Effect of the Ovsynch protocol on ovarian changes and pregnancy rates after first insemination in lactating cows with or without puerperal metritis

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ABSTRACT. The aim of this study was to determine how puerperal metritis affects pregnancy rates and ovarian changes in dairy cows synchronised with the Ovsynch protocol. The study included 72 cows, which were divided into two groups. Cows treated for puerperal metritis were defined as Group M (n = 34), and those without signs of puerperal metritis as Group H (n = 38). Cows were synchronised for the first artificial insemination (AI) on day 49 ± 3 postpartum using a modified Ovsynch protocol. The number of cows that ovulated after the first injection of gonadotropin-releasing hormone (G1) was similar between Group H and Group M (36.84 and 23.53%, respectively; $P > 0.05$). At G1, the size of dominant follicles was smaller in healthy non-ovulated subgroup (HNO) compared to metritic an-ovulated cows (MNO) ($P < 0.05$). Furthermore, the volume of corpus luteum was lower in the subgroup of metritic ovulated cows (MO) compared to healthy ovulated cows (HO) ($P < 0.05$). At the time of prostaglandin F2α treatment, progesterone (P4) levels in subgroups HO and MO were > 1 ng/ml, and they were different compared to subgroups HNO and MNO ($P < 0.05$). The study showed that at the time of the second injection of gonadotropin-releasing hormone (G2), follicle size was larger in Subgroup MO compared to Subgroup HO ($P < 0.05$). In conclusion, at the time of synchronisation, P4 concentration and luteal volume size was lower in metritic cows compared to healthy counterparts. However, pregnancy and embryo mortality rates were similar between metritic and healthy cows.

Introduction

Despite improvements in animal welfare and management, there are still certain postpartum processes that compromise the fertility of dairy cows, and one such processes is puerperal metritis. It is a common disease in the postpartum period that leads not only to milk production losses, but also to reduced fertility compared to healthy herdmates (Wittrock et al., 2011; Giuliodori et al., 2013).

Puerperal metritis has been well characterized by Sheldon et al. (2009a). According to these authors, cows with 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 (Sheldon et al., 2009a). The condition can be highly prevalent in some dairy farms and can range from 7 to 20% in multiparous dairy cows (Benzaquen et al., 2007; Giuliodori et al., 2013; Armengol and Fraile, 2015).

Another important event after calving is the recovery of cyclicity. Studies have shown that the first ovulation normally occurs within the first month postpartum and such cows are likely to achieve reproductive success (Galvão et al., 2010). However, there is evidence that puerperal metritis interferes with ovarian function and reduces circulating progesterone (P4) levels during the first oestrous cycles after calving (Sheldon et al., 2009b; Williams, 2013). The widespread adoption of ovulation synchronisation protocols for timed artificial insemination (TAI) has increased reproductive performance and economical improvement in the dairy industry (Caraviello et al., 2006). Currently, the use of hormones in the management of dairy cow reproduction is very common. The most popular TAI protocol is a combination of two hormones – gonadotropin-releasing hormone (GnRH) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) – and is known as Ovsynch (Pursley et al., 1995). Most of the studies on TAI are conducted with cows that do not have postpartum disorders or these disorders are not listed. In general, studies are carried out after analysing TAI programs based on cows' age (i.e., multiparous and primiparous cows) and productivity (i.e., daily milk yield). There is very limited information on how dairy cows react to the TAI protocol after treatment of puerperal metritis and at what level of fertility it could be expected.

The objective of the study was to determine pregnancy rates and ovarian changes in dairy cows synchronised with the TAI protocol after puerperal metritis and compare affected cows with healthy herdmates.

