array and fluorescence detectors. The type of used column was a Primesphere C18-HC, 250 x 4.6 mm, 5 μ m paticle size (Phenomenex), eluted with acetonitrite : tetrahydrofuran : methanol 1:1 % (w : v) ammonium acetate (684: 220: 68: 28) at a flow rate of 0.7 mL/min. Serum selenium was quantified according to Ericson *et al.* (1986) using graphite furnace atomic absorption spectrophotometer (BE 744A).

4. Hormonal assay.

Progesterone (P₄) and estradiol-17 β (E₂) concentrations in the blood serum were determined by commercial ELISA kits (BIOSURCE, EUROPS S.A) and automatic ELISA reader Anthos, 2000.

5. Statistical analysis.

Data were expressed as the mean \pm SEM for all parameters. The data were analyzed by using Analysis of Variance (ANOVA) with Bonferroni's post-test. The data of animals in prepartum and non-pregnant were analyzed using ANOVA and post-test Dunnett for multiple comparisons with confidence intervals at 90% as appropriate. The correlation coefficient with Pearson test were done between LPO, NO and other parameter. Results were considered significant at P > 0.05 or less. The graphs of these parameters were done using Prism 3 Graph Pad computer programmed.

RESULTS

The obtained results are presented in Tables 1, 2 and Figures 1 and 2. Table 1 shows that LPO levels were increased significantly (P < 0.01 and P < 0.05) in G2 and G3 during 2nd and 1st week before

parturition when compared with non-pregnant cows. While it was elevated significantly ($P < 0.05$) in 1st week before parturition in G1 but increased non-significantly in G2 when compared with non-pregnant cows. The nitric oxide (NO) levels were increased significantly ($P < 0.01$) in animals of G3 before parturition by 2nd and 1st weeks, however it increased significantly ($P < 0.05$) in G1 and G2 in comparison with non-pregnant animals. SOD levels were decreased significantly ($P < 0.5$, $P < 0.01$) in animals of G3 at 2nd and 1st week before parturition respectively. Moreover, it was increased significantly ($P < 0.05$) at both 2nd and 1st week before parturition in the animals of G2. In G1, SOD levels were decreased non-significantly in comparison to non-pregnant animals. Looking to the same table, vitamin E and selenium levels recorded a significant ($P < 0.01$) decreased in all groups at 1st week before parturition.

Table 2 shows that the correlation between oxidative stress (LPO, NO) and antioxidants (SOD, Selenium and vitamin E) with progesterone (P₄) and estradiol-17 β (E₂) in groups of animals during postpartum. LPO were negative correlated with SOD activities, vitamin E and selenium in all groups of animals. In G1 and G2, LPO had a non-significantly negative correlation ($r = -0.1$ and $r = -0.2$) with SOD, however, in G3, it became significantly ($P < 0.01$) negative correlation ($r = -0.65$). There were a significantly ($P < 0.01$) negative correlation ($r = -0.71$, -0.64 , -0.77) between LPO and vitamin E, selenium and E₂ in G3 respectively, but the correlation between LPO and P₄ was positively significant ($P < 0.05$) correlation ($r = 0.58$) in G3. The same recorded results were noticed between NO and SOD activities, vitamin E, selenium, E₂ and P₄ in G3 expect, the significant results between NO with selenium became $P < 0.05$ and $P < 0.01$ in E₂. Moreover, it became $P < 0.01$ in case of P₄.

Fig. 1 shows that the levels of LPO, NO, SOD, vitamin E and selenium hormone in all the three groups of animals (G1, G2 and G3) from the first week postpartum ended by the week of ovarian resumption. Generally, LPO levels were significantly increased ($P < 0.01$) in G3 versus G1 and G2. At the first week after parturition, the levels of LPO were 0.97 ± 0.12 (in G1), 1.89 ± 0.1 (in G2) and 3.76 ± 0.45 nM/ml (in G3). These obtained results decreased significantly ($P < 0.01$) near the weeks of ovarian resumption in each group (8th and 9th week in G1, 11th and 12th week in G2 and 14th and 15th week in G3). The same recorded results of NO were in each group were observed. The levels of antioxidant system in this study (SOD, vitamin E and selenium) were

decreased significantly ($P<0.05$) at 1st week after delivery. Furthermore, these recorded results were increased significantly ($P<0.01$) near the weeks of ovarian resumption in each group (8th and 9th week in G1, 11th and 12th week in G2 and 14th and 15th week in G3).

