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Original article

Influence of puerperal metritis on the first ovulation after calving in dairy cows

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Abstract

The aim of this study was to determine how puerperal metritis influences the resumption of estrous cycle in dairy cows. The ovaries of 72 multiparous Holstein cows (38 healthy and 34 metritic – after treatment) were ultrasonographically scanned until the first ovulation postpartum and 7 days after to confirm the ovulation. All 72 cows were divided in to 4 groups: HSO (healthy with single ovulation) (n=29), MSO (metritic with single ovulation) (n=21), HDO (healthy with double ovulation) (n=9), and MDO (metritic with double ovulation) (n=13). The proportion of cows that had DO in the first ovulation postpartum was similar between M and H groups, 38.2% and 23.6%, respectively ($p>0.05$). There was a difference between HDO and MDO groups comparing the first dominant follicle ovulation postpartum (11.4±2.7 and 20±1 days, respectively $p<0.05$) and the diameter of the ovulatory follicles (15.3±1.9 mm and 17.3±1.7 mm, respectively $p<0.05$). The percentage of cows that had double follicle dominance in the first follicular wave after first ovulation was higher in the M groups (33.3% (MSO) vs. 6.9% (HSO) ($p<0.05$) and (69.2% (MDO) vs. 22.2% (HDO) ($p<0.05$)). The MSO group dominant follicle diameter was bigger for cows which had one dominant follicle ($p<0.05$). It might be concluded that dairy cows after puerperal metritis need more time until the first ovulation. Also, metritic cows have a higher risk for double dominance in the first follicular wave, after the first ovulation.

Key words: corpus luteum, dominant follicle, ovulation, metritis, progesterone

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Introduction

Fast recovery of ovarian activity postpartum is relevant for high yielding dairy cows. It greatly influences the risk of successful insemination in early lactation. However, in modern dairy cows, slow resumption of the estrous cycle postpartum leads to the negative effects which compromise fertility. The occurrence of early ovulation is associated with the probability that a cow will have frequent and normal estrous cycles, which will increase the reproductive performance of the uterine environment (Thatcher et al. 2005).

In postpartum dairy cows, metritis is a common disease. It leads to milk production losses and also to reduced fertility compared with healthy herd mates (Fourichon et al. 2000, Bell and Roberts 2007, Wittrock et al. 2011, Giuliodori et al. 2013). The definitions of metritis have been standardized by Sheldon et al. (2009). According to these authors, cows which have an abnormally enlarged uterus and a fetid, reddish brown, watery uterine discharge detectable in the vagina with the presence of pyrexia (≥39.5°C) are defined as having puerperal metritis. Puerperal metritis can be highly prevalent in some dairy farms and can vary from 7% to 20% for multiparous dairy cows (Benzaquen et al. 2007, Giuliodori et al. 2013, Armengol and Fraile 2015). Furthermore, multiparous cows have a higher risk of puerperal metritis than primiparous cows (Kawashima et al. 2006, Wittrock et al. 2011). However, there are numerous studies investigating the resumption of ovarian activity in the postpartum period (Peter et al. 1989, Kamimura et al. 1993, Sakaguchi et al. 2004, Butler et al. 2006, Tanaka et al. 2008, Rajmon et al. 2012). Most studies are conducted with cows that have no negative disorders postpartum. In the literature, the first ovulation in dairy cows is well characterized, but there is no information on what proportion of cows have problems with uterine diseases. There is a lack of information about how the dairy cow estrous cycle recovers after puerperal metritis, and how derivatives in the ovaries (follicles and corpus luteum (CL)) change in early lactation for cows after puerperal metritis.

The aim of this study was to determine how puerperal metritis and its treatment with antibiotic Ceftiofur (1 mg/kg *s.c.* Cevaxel RTU, Ceva, France) influences the first ovulation and the rate of double ovulation in healthy cows and cows after treatment for puerperal metritis, the difference between cows with single and double ovulation on progesterone (P_4) concentration and CL volume on day 7 after the first ovulation between healthy cows and cows after puerperal metritis, and the difference in the first follicular wave after the first ovulation between healthy cows and cows after puerperal metritis treatment.

