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Postpartum Ovarian Resumption in Native Dairy Cows in Upper Egypt and their Relation to Oxidant Antioxidant Status

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Abstract

The present study aimed to characterize the follicular pattern and ovarian resumption during the postpartum period in lactating dairy cows in Upper Egypt and study the impact of different components of the antioxidant system on follicular dynamics. Sixteen native dairy cows in their second lactation period were assigned for the current study. Cows were kept indoor and subjected to ultrasonographic examination and bled daily. Data regarding the change in the follicular dynamics and incidence of short cycles were collected and tabulated then statistically analyzed. Blood samples were used to determine nitric oxide (NO), Vitamin A, B-Carotene, Vitamin C and Vitamin E. results of the present study revealed that The first postpartum behavioral estrus observed 35-47 days (41.5 ± 1.86 days postpartum). The average postpartum days required for the animal to come in estrus in cows with transient CL was 44 ± 1.76 days and in cows without transient CL was 37 ± 1.18 days. In cows with transient corpus luteum, first ovulation was not accompanied by behavioral estrus (Quiet ovulation) at 23 ±1.02 days. Vit A, B-carotene, Vit E, NO, but not Vit C, increased significantly during the first observable postpartum heat in the studied cows and their level in the plasma correlated differently with the different classes of ovarian follicles. It is concluded that most antioxidants in the present study, except ascorbic acid, increases at the time of estrus and they are not affected by the day of postpartum period or the number and size of the dominant follicles. Short cycles are frequent in the postpartum cows (50% of cows had a short cycles in the present work). Follicular growth starts immediately – may be before the studied time- in postpartum dairy cows.

Keywords: Postpartum; Dairy cow; Oxidant/antioxidant; Follicle; CL

Introduction

The reproductive efficiency in dairy cattle is influenced by the postpartum ovarian activity. Therefore, it is desirable that such activity must be resumed as early as possible after parturition. Energy balance is the primary factor determining the length of acyclic period and can be reduced by increasing dietary energy [1]. The corpora lutea resulted from the first ovulation postpartum fail to develop to the normal size and had shorter lifespan than corpora lutea of normal estrus cycles [2]. The first corpus luteum formed in postpartum dairy cows following hormone-induced or spontaneous ovulation was frequently short-lived resulting in a luteal phase shorter than normal duration [3]. Cows had short luteal phases after first ovulation with an average interval of 8.5 ± 0.2 days between first and second ovulation [4]. Antioxidants are enzymes or compounds that scavenge and reduce the presence of free radicals. Normally, a balance exists between concentrations of reactive oxygen species and antioxidant scavenging systems [5]. The transition period is critical for the health of dairy cattle [6]. It has been observed that during the transition period cows can experience oxidative stress [7-9], which may contribute to periparturient disorders [10,11], and may be associated with metabolic diseases [9]. Oxidative stress can be monitored with several biomarkers (antioxidants and pro-oxidants) which can be assessed in plasma and/or erythrocytes [12]. Nitric oxide (NO) is an inorganic, short-lived (a few seconds) free radical gas that, due to its high solubility, freely diffuses through biological membranes. Parenteral vitamin E also helps prevent reproductive disorders in periparturient cows [13]. Therefore, adequate Se nutrition is critical for managing oxidative stress in infected mammary glands of dairy cows. Vitamin C is normally produced by the liver of adult cows and is active both in blood plasma and in the cytoplasm of cells. Its function is to scavenge free radicals and regenerate plasma membrane-bound vitamin E and cytosolic glutathione peroxidase [14]. The physiological events, during the postpartum period in dairy cows, regarding the role of these antioxidants and their levels relative to the ovarian changes are lacking. Hence, the present study aimed to outline the ovarian changes during the postpartum period in cows and correlate them with the oxidant/antioxidant status.

Materials and Methods

Animals

The present study was carried out on 16 native pluriparous cows (average age, 2-6 years, weight, 250-400 Kg) belonging to the veterinary hospital of the faculty of veterinary medicine, Assiut university. Cows kept indoor with daily exercise outdoor. Animals fed on Barseem (Trifolium alexandrenum) in addition to concentrate mixture 1-2 Kg / head /day during the time with free access to water.