Fig. 2 shows that the concentrations of E₂ and P₄ were fluctuated during postpartum period. The concentrations of E₂ were increased significantly ($P<0.01$) in animals of G1 and G2 and significantly ($P<0.05$) in G3 near the weeks of ovarian resumption in each group. While P₄ decreased significantly ($P<0.01$) in all groups near the weeks of ovarian resumption.

Table 1: The levels of oxidative stress (lipid peroxidation LPO, nitric oxide NO) and antioxidants (SOD, Selenium and vitamin E) in groups of animals during the last two weeks of prepartum and its comparison with levels in non-pregnant cows (n=10).

	non-pregnant cows	2 nd week prepartum			1 st week prepartum		
		G1 (n=16)	G2 (n=13)	G3 (n=10)	G1 (n=16)	G2 (n=13)	G3 (n=10)
LPO (nM/ml)	1.08±0.07	1.82±0.07	2.34±0.11*	2.55±0.25*	2.17±0.14*	2.64±0.35**	2.8±0.58**
NO (nM/ml)	23.63±0.91	29.31±0.31*	27.58±0.57*	30.55±0.65**	29.74±0.48*	27.95±0.65*	31.39±0.37**
SOD (U/ml)	10.63±1.01	10.26±0.36	9.27±0.53*	8.39±0.58*	10.58±0.55	9.06±0.58*	4.97±0.12**
Vitamin E (µg/100ml)	60.8±1.55	59.57±0.76	58.87±0.25*	58.06±0.21*	56.18±0.25**	53.94±0.55**	53.21±0.23**
Selenium (µg/100ml)	8.9±0.12	8.28±0.41	7.82±0.32*	6.40±0.83**	5.54±0.45**	4.66±0.65**	4.07±0.32**

* significant at $P<0.05$. ** significant at $P<0.01$.
No letter means non-significant.

Table 2: The correlation between oxidative stress (lipid peroxidation LPO, nitric oxide NO) and antioxidants (SOD, Selenium and vitamin E) with progesterone (P₄) and estradiol-17β (E₂) in groups of animals during postpartum.

	LPO			NO		
	G1	G2	G3	G1	G2	G3
SOD	- 0.10	- 0.20	- 0.65**	- 0.04	- 0.15	- 0.56*
Vit. E	- 0.01	- 0.51*	- 0.71**	- 0.16	- 0.39*	- 0.77**
Sel.	- 0.02	- 0.41*	- 0.64**	- 0.25	- 0.58*	- 0.46*
E ₂	+ 0.13	+ 0.51	- 0.79**	- 0.17	- 0.32	- 0.7**
P ₄	+0.11	- 0.30	+ 0.58*	+ 0.40	+ 0.41	+ 0.6**

* significant at $P<0.05$. ** significant at $P<0.01$.
No letter means non-significant.

