Inflammatory status and its relationships with different patterns of postpartum luteal activity and reproductive performance in early lactating Holstein cows

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ABSTRACT

This study was undertaken to determine the effect of inflammatory status on different patterns of postpartum luteal activity and reproductive performance of Holstein cows during early lactation. The cows (n = 75) averaged 3.4 ± 1.2 (mean ± SEM) in parity and 3.1 ± 0.2 (mean ± SEM) in body condition score at calving. Transrectal ultrasonography was performed twice weekly from day 10–60 postpartum to consider ovarian dynamics. Plasma concentrations of progesterone (P4) and estradiol (E2) were measured twice weekly and plasma levels of tumor necrosis factor alpha (TNF-α) and lipopolysaccharide binding protein (LBP) were measured weekly (week 3–6 postpartum). Based on plasma P4 and E2 results, 34 (45.3%) cows had normal luteal activity (NLA), whereas 19 (25.3%), 12 (16.0%), 7 (9.3%) and 3 (4.0%) cows had prolonged luteal phase (PLP), delayed first ovulation (DO), anovulation (AO) and short luteal phase (SLP), respectively. Plasma TNF-α and LBP concentrations were affected by postpartum luteal activity (NLA, SLP and PLP), ovulatory status (DO and AO) and number of weeks postpartum (P < 0.05). These concentrations were greater in cows with PLP (P < 0.05) as compared to NLA cows, and in cows that had delayed ovulation or anovulation compared to ovulated cows (P < 0.05). Cows with PLP had greater open days and lower conception rate as compared to NLA cows (P < 0.05). Healthy cows had a larger CL and greater plasma estradiol and progesterone concentrations at first and second cycle postpartum compared to inflamed cows and followed it with greater fertility (P < 0.05). In conclusion, inflammatory statuses were different in high-producing dairy cows showing PLP, AO and DO in comparison with the postpartum normal luteal activity (NLA) cows that influenced reproduction outcomes.

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1. Introduction

Inflammation is an adaptive response triggered by adverse conditions e.g., infection and tissue injury [1]. Recently, there has been a growing interest in the study of postpartum immunity and inflammation in dairy cattle because of the increased inflammation observed during postpartum as a result of physiological stress and metabolic changes caused by parturition and initiation of lactation [2]. However, the magnitude of this inflammatory condition varies greatly between cows. Inflammation due to uterine infections during early lactation affects ovarian follicular development and function, which can eventually lead to delayed ovulation and impaired fertility [3,4]. Thus, Dourey et al. [3] found that cows with >8% PMN at 25 days postpartum had a longer interval from calving to first ovulation (45 vs 32 days) and tended to have fewer cumulative pregnancies by 270 days after calving (58 vs 80%) as compared to those with <8% PMN. Most cows will have bacterial contamination of the uterus which is eventually cleared by the immune system in healthy cows. While virtually all cows have intrauterine inflammation in the early postpartum period, a smaller proportion of cows will have a systemic immune response characterized by elevation of acute phase proteins such as haptoglobin [5].

Delayed resumption of ovarian cyclicity during postpartum period contributes to a decline in subsequent reproductive performance in dairy cows leading to significant economic losses [6,7]. Galvao et al. [6] showed that cows ovulating before 21 days postpartum had greater fertility than those ovulating between 21 and 49 days postpartum [6]. Follicular activity, which stops in late pregnancy, resumes shortly after calving because clearance of...
pregnancy hormones induces the secretion of follicle-stimulating hormone (FSH) [7]. The first dominant follicle is detectable morphologically within the first two weeks postpartum but only 40% of these dominant follicles proceed to ovulation [8,9].

