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

Derar Refaat Derar*, Hamdy Salah Hasab-Enaby, Hasan Hussein Ali, Alaa-Edin Zain, Shehata Hasan Shehata

Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt

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 ± 0.02 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 corpus luteum resulted from the first ovulation postpartum fail to develop to the normal size and had shorter lifespan than corpus 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 cow [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...

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 (R²) 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 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

![Figure 1: change in the No. (Y axis) of ovarian follicles and CLs during the post partum period.](image1.png)

![Figure 2: Follicular pattern in 4-waves animals during the post-partum period (Y axis is the size of the dominant follicle in mm).](image2.png)

![Figure 3: Follicular changes during the post partum period No CL group (Y axis refers to size of the dominant follicle in mm).](image3.png)

![Figure 4: Follicular changes during the post partum period in CL group (Y axis refers to size of the dominant follicle in mm).](image4.png)
There is follicular development in both ovaries of postpartum cows; ovulation was reported to be 2.1 ± 0.6 [20]. The emergence of a wave is 9.6 ± 0.6 days in beef cows and the number of DF before the first ovulation and their plasma concentration was not affected by the production decreased gradually throughout the postpartum period till 0 (Parturition) 20 days post partum 2.15 ± 0.16

<table>
<thead>
<tr>
<th>Day of the studied period</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parturition</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 days post partum</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Estrus</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Letters with different superscripts are significantly different (P<0.05).

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 progastadulin 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 anestrus 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

<table>
<thead>
<tr>
<th>Days around parturition</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 days post partum</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Estrus</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Endocrinol Metab Hormonomics ISSN:2161-1017 EMS, an open access journal

<table>
<thead>
<tr>
<th>β- Carotene (ug/dl)</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>84.19 ± 13.96</td>
<td>110.52 ± 17.22</td>
<td>146.00 ± 13.83</td>
<td></td>
</tr>
<tr>
<td>56.30 ± 5.87</td>
<td>58.18 ± 3.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>193.87 ± 19.07</td>
<td>205.60 ± 23.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vit. A (ug/dl)</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.59 ± 0.03</td>
<td>54.49 ± 5.45</td>
<td>56.30 ± 5.87</td>
<td></td>
</tr>
<tr>
<td>56.30 ± 5.87</td>
<td>58.18 ± 3.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>205.60 ± 23.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vit. C (mg/dl)</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.29 ± 0.48</td>
<td>2.59 ± 0.28</td>
<td>2.15 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>2.51 ± 0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NO. (nmol/ml)</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>64.33 ± 9.88</td>
<td>40.28 ± 7.61</td>
<td>36.80 ± 4.58</td>
<td></td>
</tr>
<tr>
<td>45.44 ± 0.88</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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