Experimental design

Animals in this study were assigned for daily clinical examination and blood sampling during the postpartum period in regular basis starting on d 5 postpartum till the first postpartum behavioral estrus. Cows were categorized according to the number of follicular waves, presence of short cycles or presence of transient CL.

Sampling

Blood samples were obtained by jugular venipuncture from all animals at day 5 postpartum then day after day till the first postpartum

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heat. Samples were collected into heparinized vacutainer tube and were centrifuged at 3000 rpm for 15 min, plasma separated and stored at -20°C till the time of biochemical assay.

Ultrasonographic examination

Cows were examined ultrasonographically using a real-time B-mode 100 LC-scanner (Pie Medical, Maastricht, Netherlands) connected to a 6/8 MHz changeable transrectal linear array transducer. At each examination, the number, diameter and relative position of all follicles > 5 mm in diameter and corpora lutea (CL) were recorded and sketched on ovarian charts to analyze the pattern of growth or/and atresia. When a follicle or CL was not spherical a mean diameter was taken. Follicles were classified into three size classes: small follicle < 5 mm, medium sized follicle 5-10 mm and large follicles >10 mm.

Follicular data analysis

The growth and atretic rates (mm /day) of ovarian follicles and CL with their day of maximum diameter were regarded. In addition, number of follicular waves for each cow was determined.

Determination of blood plasma oxidant/ antioxidant levels

Nitric oxide, vitamin A and β- Carotenes, Ascorbic acid and vitamin E levels in the plasma of these animals were measured according to the procedures of previous studies [15-18].

Statistical analysis

The packaged SPSS program for windows version 10.0.1 was used for statistical analysis [19]. Data were expressed as mean ± standard error (SE). Pearson's correlation (r) and linear regression analysis (R^2) and the linear regression equation (Y= X+K; where K is the soap of regression) were performed on the paired data obtained by the individual infected cases. Significance level was set at P≤0.05.

Results

Ultrasonographic findings

Data regarding the number of different classes of the ovarian follicles and corpora lutea during the postpartum period is presented in Figure 1. Day of the studied period had no effect on the number of large follicles and CLs (P< 0.05). Four waves (Figure 2-4) were recorded in 5 animals during the studied period (31.25%) while three waves were recorded in 11 out of 16 studied cows (68.75 %) with or without short

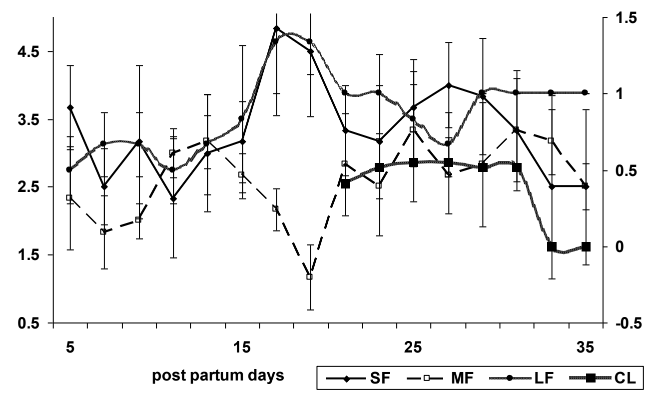

Figure 1: change in the No. (Y axis) of ovarian follicles and CLs during the post partum period.

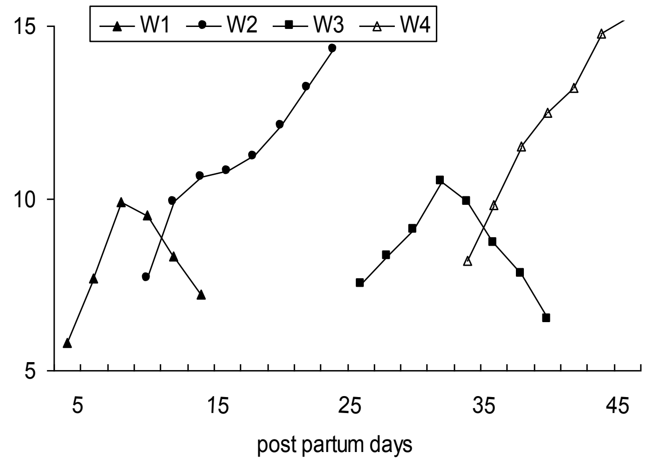

Figure 2: Follicular pattern in 4-waves animals during the post-partum period (Y axis is the size of the dominant follicle in mm).