Measurement of progesterone level can be used as a tool to detect the commencement of luteal activity since early establishment of ovarian activity is important for fertility [10]. Using milk or serum progesterone (P4) profiles, it has been shown that negative energy balance is associated with irregular patterns of luteal activity such as delayed first ovulation (DO), anovulation (AO), prolonged luteal phase (PLP) and short luteal phase (SLP) [11–13]. In addition, nutrition and genetic background, greater negative energy balance, uterine infections and heat stress can all cause abnormal patterns of luteal activity in postpartum cows [11,14]. It is not known, however, if the pattern of inflammation during early lactation impacts the luteal activity and ovulation eventually resulting in infertility in dairy cows. This study was undertaken to determine the relationship between inflammatory statuses and different patterns of postpartum luteal activity and ovulation in multiparous Holstein cows during early lactation. We hypothesized that the magnitude of inflammation during early lactation will influence fertility by affecting luteal activity and ovulation.

2. Materials and methods

2.1. Animals

This study was carried out in a large commercial dairy herd with 2700 milking cows in the north of Iran (Longitude – E 53.06 and Latitude – N 36.33). During the study, the ambient temperature and the relative humidity of the region were 11–27 °C and 52–75%, respectively.

Multiparous Holstein dairy cows (n = 75) were enrolled into the study at d 10 postpartum. Cows averaged 3.4 ± 1.2 (mean ± SEM) in parity and 3.1 ± 0.2 (mean ± SEM) in body condition score at calving [(based on a 1 (thin) to 5 (obese) scale)]. Throughout the year, the cows were housed in covered free stalls and washed sand for bedding, and were fed a total mixed ration formulated to meet or exceed all National Research Council (NRC) 2001. The diet consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or brain grain, corn gluten feed, vitamins, and minerals. Cows were milked three times daily at 0030, 0730 and 1430 h. Only healthy cows free of detectable reproductive disorders and free of any clinical disease during 1st to the 8th week after calving were used in this study. Transrectal ultrasonography for uterine involution starting on Day 21 after calving (first measurement of LPS and TNF) was performed on all cows every 3 days up to Day 45 postpartum. The uterus was considered fully involuted when the cervix and previous gravid horn diameter after calving decreased to unchanged and were similar to that of a non-gravid uterus [23].

All cows had natural calving without dystocia and retained fetal membrane. Cows were considered to have retained fetal membranes if the fetal membranes were not passed within the first 24 h postpartum. The herd veterinarians examined all cows once daily for Rectal temperature (fever was defined as a rectal temperature > 40.2 °C for 20 min at 4 °C and plasma was decanted and stored at −20 °C until assayed for E2, P4, TNF-α, LBP, NEFA and BHBA concentrations. Plasma concentrations of P4 and E2 were determined using ELISA (Diaplus, North York, Ontario, Canada). Intra- and interassay coefficients of variation were <5%. Plasma concentrations of TNF-α and LBP were determined by the ELISA method using Bovine commercial kit (Eastbiopharm Inc, Lake Forest, CA, China) according to manufacturer’s instructions. Plasma concentration of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) were determined by calorimeter-ric method (Randox Laboratories Ltd., Ardmore, UK). Intra- and interassay coefficients of variation were <3%.

2.4. Definition

Stevenson [16] demonstrated that cows with serum P4 concentration of ≥1 ng/ml on two consecutive blood samplings had luteal activity. Cows have been classified into various groups on the basis of their P4 profile [11,17,18]. The resumption of luteal activity is defined as normal luteal activity (NLA) if the first P4 rise occurs ≤45 days after calving followed by regular ovarian cycles (n = 25). If ovulation does not occur in ≤45 days after calving or if irregular ovarian cycles are observed in less than 45 days of calving, the cows are classified as having abnormal luteal activity. These cows are then further divided into the following types. The cows showing one ovarian cycle with luteal activity of ≥19 days are defined as PLP (n = 30). The cows showing one or more ovarian cycles with luteal activity of less than 10 days (excluding the first cycle) are defined as SLPL. Finally, ovarian cysts (follicular or luteal cysts) were defined as a non-echogenic area at least 25 mm in diameter persistent for more than 10 days in the absence of a mature corpus luteum [19]. On days 5 ± 1 and 11 ± 2 of cycle, the presence of a CL was verified sonographically. To determine the occurrence of the second ovulation, monitoring (three times per week) started on day 14, and for detection of the third postpartum ovulations, monitoring (three times per week) started on day 18 of the previous cycle. Luteal size was measured once between days 9 and 13 of the first, second and fourth cycles. Blood samples were also collected at the same time for measuring plasma P4 and estradiol.