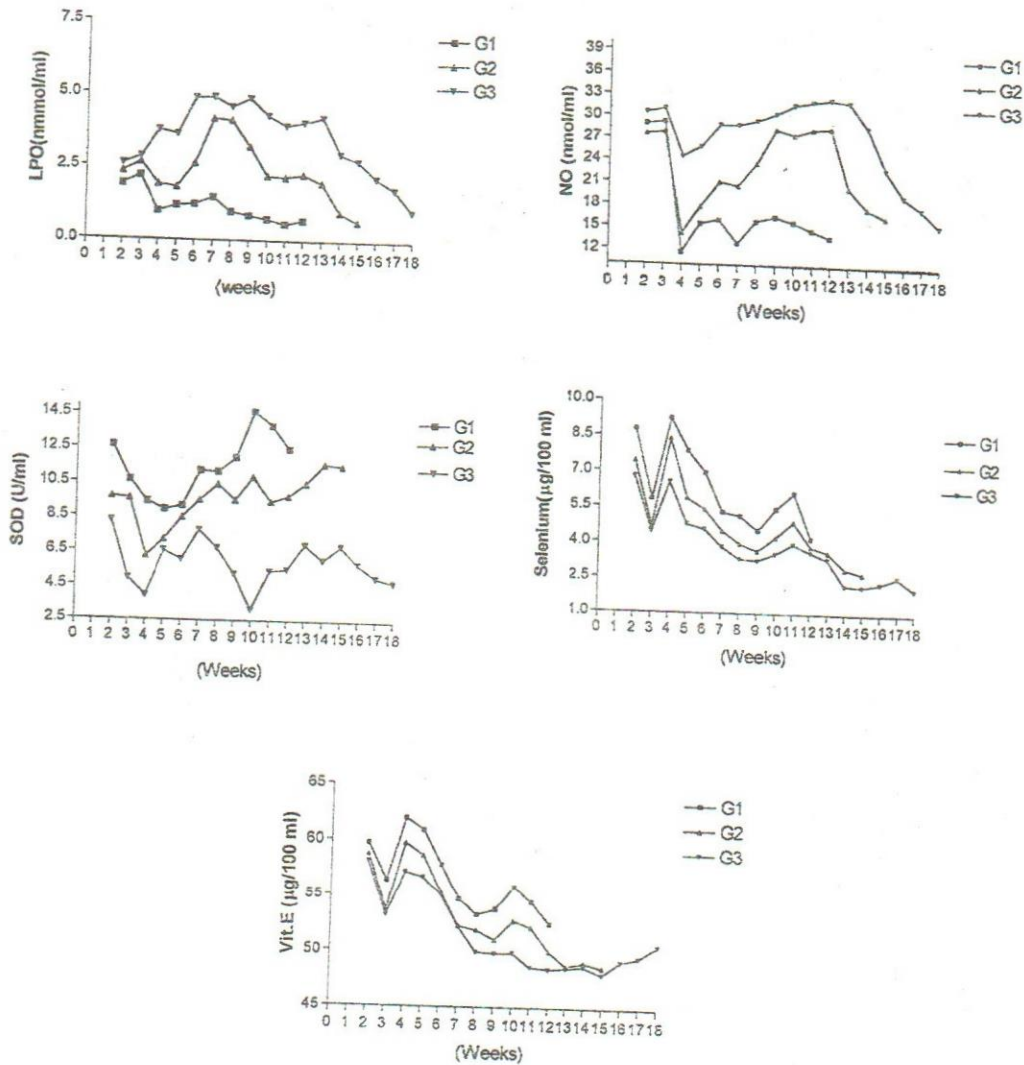


Fig. 1: Comparison between oxidative stress (lipid peroxidation LPO, nitric oxide NO) and antioxidants (SOD, Selenium and vitamin E) in groups of animals during the last two weeks of prepartum and postpartum period.

* Weeks 2,3 means a 2nd and 1st week before parturition.

* Weeks 4 to 18 means 1st to 15th week postpartum.

* Ovarian resumption completed after 56-63 days in G1, 70-84 days in G2 and more than 90 days in G3.