Materials and Methods

The study was carried out on lactating Holstein cows from a commercial dairy farm located in Lithuania, milking approximately 1100 cows, and averaging approximately 33 kg of milk/d. This study was performed from 2018 September to July 2019. The cows were housed in freestall barns with access to fresh water *ad libitum* and were fed a total mixed ration supplemented with the concentrate based on milk yield. The cows were milked with Lely Astronaut® A3 milking robots with free traffic. To motivate the cows to visit the robot, 2 kg/day of concentrate was fed to them by a milking robot. Data for daily milk yield and lactation days data were collected from the Lely T4C management program for analysis.

The study was carried out in compliance with EU legislation. The procedures complied with the criteria given by the Lithuanian animal welfare regulations (No. B1-866, 2012; No. XI-2271, 2012) and the decree of the director of the State Food and Veterinary Service, Republic of Lithuania (No. B6-(1.9)-855, 2017).

For this study, 85 cows (41 H and 44 M) were enrolled, and 72 (38 H and 34 M) cows were used at the end of the analyses. The 13 cows not used were excluded from the study because of the following disorders until first ovulation: mastitis (n=7), lameness (n=2) and left displaced abomasum (n=4). Puerperal metritis was diagnosed in 10 of the 13 cows. These cows were not included in our data analyses. All 72 cows were divided into groups after their first ovulation: HSO (n=29) – cows without signs of puerperal metritis and with a single ovulation; MSO (n=21) – cows after puerperal metritis treatment with a single ovulation; HDO (n=9) – cows without signs of puerperal metritis and with a double ovulation; and MDO (n=13) – cows after puerperal metritis treatment with a double ovulation.

In the present study, the cows were selected between day 5 to 14 after calving (day 0 = day of calving). The cows were divided into two different groups: multiparous cows after puerperal metritis treatment (M) and multiparous cows without signs of puerperal metritis (H). There was no significant difference in parity among the groups, H group – 2.38±0.55 days and M group – 2.32±0.58 days.

Information about the reproductive history of the experimental cows was also recorded. M group cows delivered 58.8% (20/34) male calves before the study, while 36.8% (14/38) of male calves were delivered by H group cows. 17.6% (6/34) of M group cows had the incidence of dystocia compared to 5.3% (2/38) of H group cows. Furthermore, M group cows had more stillbirths than H group cows (11.8% (4/34) and 5.3% (2/38), respectively) and there were more cows with retained placenta in the M group compared to the H group (20.6% (7/34) and 0% (0/38), respectively).

Puerperal metritis was diagnosed by rectal temperature and if the relevant uterine discharge was present (Sheldon et al. 2009). All the cows with signs of puerperal metritis (abnormally enlarged uterus and a fetid, reddish brown, watery uterine discharge detectable in the vagina with the presence of pyrexia (≥39.5°C) were treated with Ceftiofur (1 mg/kg *s.c.* Cevaxel RTU, Ceva, France) for 5 days. All the cows from the M group were checked after the treatment with Ceftiofur to evaluate the process of recovery. All these cows were checked again on day 30. After uterus massage, cows which had purulent uterine discharge (>50% of pus) (20.6% (7/34) of M group cows) seen in the vagina were treated with Clamoxyl metritis® intra-uterine antibiotic infusion (Amoxicillin 0.84 g, Pfizer animal health, Belgium). At the end of the voluntary waiting period on this farm (50 days postpartum), all M group cows (also, additionally treated with Clamoxyl metritis® intra-uterine infusion) were considered to be healthy if they had no vaginal discharge on day 50.

All cows were subdivided into two subgroups after the first ovulation as follows: cows which ovulated one dominant follicle (SO) and cows which had double ovulation (DO). All cows were examined from day 5 (day 0 = day of calving) three times per week (Monday, Wednesday, Friday) until the first ovulation and on the 7th day after this ovulation to evaluate cyclicity.

The changes in ovaries were examined using a digital diagnostic ultrasound scanner (Dramiński iScan, Dramiński S.A., Olsztyn, Poland) at a frequency of 7.5 MHz, using a linear rectal transducer.