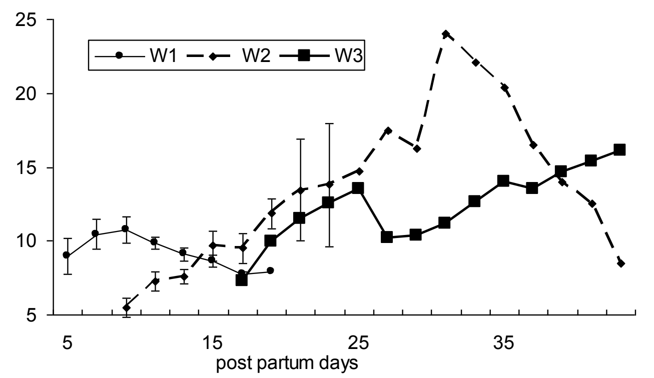

Figure 3: Follicular changes during the post partum period No CL group (Y axis refers to size of the dominant follicle in mm).

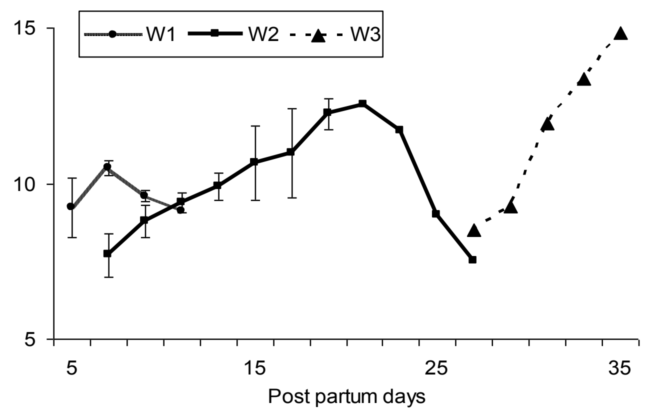

Figure 4: Follicular changes during the post partum period in CL group (Y axis refers to size of the dominant follicle in mm).

cycles and transient CL. Dominant follicle of the first follicular wave reached its maximum diameter (10.42 ± 0.34 mm /day) at 9.3±0.06 days postpartum. The first postpartum behavioral estrus observed 35-47 days

(41.5 ± 1.86 days postpartum). The first, second, third and fourth waves averaged 9.7, 18.7, 15 and 8 days respectively. The average diameters of the dominant follicle in cow having three waves were 10.95 ± 0.51, 13.6 ± 0.95 and 15.9 ± 0.11 mm /day for the 1st, 2nd and 3rd follicular waves respectively. For cows having four waves were 10.95 ± 1.07, 13.7 ± 0.66, 10.25 ± 0.25 and 15.1 ± 0.1 mm /day for the 1st, 2nd 3rd and 4th follicular waves respectively. The growth rate of the dominant follicle in cow having three waves were 0.61 ±0.14, 0.63 ± 0.14 and 0.59 ± 0.64 mm /day for the 1st, 2nd and 3rd follicular waves, respectively and for cows having four waves were 0.48 ± 0.05, 0.41 ± 0.04, 0.42 ± 0.09 and 0.55 ± 0.03 mm /day for the 1st, 2nd, 3rd and 4th follicular waves, respectively. The atretic rate of dominant follicle in cow having three waves were 0.39± 0.06, 0.32 ± 0.1 for the 1st, 2nd follicular waves while for cows having four waves were 0.48 ± 0.03, 0.66 and 0.47 ±0.21 for the 1st, 2nd and 3rd follicular waves, respectively. Transient corpus luteum average diameter was (13.1± 0.32 mm/day, mean life span was 7.3 ± 0.58 days and the atretic rate was (0.48 mm /day). Atretic rate of CL graviditatis (CL of pregnancy) was 0.61 mm /days. The average postpartum days required for the animal to come in estrus in cows with transient CL was 44 ± 1.76 days and in cows without transient CL was 37 ± 1.18 days. In cows with transient corpus luteum, first ovulation was not accompanied by behavioral estrus (Quiet ovulation) but observed ultrasonically at 23 ±1.02 days. Maximum diameter of the ovulatory follicle resulting in transient CL was (14.03 ± 0.03mm) during the second wave. The growth rate of the dominant ovulatory follicle of this group was (0.54±0.07 mm /days) and the atretic rate was 0.47 ± 0.053 mm / days. The diameter of ovulatory follicle resulting in cyclic CL of the first postpartum behavioral estrus was 15.65 ± 0.18 mm/day. Ovulation took place at the right ovary in 10 cows and the left ovary in 6 cows. All cows showed ovulation from the ovary contra lateral to CL graviditatis.