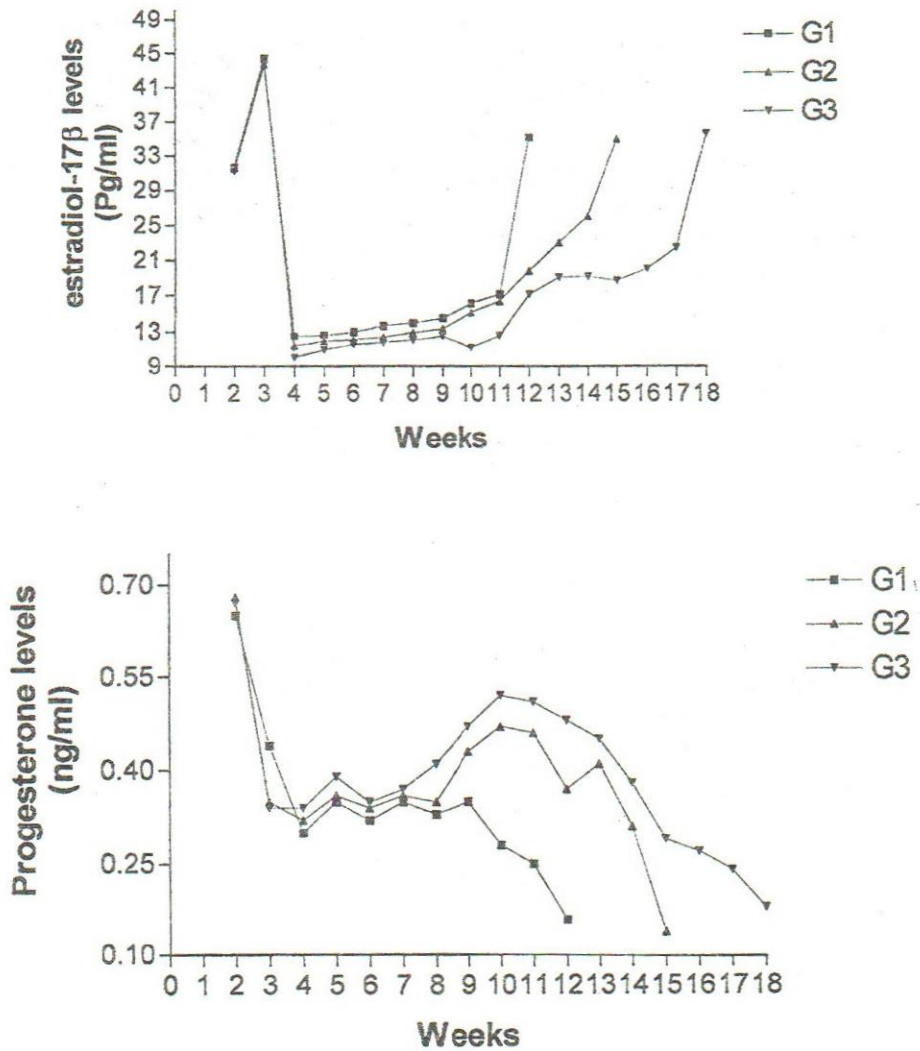


Fig. 2: Levels of estradiol-17β (pg/ml) and progesterone (ng/ml) in groups of animals during the last two weeks of prepartum and postpartum period.

* Weeks 2,3 means a 2nd and 1st week before parturition.

* Weeks 4 to 18 means 1st to 15th week postpartum.

* Ovarian resumption completed after 56-63 days in G1, 70-84 days in G2 and more than 90 days in G3.

circulation levels of gonadotropine and steroidogenesis (Abou-Seif, and Youssef, 2001).

Free radicals stimulate apoptosis in ovarian tissues and causes follicular atresia in ovary and the antioxidants including estradiol reduced the apoptotic cells by scavenger of the free radical (Murdoch, 1998). The relation between apoptosis and oxidants/antioxidants imbalance were established. Caspase- 3 apoptotic enzymes were induced by free radical production and it stimulate apoptosis in follicular cells on ovarian tissues (Boone and Tsang 1998). There is a link between bcl-2 antiapoptotic gene and oxidative stress in cells. Oxygen free radicals involved in the process of granulosa cells apoptosis during atresia (Tilly and Tilly, 1995). They reported that oxidative stress inhibited the expression of bcl-2 gene. Moreover, they found that gonadotropin mediated follicular survival through enhanced expression of antioxidants. Oxidative stress to follicles inhibited the ability of FSH to prevent apoptosis in granulosa cells (Tilly and Tilly, 1995).

One could be concluded that the prolongation of postpartum anoestrus in dairy cows controlled by many factors and one from these factors may be oxidative stress and exhaustion of the antioxidants, particularly in dairy cows. This may impair of follicular growth and failure to produce fertile cycle. Therefore it was advise to supplement the dam with antioxidants during the late stage of pregnancy and in the postpartum period to stimulate the normal ovarian activity and cyclicity.

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