The first dominance of the follicle postpartum was recorded when at least one of the follicles reached 8.5 mm in diameter. To detect follicle ovulation, the cows were monitored by ultrasound machine three times a week (Monday, Wednesday, Friday). Ultrasonography was started on day 5 postpartum and was continued until the follicle ovulation was diagnosed. The follicle ovulation was diagnosed when a dominant follicle (DF), which had been seen at previous examination, was no longer visible. A CL with a diameter of more than 7 mm was deemed to be the result of ovulation two days previously (day of ovulation = day 1), and the cycle stage was designated as day 3. When the DF was no longer present, and CL was not visible or was smaller than 7 mm, the previous day was considered the day of ovulation (Struve et al 2013). The last size measure of an ovulated follicle was recorded, and ultrasonography was repeated on day 7 after ovulation (day of ovulation = day 0) to measure the volume of CL and the size of the follicles. Ultrasound measurements of CL were used to calculate average diameters (average of length (L) and width (W) and volume (V). The volume of CL was calculated using the formula $V=4/3\times\pi\times R^3$ using a radius (R) calculated by the formula $R=(L/2+W/2)/2$ and $\pi=3.14$. For CL with a fluid-filled cavity, the volume of the cavity was calculated and subtracted from the total CL volume (Sartori et al. 2004). Results from mm^3 were converted to cm^3.

Blood samples were collected into tubes without anticoagulants via a puncture from the median caudal blood vessels. The first blood sample was collected on day 7 postpartum and then every 7 days until ovulation, and on day 7 after ovulation (day of ovulation = day 0). To take the blood samples at the correct time, the farm was visited every day. Ovulation was determined by ultrasonography. After collection, blood samples were taken to the laboratory and centrifuged (2000 × g, 20 minutes at 4°C), and serum was collected and stored at –20°C until analysis. The serum progesterone (P_4) concentration was analyzed using chemiluminescent assay (Immulite, Siemens, Wales, UK) in an accredited laboratory (Segalab, Portugal). The minimum detection level for P_4 was 0.2 *n*g/mL.

Statistical analysis was performed using SPSS 22 computer software. Averaged experimental results are reported as mean ± standard error of the mean. All the results between the groups were compared using the independent *t*-test. The level of significance was set at $p<0.05$.

Results

In the present study, the proportion of cows that had SO in their first ovulation postpartum was similar between M and H groups, 61.8% and 76.4%, respectively ($p>0.05$). Also, the same tendency was observed in the cows with DO after the first ovulation, M group 38.2% and H group 23.6% ($p>0.05$).

The mean time to the first follicle deviation (selection of the DF during follicular wave from the size of 8.5 mm) postpartum was higher in the MSO group compared with the HSO group, 8.9±1.6 and 6.8±1.8 days postpartum, respectively ($p<0.05$). The same tendency was observed in the MDO and HDO groups, 9.5±1.3 and 7.0±1.4 days postpartum, respectively ($p<0.05$). Overall, the mean time to the first follicle deviation inside M groups was longer (9.2±1.5 days), compared to H group cows (6.9±1.6 days) ($p<0.05$).

There was no significant difference between HSO and MSO groups at the resumption of cyclicity on the days with regard to the first DF ovulation postpartum and on the diameter of the ovulatory follicle at the first follicular wave follicle ovulation (Table 1). Meanwhile, HDO group cows ovulated their follicle during the first

Table 1. First ovulation after calving in cows.

Category	Groups			
	H		M	
	HSO (n/n)	HDO (n/n)	MSO (n/n)	MDO (n/n)
1st follicular wave follicle ovulation				
% of cows which ovulate during their 1 st follicle wave (n/n)	41.4 (12/29)	55.6[a] (5/9)	47.6 (10/21)	23.1[b] (3/13)
Days	15.9±3	11.4±2.7[c]	18.8±6.3	20±[1]d
Diameter of the ovulatory follicle (mm)	22.5±3.5	15.3±1.9[e]	19.2±3.5	17.3±1.7[f]
2nd and later follicular waves ovulation				
% of cows which ovulate during their 2 nd and later follicle waves (n/n)	58.6 (17/29)	44.4 (4/9)	52.4 (11/21)	76.9 (10/13)
Days	37±8.9	30.5±7.8	35.9±10	32.9±6.5
Diameter of the ovulatory follicle (mm)	19.8±3.9	15.3±1.9	20.1±4.7	16.5±2.7