Material and methods

Farm

At the time of the study, approximately 1 100 cows were milked in the farm and average milk yield was approximately 33 kg milk/day per cow. Cows were fed twice daily with a total mixed ration supplemented with concentrate (Table 1) depending on milk yield,

Table 1. Total mixed ration with 27.3 kg dry matter

Ration composition	Dry matter, kg
Corn silage	4.7
Grass silage	8.13
Crushed corn grains	3.2
Sugar beet pulp silage	1.37
Grain concentrate mash	8.5
Wheat straw	0.36
Molasses	0.77

and housed in free stall barns with access to fresh water *ad libitum*. Cows were milked using Lely Astronaut® A3 milking robots (Lely Holding B.V., Maassluis, The Netherlands) with free traffic. To motivate the cows to visit robots, 2 kg of concentrate per day were offered by a single milking robot. Data on daily milk yield and lactation days were collected from the Lely T4C management program (Lely Holding B.V., Maassluis, The Netherlands) and used for analysis.

The study was carried out in compliance with European Union (EU) legislation. The procedures met the criteria set out in animal welfare regulations of the Republic of Lithuania (No B1-866, 2012; No XI-2271, 2012) and those established by the decree of the Director of the State Food and Veterinary Service of the Republic of Lithuania (No B6-(1.9)-855, 2017).

Study design

Cows. Seventy-two multiparous cows were enrolled in the study. All 72 cows were divided in two groups, according to their postpartum uterine health. Cows treated for puerperal metritis were defined as Group M (n = 34) and those without signs of puerperal metritis as Group H (n = 38). For Group M, cows were selected based on signs of puerperal metritis between day 5 to 14 postpartum (day 0 = day of calving) (Sheldon et al. 2009a). There were no differences in parity between the groups: for Group H, it was 2.38 ± 0.55 days, and for Group M – 2.32 ± 0.58 days. All 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 sc Cevaxel RTU, Ceva, France) for 5 days, whereas Group H did not receive any treatment. Cows in both groups were examined three times per week (Monday, Wednesday, and Friday) from day 5 ± 2 until day 49 ± 3 postpartum, and the modified Ovsynch protocol (GPPG) was initiated on day 49 postpartum. To assess cyclicity, ovaries were examined with a digital diagnostic ultrasound scanner

(Dramiński iScan, Dramiński S.A., Olsztyn, Poland) at a frequency of 7.5 MHz using a linear rectal transducer. All cows were synchronised for first insemination 49 ± 3 days in milk (DIM) using the modified protocol Ovsynch (GPPG). All 72 cows were cycling at the beginning of the protocol and did not show any signs of uterine disease. These cows had at least one corpus luteum (CL), which was > 15 mm in diameter, and at least one follicle ≥ 10 mm in diameter. Cows showing signs of oestrus midway through the protocol were inseminated accordingly.

Synchronisation protocol

Cows were synchronised for the first artificial insemination (AI) on day 49 ± 3 postpartum using the modified Ovsynch protocol described by Brusveen et al. (2009), following the administration of the second dose of $PGF_2\alpha$ 24 h after the first. Only cows that were cycling and had follicles larger than 10 mm were enrolled in this study. The first injection was GnRH (G1) (100 µg/dose of gonadorelin diacetate, Ovarelin, Ceva, France), followed by two $PGF_2\alpha$ (25 mg/dose of dinoprost tromethamine, Enzaprost, Ceva, France) treatments administered after 7 and 8 days, and the last GnRH (G2) injection was administered 56 h after the first $PGF_2\alpha$ treatment. AI was carried out by the same person, 16–18 h after G2 treatment. Cows were re-examined 24 h after insemination to determine the occurrence of ovulation after insemination. All cows from Groups H and M were subdivided into four subgroups after G1 injection: HO – healthy cows that ovulated their dominant follicle, MO – metric cows that ovulated their dominant follicle, HNO – healthy cows that did not ovulate their dominant follicle and MNO – metric cows that did not ovulate their dominant follicle.

Ovary measurements and pregnancy diagnosis

The ovaries during the study were scanned using an ultrasound scanner and the derivatives (follicles and CLs) in the ovaries were measured. The corpus luteum (CL) values obtained during ultrasound testing were used to calculate the average values (length (L) and width (W) and volume (V). The volume of CL was calculated using the following formula: $V = 4 / 3 \times \pi \times R^3$, where the radius (R) was calculated using the following 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). The results in mm^3 were converted to cm^3.