Once a follicle grew to 10 mm in diameter, it was considered to be the first dominant follicle postpartum. Ovulation was confirmed if circulating estradiol was >2 pg/ml and with a follicle >10 mm in diameter (n = 52). The cows were classified into DO if the first
ovulation did not occur until 45 days after calving (n = 12) and AO if the diameter of the first dominant follicle reached 15 mm and circulating estradiol did not reach a concentration of ≥2 pg/ml by the following day (n = 7); or if the follicle failed to grow or regressed in size before achieving 15 mm in diameter (n = 8).

To monitor systemic inflammation, plasma concentrations of the TNF-α and LBP were determined in all cows moreover uterine samples were collected on d 21 postpartum for each cow using low-volume uterine lavage as previously described for endometrial cytology [20]. Uterine inflammatory status was determined by evaluating the proportion of inflammatory cells in uterine samples by cytology and by using a reagent strip test. Uterine cytology was performed on the uterine samples as previously described [21]. Systemic inflammation characterized by elevated TNF-α (≥1.5 ng/ml [22]) and LBP (≥10 [μg/mL] [32]) concentrations, moreover cows that had a strong initial uterine inflammatory response (robust recruitment of polymorphonuclear leukocytes of ≥35%) were defined as a inflamed cows and cows with PMN content of <8% were defined as healthy [23].

2.5. Productive and reproductive performances

Milk production was recorded at each milking. Reproductive performance of cows in this study was evaluated using the following parameters: days from calving to first service (DFS), open days (days from calving to conception as defined by pregnancy detection on transrectal ultrasonography at day 32 after AI, OD) and conception rate (CR).

2.6. Statistical analyses

The MIXED procedure of the SAS System (SAS Institute, Cary, NC, USA) was used to perform repeated measures ANOVA. Cows were considered as subject effect and time (weeks) as repeated effect in the model. Time trends in milk yield, plasma hormones concentrations (P4, E2, TNF-α, LBP) during the period of the study were compared between different groups of luteal activity patterns. The interaction term for time by luteal activity groups was also investigated in the model. When the effect of group was significant, the difference between groups at each time interval from calving (weeks) was investigated using the ‘estimate’ statement. First of all we considered the 5 parameters studied (normal luteal phase, short luteal phase, prolonged luteal phase, delayed ovulation, and anovulation) within the same variable by repeated measures ANOVA and after that we used t-test to compare healthy vs. inflamed cows. Conception rate analyzed by logistic regression.

3. Results

Milk yield was not affected by postpartum luteal activity (P > 0.05, Fig. 1) but affected by inflammatory status. Milk yield (mean ± S.D) was lower in inflamed cows compared to healthy cows (49.60 ± 0.3 vs. 53.20 ± 1.3 kg/d). Milk yield increased during the second to eight weeks postpartum (P = .001) in all groups.

Different patterns of the postpartum luteal activity have shown in Table 1 as the percentage of the total. Based on progesterone concentrations and ultrasonography findings of the 75 cows monitored, 34 (45.3%) had normal luteal activity (NLA), 19 (25.3%) had prolonged luteal phase (PLP), and 3 (4%) had short luteal phase (SLP). Furthermore, 12 (16%) and 7 (9.3%) had delayed first ovulation (DO) and anovulation (AO), respectively (Table 1).