HSO – cows without signs of puerperal metritis – single ovulation, MSO – cows after puerperal metritis – single ovulation, HDO – cows without signs of puerperal metritis – double ovulation, MDO – cows after puerperal metritis – double ovulation. Significant difference between groups with the letters a, b; c, d; e, f ($p<0.05$).

Table 2. Differences in the follicle size, corpus luteum volume and progesterone concentration between H and M groups of cows on day 7 post ovulation.

Item	Groups			
	H		M	
	HSO (n/n)	HDO (n/n)	MSO (n/n)	MDO (n/n)
Dominant follicle size at day 7 post ovulation (mm)				
One dominant follicle	14.6±1.1 (27/29)	14.3±0.9 (7/9)	14.1±0.7[a] (14/21)	13.8±0.4 (4/13)
Two dominant follicles	13.5±0.6 (2/29)	13.5±0.6 (2/9)	13.4±0.5[b] (7/21)	13.6±0.9 (9/13)
Corpus luteum volume at day 7 post ovulation (cm^3)				
One dominant follicle	9.51±4.01 (27/29)	12.14±4.83 (7/9)	8.29±2.59 (14/21)	9.44±3.72 (4/13)
Two dominant follicles	10.95±6.50 (2/29)	13.78±0.33 (2/9)	7.55±2.04 (7/21)	11.56±3.71 (9/13)
Progesterone concentration at day 7 post ovulation (*ng*/mL)				
One dominant follicle	2.8±1.6 (27/29)	3.1±0.9 (7/9)	2.8±1.3 (14/21)	3.1±0.6 (4/13)
Two dominant follicles	2.6±1.1 (2/29)	2.7±0.8 (2/9)	2.5±0.7 (7/21)	3.3±0.9 (9/13)

HSO – cows without signs of puerperal metritis – single ovulation, MSO – cows after puerperal metritis – single ovulation, HDO – cows without signs of puerperal metritis – double ovulation, MDO – cows after puerperal metritis – double ovulation.
H – cows without signs of puerperal metritis, M – cows after puerperal metritis. Significant difference between subgroups with the letters a, b; ($p<0.05$).

follicular wave faster compared to the MDO group ($p<0.05$) (Table 1). Also, HDO group cows had wider diameter of the ovulatory follicle compared to the MDO group ($p<0.05$) (Table 1). Furthermore, we found that MDO cows ovulate their first dominant follicle less frequently compared with the HDO group ($p<0.05$) (Table 1). Cows that did not ovulate their first DF postpartum, ovulated their DF's from the second or later follicular waves. We did not find any significant difference in later ovulations between HSO and MSO, nor between HDO and MDO groups at the time of ovulation and the diameter of the ovulatory follicle ($p>0.05$) (Table 1).

Comparing HSO and MSO groups and HDO and MDO groups, there were no significant differences between the diameter of the ovulatory follicles, CL volume at day 7 and P4 concentration at day 7 ($p>0.05$) (Fig. 1).

Comparing total 1st follicular and 2nd and later follicular waves ovulatory follicle diameters, in H and M groups , we found that HSO and MSO group cows had significantly larger ovulatory follicles than the cows in the HDO and MDO groups, respectively ($p<0.05$) (Fig. 1). We did not find any significant difference in the CL volume between HSO and HDO groups at day 7 after ovulation. Meanwhile, there was a significant difference in the CL volume between cows from the MSO and MDO groups at day 7 after ovulation (8.05±2.40 cm^3 and 10.91±3.71 cm^3, respectively, $p<0.05$). Comparative analysis revealed no difference in the P4 concentration between SO and DO cows in both H and M groups ($p>0.05$) (Fig. 1).