Oxidant /antioxidant status

Similar to vitamin A and B- carotene, there was a constant and gradual increase in vitamin E during the postpartum period. Peak levels for these antioxidants were found at the day of first postpartum observable estrous (Table 1). No relation was found between number of follicle and level of vitamin E in plasma. Vitamin C and NO production decreased gradually throughout the postpartum period till first ovulation and their plasma concentration was not affected by the number of different classes of ovarian follicles (Table 2). Diameter of the ovarian follicles significantly (P < 0.05) affected the level of Vitamin E, C and NO (Table 3).

Discussion

Follicular growth was detected as early as D5 postpartum. The interval to detection of the first postpartum DF was reported to be 9.6 ± 0.6 days in beef cows and the number of DF before the first ovulation was reported to be 2.1 ± 0.6 [20]. The emergence of a wave is associated with a surge in FSH concentrations in cycling cows [20,21]. There is follicular development in both ovaries of postpartum cows; however, postpartum follicular activity in the ovary ipsilateral to the previously gravid uterine horn was reported to be lower than that in the contralateral ovary [22]. As the follicular growth is a continuous process we think that the ovarian resumption may be active before that day but the ovaries were unreachable. In the present study, interval from parturition till first heat averaged 35 + 0.56. The postpartum anestrus in the study is significantly shorter than reported previously [23], 80.8 ± 8.6 days for Holsteins and 104.8 ± 7.6 days for crosses. Short cycles (transient CLs resulting from silent ovulations) were recorded in 8 cows (50%) in the present study. The occurrence of a short luteal phase following first ovulation in the postpartum period of cattle reported previously [24]. The first ovulation postpartum generally occurs with silent estrus and is followed by a short estrous cycle of 8 to 12 days of duration in the majority of cows [25-28] Occurrences of short estrous cycles frequently appear during the first 30 to 40 days postpartum. The oocyte released during this short estrous cycle in cattle can be fertilized [29]. However, pregnancy is not maintained, apparently because the corpus luteum is regressing before the ovary receives the uterine signal that a pregnancy exists [30,31]. Short cycles are also common after induced ovulation in the postpartum period by weaning, weaning plus GnRH injection, a single injection of GnRH, intermittent injection of GnRH and continuous infusion of GnRH as well as after the first ovulation at puberty [32-37]. Premature release of prostaglandin F2α (PGF2α) from the uterus on day 5 of a short estrous cycle is probably the mechanism involved in subnormal luteal function in sheep and cattle [38]. Similar conclusions were obtained when premature release of PGF2α (from the uterine endometrium) resulted in premature luteolysis when suckling induced an oxytocin release [37,38]. The CL that is formed during a short cycle is smaller and secretes less P4 than a CL during a normal cycle [39,40]. Short estrous cycles prevent fertility during the first 20 days after parturition by causing the cow to return to estrus before pregnancy recognition occurs [29]. The duration of the postpartum anestrus is affected by four major factors: season, nursing, nutrition and cow age [41,42]. Day of the studied period had no effect on the number of large follicles and CLs (P< 0.05). The duration of postpartum anestrus is not determined by emergence of follicular waves, but rather by follicular deviation and/or the fate of the dominant follicle [43]. The emergence of the first follicle wave occurred within 10–14 days [44] of parturition and is associated with the early resumption of recurrent FSH increases [20], presumably due to the abrupt withdrawal of the negative feedback effect of estrogen and progesterone before parturition. Prolonged postpartum anoestrus is due to failure of early dominant follicles to ovulate [44,45]. Dairy cows that are not under nutritional stress generally ovulates the first dominant follicle [26], earlier than beef suckler cows with a good body condition score [44]. However, beef cows in poor body condition can have 8–14 follicle waves before the first ovulation [27]. Failure of the early dominant follicle to ovulate is thought to be due to inadequate LH pulse frequency [41,45,46], which results in low androgen production in the follicle [47] and inadequate estradiol positive feedback to induce a preovulatory gonadotrophins surge [48]. On the contrary to our