Mean plasma BHBA (mmol/L) and NEFA (mmol/L) concentrations did not affect by health status (P = .34) but decreased with increasing days in milk (P = .04). There were 6.6% (3/45) and 13.3% (4/30) cows that had NEFA and BHBA concentrations greater than standard value (NEFA ≥ 0.7 mmol/L and BHBA ≥ 1.2 mmol/L [24]) in healthy and inflamed groups respectively. Plasma TNF-α concentrations differed among postpartum luteal activity (NLA, SLP and PLP), ovulatory status (O and AO) and weeks postpartum (2–8, Fig. 2; P < .05). Plasma TNF-α concentrations was higher in cows with PLP (P = .003) compared to NLA cows, and in AO compared to ovulated cows (P = .03, Fig. 2). Plasma TNF-α concentration was lower in NLA cows compared to AO cows (P = .03). Plasma TNF-α concentrations did not change (P = .05) from the d 14 to 60 postpartum in cows with NLA, whereas increased during same period in cows with PLP (P = .01). Plasma TNF-α concentrations was higher in AO cows compared to those had ovulation from 3rd to 8th week (Fig. 2).

Mean Plasma concentrations of tumor necrosis factor alpha (TNF-α, ng/ml), and lipopolysaccharide binding protein (LBP, μg/ml) of healthy were lower in healthy cows compared with inflamed cows at different time intervals (days) from calving (P < .05, Fig. 3).

Mean plasma concentrations of LBP were affected (P = .03) by postpartum luteal activity (P = .03), ovulation status (P = .03) and weeks postpartum (P = .04). The mean plasma concentrations of LBP were lower in cows with NLA compared to PLP and AO cows, also were lower in ovulated cows compared to AO (P = .03). Plasma LBP concentrations did not change (P = .05) from d 14 to 60th week postpartum and decreased from 6th to 8th weeks postpartum in cows with NLA, whereas in cows with PLP increased from 3rd to 8th weeks postpartum (P = .01, Fig. 2).

 Corpus luteum of the first postpartum estrous cycle was smaller compared to CL of second estrous cycle in healthy and inflamed cows (P = .01, Table 2). Luteal size of the first postpartum estrous cycle affected by inflammatory status and was larger in healthy cows than inflamed cows (P = .01, Table 2). The mean P4 and E2 area under curve (AUC) were greater in healthy cows compared to inflamed cows during first (P = .04) and second (P = .03) postpartum estrous cycle (Table 2).

Postpartum luteal activity affected day to first service (DFS), open days (OD) and conception rate (P = .03, Table 3). Cows with PLP had greater DFS and OD and lower conception rate compared to NLA cows (P < .05, Table 3), moreover cows with abnormal luteal activity (DO and AO) had a greater DFS, OD and lower conception rate (P < .05, Table 3). Moreover uterine inflammatory status was significantly associated with ovulatory follicle diameter, DFS, OD and conception rate (P = .01). Inflamed cows had smaller ovulatory follicle (12.2 ± 1.2 mm vs. 12.2 ± 1.2), greater DFS (12.2 ± 1.2 vs. 12.2 ± 1.2) and lower conception rate (12.2 ± 1.2 vs. 12.2 ± 1.2).

4. Discussion

Results of the present study showed an association between inflammatory status and different pattern of luteal activity, ovulation and reproductive performance in early lactating Holstein dairy cows. The effect of inflammation activation (increased plasma TNF-α and LBP concentrations) is decreased luteal size, abnormal growth of largest follicle, declined estradiol and progesterone concentrations, delayed ovulation, shortened or extended luteal phase after ovulation, increased time to first insemination, decreased conception rates and increased open days.