A double-dominance of the first follicle wave after the first ovulation was also observed. It was found that MSO and MDO group cows had a significant difference

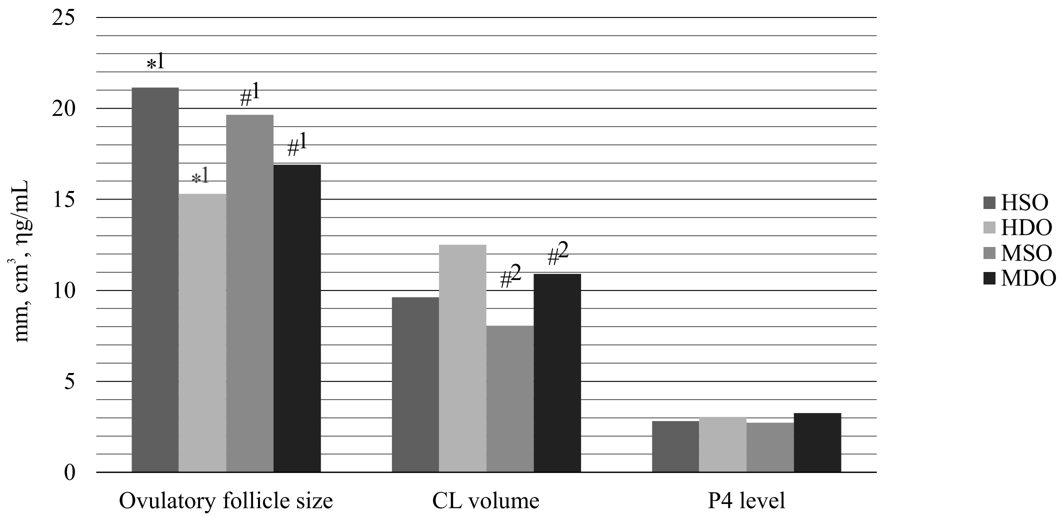

Figure 1. Comparison within the H and M groups of cows. HSO – cows without signs of puerperal metritis – single ovulation, MSO – cows after puerperal metritis – single ovulation, HDO – cows without signs of puerperal metritis – double ovulation, MDO – cows after puerperal metritis – double ovulation. Significant difference between groups with the same tags ($p<0.05$)

for double follicle dominance on day 7 after the first ovulation compared to HSO and HDO groups, respectively; between MSO and HSO groups (33.3% and 6.9%, respectively, $p<0.05$), and between MDO and HDO groups (69.2% and 22.2%, respectively, $p<0.05$).

The diameter of the follicles on day 7 after ovulation was compared in all four groups (HSO, HDO, MSO and MDO). There was no significant difference in follicle diameter between groups with one or two dominant follicles in groups HSO, HDO and MDO ($p>0.05$) (Table 2). Meanwhile, dominant follicle diameter was wider for cows which had one dominant follicle in the group MSO ($p<0.05$) (Table 2). Comparative analysis revealed no difference at day 7 in all the groups on CL volume between cows with one and two dominant follicles ($p>0.05$) (Table 2). Furthermore, there was no significant difference on progesterone concentration on day 7 in all the groups between cows with one dominant follicle and two dominant follicles ($p>0.05$) (Table 2).

Discussion

In the postpartum period, dairy cows face many challenges. Fast recovery of ovarian activity at the time of postpartum in high-producing dairy cows is critical for good reproduction. One of the criteria for a postpartum cow to have good reproductive health is the time of the first ovulation. A shorter interval to the first ovulation leads to a reduced interval to conception and increased conception rates (Tanaka et al. 2008). However, such disease as puerperal metritis leads to negative effects which can compromise fertility.

Puerperal metritis is highly prevalent in some dairy farms and can vary from 7% to 20% for multiparous dairy cows (Benzaquen et al. 2007, Giuliodori et al. 2013, Armengol and Fraile 2015). It is well known that the impact of puerperal metritis on dairy cow reproduction can cause significant losses in production (Benzaquen et al. 2007, Dubuc et al. 2010, Martinez et al. 2012, Giuliodori et al. 2013, Lima et al. 2014, Armengol and Fraile 2015, Lopez-Helguera et al. 2016). In the present study, the incidence of puerperal metritis for multiparous cows in the herd was 9.2%. Our results ware those obtained by Benzaquen et al. (2007) and Armengol and Fraile (2015). They found that the cases of puerperal metritis were 7.5% and 7.9%, respectively (Benzaquen et al. 2007, Armengol and Fraile 2015).