Pregnancy diagnosis, aimed at assessing pregnancy and pregnancy loss was performed by the same person on day 33 ± 3 and 63 ± 3 after insemination. Pregnancy diagnoses were carried out using transrectal ultrasonography (Dramiński iScan, Dramiński S.A., Olsztyn, Poland). Cows were considered pregnant if CL was present on the ovary ipsilateral to the uterine horn containing an embryo with a heartbeat. Cows that were diagnosed pregnant on day 33 ± 3 after AI and subsequently diagnosed non-pregnant on day 63 ± 3, were considered to have lost their pregnancy.

Blood sampling and P4 analysis

Blood samples were collected at each treatment, G1 injection, first $PGF_2\alpha$ injection, and G2 injection. Blood samples taken between G1 and $PGF_2\alpha$ were used to determine the impact of ovulation on the increase in P4 levels after G1 injection. Blood samples taken at $PGF_2\alpha$ were necessary to ensure that cows had functional CL, and those drawn between the first $PGF_2\alpha$ and G2 were used to determine complete luteolysis.

Blood samples were collected into tubes without anticoagulants by puncture from the median caudal vein or artery. After collection, blood samples were transported to the laboratory and centrifuged (2 000 g, 20 min at 4 °C); blood serum was stored at −20 °C until analysis. Serum P_4 concentration was analysed in an accredited laboratory (Segalab, Portugal) using a chemiluminescent assay (Immulite, Siemens, Wales, UK). The minimum level of detection for P_4 was 0.2 ng/ml.

Statistical analysis

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

Results

Ovulatory response after G1 treatment

In the present study, the number of cows that ovulated after G1 was similar between Group H and Group M – 36.84% (14/38) and 23.53% (8/34), respectively ($P > 0.05$). It was found that after G1, the highest risk of treatment effect on ovulation occurred between days 5 and 9 of the new oestrous cycle. Cows that received G1 treatment at this time

ovulated their dominant follicle in 93.33% (14/15) (Group H) and 100% (6/6) (Group M).

Group H and Group M at the time of G1

No differences were observed between Subgroup HO and Subgroup MO during G1 treatment with respect to ovulated follicle size, P4 concentration, and CL volume (Table 2).

at the time of $PGF_2\alpha$ treatment (seven days after G1) between Subgroup HO and MO, as well as between Subgroup HNO and MNO (Table 3).

CL volume was found to be lower in Subgroup MO compared to Subgroup HO. However, no significant differences in CL volumes were recorded between Subgroup HNO and Subgroup MNO (Table 3).

Table 2. Effect of G1 treatment in the experimental groups of cows

Item	Ovulation after G1			
	Group H (n = 34)		Group M (n = 38)	
	HO	HNO	MO	MNO
G1				
follicle size, mm	12.13 ± 0.26	13.74 ± 0.58$^\triangle$	13.50 ± 0.89$^\#$	15.50 ± 0.43$^{\#\triangle}$
progesterone (P4), ng/ml	2.65 ± 0.31	3.06 ± 0.38$^\triangle$	2.55 ± 0.19$^\#$	5.18 ± 0.32$^{\#\triangle}$
cows with progesterone > 0.5 ng/ml, % (n =/n =)	100 (13/13)	100 (25/25)	100.0 (8/8)	100.0 (26/26)
CL volume, cm^3	9.28 ± 1.12	8.81 ± 0.96	7.97 ± 0.75	9.54 ± 0.56

G1 – first GnRH injection, GnRH – gonadotropin-releasing hormone, Group H – cows without signs of puerperal metritis, Group M – cows treated for puerperal metritis, HO – subgroup of healthy ovulated cows, HNO – subgroup of healthy non-ovulated cows, MO – subgroup of metritic ovulated cows, MNO – subgroup of metritic an-ovulated cows, CL – corpus luteum; data are presented as mean value ± SEM (standard error of the mean); $^{\#*\triangle}$ – means within a row with different superscripts are significantly different at *P* < 0.05

At the time of G1, the size of dominant follicles was smaller in Subgroup HNO compared to Subgroup MNO (*P* < 0.05). Moreover, P4 concentration at G1 (*P* < 0.05), tended to be lower in Subgroup HNO compared to Subgroup MNO. No differences were observed in CL volume between those two subgroups (Table 2).