The incidence of PLP (25.3%) was the most common abnormal pattern of luteal activity in healthy dairy cows. In agreement with our results, the incidence of PLP in lactating dairy cows in previous studies was 20% [17], 23.8% [13] and 28.3% [25]. The concentrations of inflammatory markers (TNF-α and LBP) were higher in cows with PLP compared to NLA and in inflamed cows compared to healthy cows. One of the responses in cows having uterine disease is a prolonged luteal phase, which occurs when the first ovulation postpartum occurs in the presence of a heavily contaminated
uterus [25], or when A. pyogenes is infused into the uterine lumen [26]. In a study of 82 clinically normal postpartum cattle with no risk factors for uterine disease, 75% were found to have a high number of uterine pathogens on day 7 postpartum, the predominant isolate being *Escherichia coli*. Within the uterus, *E. coli* may disrupt the mechanism of PG-induced luteolysis in cyclic cows and may prolong the luteal phase by switching PG synthesis away from PGF2α [27,28]. If cows with uterine infections ovulate, the plasma concentration of progesterone is lower than in normal fertile animals leading to the extension of the luteal phases. The follicular fluid of cattle with uterine inflammation also contains LPS [29]; animals with clinical disease had LPS concentrations of up to 0.8 mg/ml while normal animals did not have any measurable concentration of LPS in their ovarian follicular fluid.

There was an association between inflammatory status and ovarian activity in present study. Uterine inflammation negatively

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Table 1

<table>
<thead>
<tr>
<th>Postpartum luteal activity</th>
<th>Incidence % (number/total)</th>
<th>Parity</th>
<th>milk yield (Kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal luteal activity (NLA)</td>
<td>45.3 (34/75)</td>
<td>4.0 ± 0.6</td>
<td>53.0 ± 3.4</td>
</tr>
<tr>
<td>Prolonged luteal activity (PLP)</td>
<td>25.3 (19/75)</td>
<td>3.2 ± 0.8</td>
<td>51.0 ± 2.6</td>
</tr>
<tr>
<td>Short luteal activity (SLP)</td>
<td>4.0 (3/75)</td>
<td>4.0 ± 1.1</td>
<td>51.2 ± 2.8</td>
</tr>
<tr>
<td>Anovulation (AO)</td>
<td>9.3 (7/75)</td>
<td>3.2 ± 0.6</td>
<td>52.2 ± 2.9</td>
</tr>
<tr>
<td>Delayed ovulation (DO)</td>
<td>16.0 (12/75)</td>
<td>3.6 ± 1.4</td>
<td>51.1 ± 3.1</td>
</tr>
</tbody>
</table>

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Fig. 1. Milk yield (Kg/d) in healthy and inflamed cows during week 2–8 postpartum (LEFT). Healthy cows had greater milk yield compared to inflamed cows (P < .05). Milk yield (Kg/d) in cows with prolonged luteal phase (PLP), short luteal phase (SLP), delayed ovulation (DO), anovulation (AO) and normal luteal activity (NLA) during week 2–8 postpartum (RIGHT). Milk yield was not affected by postpartum luteal activity (P > .05). Values are Means ± standard error of means.

Fig. 2. Mean Plasma concentrations of tumor necrosis factor alpha (TNF-α, ng/ml, Left) and lipopolysaccharide binding protein (LBP, μg/ml, Right) of high-producing dairy cows with normal luteal activity (n = 34, ■), prolonged luteal phase (n = 19, ●), short luteal phase (n = 3, ▲), delayed first ovulation (n = 12, ○) and anovulation (n = 7, ▼) at different time intervals (days) from calving. Values are Means ± standard error of means. The mixed procedure indicated a significant statistical difference between the highlighted black lines and the line of normal luteal activity ( ■, P < .05). Asterisks indicate a significant statistical difference in the specific week between the highlighted black symbols (P < .05).
affects ovarian activity; in cows with inflammation, the first postpartum dominant follicle was smaller and secreted less estradiol 17β compared with healthy cows. This is in agreement with the results of previous studies [4,30]. Sheldon et al. [4] reported cows with uterine infection postpartum, had slower growth of the first postpartum dominant follicle and lower plasma estradiol concentrations around the time of maximal follicle diameter [28,30]. The specific mechanisms by which uterine infection disrupts ovarian function are many and diverse, but there is substantial evidence that the endotoxin lipopolysaccharide (LPS) is a key disruptor of ovarian function [31] as we showed in present study LPS concentrations was greater in cows had AO or DO. In addition to uterus and peripheral circulation, LPS has been detected in follicular fluid of cattle with uterine disease; unsurprisingly, the concentrations of LPS are directly correlated with bacterial load [28,32,33].