Nowadays, modern dairy cows produce great quantities of milk because of genetic improvement and nutritional management optimized through lactation (Friggens et al. 2010). Such changes in production are related to changes in reproduction physiology. The greatest incidence of double ovulation is when cows produce >40 kg/d. It is clear that such cows have increased metabolism of hormones which leads to double ovulation (Lopez et al. 2005). However, in relation to the first ovulation after calving when preovulatory follicles are growing in sub-luteal P4 concentrations, there is no difference between cows with single or double ovulation on milk production (Lopez-Helguera et al. 2016, Macmillian et al. 2018). A possible reason for double ovulations at the first ovulation after calving for cows with uterine disease could be lipopolysaccharides (LPS) in the uterus. LPS decreases LH pulse frequency and decreases estradiol, and such cows, by the time of deviation, have higher levels of FSH than normal, which leads to co-dominance of two or more follicles in the follicular wave (Wiltbank et al. 2000, Lavon et al. 2008). This explains our results, as M group cows had more double ovulation at the time of first ovulation compared to H group cows, but the difference was not significant. Both M and H group results are in agreement with other studies where incidence of DO following anestrus is from 29 to 58.8% (Lopez et al. 2005, Stevenson 2016, Kusaka et al. 2017, Macmillan et al. 2018). However, Stevenson et al. (2006) claim that DO incidence in the first ovulation following anestrus is lower and reaches 15%.The results of the present study support the idea that infection slows down follicular growth for cows with uterine disease by reducing circulating estradiol (E_2) and perturbing prostaglandin signaling (Sheldon et al. 2002, Herath 2007). Probably, for this reason, M group cows showed the first DF approximately 2 days later than the healthy cows ($p<0.05$). We also observed that cows with uterine disease emerge follicle waves at the same time as healthy cows as in other studies (Sheldon et al. 2002, Herath 2007). The present study results from groups H and M are in agreement with previous studies, where deviation occured between 5 to 10 days postpartum (Savio et al. 1990, Crowe 2008, Tanaka et al. 2008). On the other hand, Rajmon et al. (2012) found that the first follicle deviation in multiparous cows was seen from day 15±3.

According to Savio et al. (1990), Sakaguchi et al. (2004) and Kawashima et al. (2006), 38–73% of a herd has first wave follicle ovulation. We can confirm this statement, as the findings in the HSO, MSO and HDO groups were similar. According to Crowe (2008), the first wave follicles could ovulate in 50–80% of a herd by day 20. Tanaka et al. (2008) claim that 83% of multiparous dairy cows ovulated their first wave DF. However, Rajmon et al. (2012) claim that only 29% of multiparous cows ovulated their first DF. Our study results of group MDO support the results of Rajmon et al. (2012). However, in the above studies, there was no information about cows which had double ovulation at the time of the first DF ovulation or which had puerperal metritis.

The mean time of the first DF ovulation postpartum was observed in the groups by day 20. According to Butler et al. (2006), the first DF ovulation in mature Holstein cows was on day 16.6±1.6 postpartum. Kamimura et al. (1993), Kawashima et al. (2006), Sakaguchi et al. (2004) and Tanaka et al. (2008) reported the first ovulation on day 17±1, 17±4, 18.1±1 and 17.3±6.3, respectively. Similar results were observed during our study in groups HSO, MSO and MDO. HDO group cows had their first DF ovulation on day 11.4±2.7, probably due to the high feed intake and low negative energy balance after calving which ensure appropriate LH pulse frequency and plasma IGF-1 level. This leads to the fast recovery of ovarian activity and first ovulation after calving (Beam and Butler 1999). The second and later DF ovulation usually occurs between 36±4 and 38±7 days postpartum as revealed in the HSO and MSO groups during our study (Kamimura et al. 1993, Kawashima et al. 2006, Rajmon et al. 2012). According to the results, it is clear that most cows ovulate their first wave DF postpartum by day 20.