Days around parturition	0 (Parturition)	10 days post partum	20 days post partum	Estrus
β- Carotene.(ug/dl)	84.19 ± 13.96^{a}	110.52 ± 17.22^{ab}	146.00 ± 13.83^{b}	167.65 ± 11.88^{c}
Vit. A. (ug/dl)	42.59 ± 6.03^{a}	54.49 ± 5.45^{ab}	56.30 ± 5.87^{ab}	58.18 ± 3.83^{b}
Vit. E.(ug/dl)	138.40 ± 17.61^{a}	170.4 ± 15.61^{b}	193.87 ± 19.07^{c}	205.60 ± 23.24^{c}
Vit. C.(mg/dl)	3.29 ± 0.48^{a}	2.59 ± 0.28^{a}	2.15 ± 0.16^{a}	2.51 ± 0.17^{a}
NO.(nmol/ml)	64.33 ± 9.88^{a}	40.28 ± 7.61^{b}	36.80 ± 4.58^{b}	45.44 ± 0.88^{b}

Letters with different superscripts are significantly different (P<0.05).
Differences are set between values in the same row.

Table 1: Mean values of measured antioxidants and the corresponding free radicals (NO) during peripartum period in subgroup B (n=10).

	B Car	Vit A	Vit E	Vit C	NO
rn	0.159	0.206	0.147	0.111	0.095
P	0.121ns	0.044*	0.153ns	0.281ns	0.355ns

rn: - Correlation between number of large follicles and biochemical parameters

Table 2: Correlation between number of large follicles and biochemical parameters.

	B Car	Vit A	Vit E	Vit C	NO
rd	0.132	0.113	0.313	0.285	0.335
P	0.200 ns	0.273 ns	0.002**	0.005**	0.001***

rd: - Correlation between diameter of large follicles and biochemical parameters

Table 3: Correlation between diameter of large follicles and biochemical parameters.

findings, in a previous study [49], the average number of small follicles (3 to 5 mm diameter) decreased before Day 25 postpartum whereas the number of large follicles (10 to 15 mm or more) increased with increasing postpartum days. Moreover, energy balance and dietary treatments influenced the number of follicles at different times after calving. In the present study, cows have 3-4 waves till first heat in accordance with previous studies [50,51]. Cows had relatively short postpartum anoestrous intervals with a maximum number of five waves were recorded [44]). On the scope of the present results, we noticed that the average diameters of the dominant follicle were increased gradually from the 1st to the 3rd follicular wave while in cows having four waves we found that the diameter decreased in 3rd wave then increased in the 4th wave. Because most dairy cows ovulate for the first time between the second and fourth weeks, any delay beyond the fourth week was defined as a delayed ovulation or anovulation [52]. The management practices that limit suckling must also avoid close cow-calf association to reduce long postpartum intervals to first ovulation [53].