Escherichia coli release the endotoxin lipopolysaccharide (LPS), which impairs the release of gonadotropin - releasing hormone (GnRH), and luteinizing hormone (LH) and decreases aromatase activity [27]. The latter is responsible for decreased follicular growth and estradiol production [28,34]. A decrease in GnRH/LH release leads to decreased ovulation rate [32]. In fact, LPS has been shown to disrupt granulosa cell oestradiol secretion via reduced expression of aromatase enzyme expression in vitro [29], while acute exposure to LPS increases follicular atresia and reduces the primordial ovarian follicle pool [35].

The effect of uterine disease on ovarian function is further enhanced by cytokines released by the endometrial cells because...

**Fig. 3.** Mean Plasma concentrations of tumor necrosis factor alpha (TNF-α, ng/ml), lipopolysaccharide binding protein (LBP, μg/ml), non-esterified fatty acids (NEFA, mmol/L) and β-hydroxybutyrate (BHBA, mmol/L) of healthy (n = 45, ■) and inflamed (n = 30, ●) high-producing dairy cows at different time intervals (days) from calving. Values are Means ± standard error of means. Asterisks indicate a significant statistical difference in the specific week between the highlighted black symbols (P < .05).

**Table 2** Mean ± standard error of Luteal size (mm), maximum follicle diameter (mm), progesterone area under curve (AUC, ng/mL) and estradiol area under curve (AUC, pg/mL) concentrations in healthy and inflamed cows in first and second cycle postpartum.

<table>
<thead>
<tr>
<th></th>
<th>First Cycle</th>
<th>Second Cycle</th>
<th>P</th>
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<tbody>
<tr>
<td>Luteal size (mm)</td>
<td><img src="image" alt="Luteal size" /></td>
<td><img src="image" alt="Luteal size" /></td>
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</tr>
<tr>
<td>Healthy</td>
<td>10.72 ± 0.19</td>
<td>12.56 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Inflamed</td>
<td>7.60 ± 0.24</td>
<td>8.82 ± 0.17</td>
<td>.01</td>
</tr>
<tr>
<td>Progesterone AUC (ng/mL)</td>
<td><img src="image" alt="Progesterone" /></td>
<td><img src="image" alt="Progesterone" /></td>
<td>.51</td>
</tr>
<tr>
<td>Healthy</td>
<td>20.13 ± 1.48</td>
<td>25.21 ± 2.50</td>
<td></td>
</tr>
<tr>
<td>Inflamed</td>
<td>11.71 ± 0.90</td>
<td>13.26 ± 1.12</td>
<td>.45</td>
</tr>
<tr>
<td>Maximum follicle diameter (mm)</td>
<td><img src="image" alt="Max follicle" /></td>
<td><img src="image" alt="Max follicle" /></td>
<td>.01</td>
</tr>
<tr>
<td>Healthy</td>
<td>11.93 ± 0.17</td>
<td>13.85 ± 0.17</td>
<td>.01</td>
</tr>
<tr>
<td>Inflamed</td>
<td>9.40 ± 0.18</td>
<td>10.50 ± 0.18</td>
<td>.01</td>
</tr>
<tr>
<td>Estradiol AUC (pg/mL)</td>
<td><img src="image" alt="Estradiol" /></td>
<td><img src="image" alt="Estradiol" /></td>
<td>.66</td>
</tr>
<tr>
<td>Healthy</td>
<td>90.24 ± 5.30</td>
<td>110.3 ± 6.46</td>
<td></td>
</tr>
<tr>
<td>Inflamed</td>
<td>74.26 ± 2.45</td>
<td>84.31 ± 4.37</td>
<td>.73</td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td>0.03</td>
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</tbody>
</table>

Luteal size was measured once between days 9 and 13 of the first and second cycles and blood samples were collected at the same time for measuring the plasma P4 concentrations and around the time of maximal follicle diameter for measuring estradiol.
pro-inflammatory cytokines impair granulosa cell steroidogenesis [36]. If animals ovulate, the cytokines secreted by the infected endometrium may also partly explain the reduced progesterone secretion from the CL because bovine luteal cells are highly responsive to a range of cytokines, which are also important in luteolysis [32,37].