We also observed the size of the first ovulating follicle postpartum. The follicles of the HSO group (22.5±3.5 mm) were larger compared to the MSO group (19.2±3.5 mm) at the time of ovulation. Butler et al. (2006) and Rajmon et al. (2012) found that multiparous cows ovulated their first DF at the size of 18.6±1.2 mm and 16.0±0.6 mm, respectively. Our results from the HDO and MDO groups are in agreement with those of Rajmon et al. (2012). Moreover, these two studies from Butler et al. (2006) and Rajmon et al. (2012) could explain the results in our groups if we combine them together (HSO, MSO, HDO, MDO – 18.5±3.9 mm), because these studies did not mention the cow's health status and the double ovulation rate. The smaller follicular size in the MSO group could be explained by deficiency of luteinizing hormone (LH) pulses caused by a more pronounced negative energy balance than in healthy cows (Beam and Butler 1999). Besides, lipopolysaccharides (LPS), which are produced by bacteria such as E. coli, could also contribute to the deficiency of LH pulses by decreasing gonadotropin realizing hormone (GnRH) secretion from the hypothalamus (Peter et al. 1989, Sheldon et al. 2009, Kassé et al. 2016). For this reason, cows with postpartum uterine disease have slower growth of the first postpartum DF and lower peripheral plasma E_2 concentrations around the time of the maximal follicle diameter (Sheldon et al. 2002, Williams et al. 2007). However, the follicle size in the MDO group was larger compared with that in the HDO group. This could be explained by the time of the first DF ovulation. In the HDO group the first DF ovulation was earlier than in the MDO group (11.4±2.7 and 20±1 days), so the DF had less time to grow.

Inflammation of the uterus is associated with smaller first CL postpartum (Williams et al. 2007, Strüve et al. 2013). The results of the present study support this idea; the size of the first CL on day 7 after the first ovulation in the MSO and MDO groups were smaller than in the HSO and HDO groups. As with the other authors, we did not notice a significant difference between the groups in the P_4 concentration on day 7 after ovulation (Strüve et al. 2013). During our study, CL volume and P_4 concentration were higher in the double ovulation group compared to the single ovulation group. According to other authors, cows which had double ovulation 7 days later had a lower P_4 concentration compared to cows after single ovulation (2.5±0.3 vs. 3.2±0.1 *n*g/mL) despite a greater CL volume (8.29±0.51 vs. 6.40±0.15 cm^3) (Lopez et al. 2005). Our results may differ, because we started to analyze cows at the beginning of lactation (until the first ovulation and 7 days after first ovulation); therefore, it is possible that at this time the metabolism of hormones is not so high.

According to Sartori et al. (2002), significantly larger follicles were found for single ovulators. The same tendency was observed between HSO and HDO, and between MSO and MDO groups in our study. Also, during our study the CL volume at day 7 after ovulation was larger in the MSO group compared to the MDO group, as in Sartori et al. (2002) ($p<0.05$). Comparative analysis revealed no difference in the P4 concentration between SO and DO cows in both H and M groups as in Sartori et al. (2002) experiment ($p>0.05$) (Fig. 1).

The double dominance of the first follicle wave after the first ovulation was also observed in MSO and MDO groups compared to HSO and HDO groups, respectively ($p<0.05$). According to the literature, the average incidence of multiple ovulations in healthy lactating dairy cows ranges from 10.3% to 22.4% (Bleach et al. 2004, Lopez et al. 2005, Lopez-Gatius et al. 2005, Stevenson et al. 2006, Stevenson 2016). However, cows which have uterine infections have a higher risk of multiple ovulations. Uterine infections soon after calving delay the return to cyclicity. Such cows have a lower P_4 environment before and after deviation, increased FSH and LH before the deviation, and increased E_2 after deviation (Macmillan et al. 2018).

Conclusions

Dairy cows which have had puerperal metritis need more time until the first ovulation. Also, metritic cows have a higher risk of double dominance in the first follicular wave, after the first ovulation.

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