Under the normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense system used by organisms to deactivate and protect them against free radical toxicity [54]. The studied antioxidants, but Vit C, increased significantly at the time of first postpartum ovulation associated with observable heat in the current study. In the contrary, Vit C and No had a peak level at calving and decreased significantly at the time of estrus. It was found that antioxidants were not cytotoxic except at high levels, and the cells appeared less damaged when incubated with antioxidants than without, particularly over long time periods of incubation. Antioxidans (Vit E and C) assisted oocyte survival in calcium-free media and extended inhibition of oocyte maturation by cAMP but did not inhibit oocyte maturation in the absence of cAMP [55]. Low levels of ascorbic acid have an antioxidant effect but higher levels were able to exert pro-oxidative effects upon the cell. This may explain their low levels during the estrus period in the studied animals. Administration of vitamin E or the combination of vitamin E and selenium has been reported to reduce the incidence of postpartum reproductive disorders such as retained fetal membranes, metritis, and cystic ovaries [56] The balanced presence of reactive oxygen species and antioxidants has a positive impact on sperm functions, oocyte maturation, fertilization and embryo development in vitro [57]. The alteration of oxidative status after calving might be related to the reduction of plasma and erythrocyte sulfhydryl concentration. Plasma protein sulfhydryl groups represent an extracellular antioxidant defense, which effectively decrease the rate of lipid peroxidation [58]. This reaction leads to a decrease of plasma thiol groups, because the major portion of peroxyl radicals is trapped by plasma sulfhydryl groups [59]. When the follicle starts growth, oxidative stress may hamper the synthesis of proteins and RNA required for further development as well as the assembly of an efficient system of antioxidant enzymatic defense. This, in turn, may jeopardize the crucial final events of follicle maturation when oocyte and granulosa cells may be required to cope with the effects of an altered follicular vascularization, such as the condition of oxidative stress induced by a reduced oxygen supply. Interference with the stress system might have a positive effect on ovarian cyclicity [60]. E_2 may play a role in the ovarian antioxidant-oxidant balance [61]. Vit C Chain breaking antioxidant competitively protects the lipoproteins from peroxyl radicals and recycles Vit E Diverse antioxidant function. Deficiency of Vit C produces ovarian atrophy, extensive follicular atresia. On the other hand, Supplementation of this antioxidant inhibits follicular apoptosis. B-Carotene Blocks DNA damage and supports cytoplasmic maturation in porcine oocytes [14]. Because vitamin E is located close to oxidase-generating enzymes in plasma membranes of cells, it is in a good position to quench free radicals before they leak into the cell. This is particularly important in neutrophils, which constantly internalize the plasma membrane during phagocytosis. There is evidence to suggest that free radicals inhibit progesterone synthesis. With regard to this, the ovary contains high levels of antioxidants and during the luteal phase these levels are seen to fluctuate indicating that these play a role in cell function [62]. Optimal concentrations of NO are necessary for the implantation of fertilized eggs into mice. In the preovulatory follicles, ascorbic acid is depleted by the presence of luteinizing hormone [14] which explains the low level of this agent during the estrus period in the studied cows (during the prevalence of preovulatory LH surge). NO has been proven to act directly at the ovarian level, where it is produced by the vasculature and neurons, as well as by various cell types, including granulosa, theca, and luteal cells. Nitric oxide production is modulated by several hormones like estradiol 17β, luteinizing hormone, follicle-stimulating hormone, and human chorionic gonadotropin [63]. Results from recent studies suggest an involvement of the NOS/NO system in ovulatory mechanism(s), mainly via its effects on vasculature and prostaglandin production [64-68]. Contradictory result were reported about a luteolytic effect of NO through its stimulation [69,70] to synthesize PGF2α in human [71] and bovine [72]. At the same time, NO decreases progesterone production in rat [73], rabbit [74,75] and bovine [76]. Alternative mechanisms by which NO participates in luteal regression involve lowering estradiol production, resulting in the subsequent demise of the CL [77], and increasing apoptosis [78]. More extensive, short intervaled, serial and regular studies should be taken to focus on this issue in the future. It is concluded that most antioxidants in the present study, except ascorbic acid, increases at the time of estrus and they are not affected by the day of postpartum period or the number and size of the dominant follicles. Short cycles are frequent in the postpartum cows (50% of cows had a short cycles in the present work). Follicular growth starts immediately –may be before the studied time- in postpartum dairy cows.

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