Present study showed healthy cows had a larger CL and greater plasma progesterone concentrations compared to inflamed cows that was consistent with results of Struve et al. [38] that reported the CLs of metritic cows was smaller than the CL of healthy cows. If animals ovulate, the cytokines secreted by the infected endometrium inhibits the responsiveness of the pituitary to GnRH [34], which in turn could affect ovulation and luteal development. In agreement with our results, Williams et al. [28] showed that bacterial contamination of postpartum uterus was associated with lower plasma P4 concentrations. The initial response to infection might be to lyses the corpus luteum [38] and less progesterone secretion than in healthy cows [39].

There is evidence that uterine infections contribute to reduced fertility via a number of mechanisms. Bacterial products or immune mediators produced in response to infection suppress pituitary LH secretion and are associated with the inhibition of folliculogenesis, decreased ovarian steroidogenesis, abnormal luteal phases and a higher incidence of cystic ovarian disease [7,33]. Result of present study showed inflammatory condition decreased progesterone concentrations during follicular development, increased percentage of cows with prolonged luteal phase and decreased ovarian steroidogenesis that all together resulted in increased time to first insemination, decreased conception rate and increased open days. In agreement with our results, Kafi et al. [13] reported healthy dairy cows with PPL had a greater calving to first service interval as compared to those with NLA. Moreover, calving to conception interval was extended in cows with PLP compared with healthy cows, moreover increased first ovulation (DO) and short luteal phase (SLP).

Acknowledgements
The authors thank owners and personnel of Mahdasht Meat & Milk Company (Northern Iran) for allowing us access to their cows and facilities to conduct this research and for covering the cost of hormonal treatments.

References
[12] Gautam G, Nakao T, Yamada K, Yoshida C. Dehiscence of postpartum uterus was associated with lower plasma P4 concentrations during follicular development, increased percent-

Table 3
Days to first service, conception rate, open days and Ovulatory follicle diameter in high-producing dairy cows with different postpartum luteal activity and ovulatory status.

<table>
<thead>
<tr>
<th>Item</th>
<th>NLA</th>
<th>PLP</th>
<th>AO</th>
<th>DO</th>
<th>SLP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to first service (d)</td>
<td>54.41 ± 0.83</td>
<td>70.21 ± 1.89</td>
<td>113.14 ± 0.09</td>
<td>79.58 ± 0.83</td>
<td>73.14 ± 0.13</td>
<td>.01</td>
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<tr>
<td>Conception rate (%)</td>
<td>58.25 ± 4.04</td>
<td>33.33 ± 4.13</td>
<td>33.33 ± 0.09</td>
<td>31.50 ± 4.04</td>
<td>30.30 ± 3.54</td>
<td>.01</td>
</tr>
<tr>
<td>Open days (d)</td>
<td>113.82 ± 0.8</td>
<td>136.41 ± 1.89</td>
<td>179.00 ± 0.09</td>
<td>151.83 ± 0.83</td>
<td>148.33 ± 0.53</td>
<td>.25</td>
</tr>
<tr>
<td>Maximum follicle diameter (mm)</td>
<td>13.85 ± 0.17</td>
<td>10.81 ± 0.17</td>
<td>9.875 ± 0.17</td>
<td>10.30 ± 0.17</td>
<td>10.00 ± 0.27</td>
<td>.01</td>
</tr>
</tbody>
</table>

Means within a row with different superscripts differ (P < .05).

1Normal luteal activity (NLA), prolonged luteal phase (PLP), anovulation (AO), delayed first ovulation (DO) and short luteal phase (SLP).