There were no significant differences between follicle size, P4 concentration, and CL volume in Subgroups HO and HNO. For Group M, it was found that the size of dominant follicles was smaller and P4 concentration was lower in Subgroup MO than in Subgroup MNO (*P* < 0.05) (Table 2).

Group H and Group M at the time of $PGF_2\alpha$ treatment

In the present study, no significant differences were identified in follicle size and P4 concentration

There was a significant difference in follicle size and CL volume between Subgroup HO and Subgroup HNO: smaller follicles but larger CL volumes were detected in Subgroup HO compared to Subgroup HNO. However, there was no difference in P4 levels (Table 3).

CL volume tended to be higher in Subgroup MO compared to Subgroup MNO (*P* < 0.05). However, no difference between follicle size and P4 concentration was observed (Table 3).

During $PGF_2\alpha$ treatment, P4 levels in all cows of Subgroups HO and MO were > 1 ng/ml, and they were significantly different from those in Subgroups HNO and MNO, where P4 concentrations > 1 ng/ml were observed only in 65.2 and 61.5% of cows, respectively (*P* < 0.05; Table 3).

Table 3. Effect of prostaglandin F2α ($PGF_{2\alpha}$) treatment in the experimental groups of cows

Item	Ovulation after G1			
	Group H (n = 34)		Group M (n = 38)	
	HO	HNO	MO	MNO
$PGF_{2\alpha}$				
follicle size, mm	12.71 ± 0.41*	15.13 ± 0.45*	13.75 ± 0.56	15.29 ± 0.71
progesterone (P4), ng/ml	5.14 ± 0.43	3.73 ± 0.65	4.42 ± 0.29	3.07 ± 0.59
cows with progesterone > 1 ng/ml, % (n =/n =)	100 (14/14)*	62.5 (15/24)*	100 (8/8)$^\#$	61.5 (16/26)$^\#$
CL volume, cm^3	17.62 ± 0.82$^{*\triangle}$	7.05 ± 1.00*	12.28 ± 0.71$^{\#\triangle}$	7.15 ± 0.85$^\#$

G1 – first GnRH injection, GnRH – gonadotropin-releasing hormone, Group H – cows without signs of puerperal metritis, Group M – cows treated for puerperal metritis, HO – subgroup of healthy ovulated cows, HNO – subgroup of healthy non-ovulated cows, MO – subgroup of metritic ovulated cows, MNO – subgroup of metritic an-ovulated cows, CL – corpus luteum; data are presented as mean value ± SEM (standard error of the mean); $^{\#*\triangle}$ – means within a row with different superscripts are significantly different at *P* < 0.05

Table 4. Effect of G2 treatment on the pregnancy rate in the experimental groups of cows

Item	Ovulation after G1			
	Group H (n = 34)		Group M (n = 38)	
	HO	HNO	MO	MNO
G2				
follicle size, mm	15.60 ± 0.30#	16.51 ± 0.48	17.75 ± 0.49#	16.65 ± 0.83
progesterone (P4), ng/ml	0.22 ± 0.10	0.23 ± 0.01	0.2	0.24 ± 0.01
Pregnancy				
P/AI at 33 d, % (n =/n =)	64.3 (9/14)	29.2 (7/24)	62.5 (5/8)	34.6 (9/26)
P/AI at 63 d, % (n =/n =)	50.0 (7/14)	20.8 (5/24)	50.0 (4/8)	23.1 (6/26)
pregnancy loss	14.3 (2/14)	8.3 (2/24)	12.5 (1/8)	11.5 (3/26)

G1 – first GnRH injection, G2 – second GnRH injection, GnRH – gonadotropin-releasing hormone, Group H – cows without signs of puerperal metritis, Group M – cows treated for puerperal metritis, HO - subgroup of healthy ovulated cows, HO – subgroup of healthy ovulated cows, HNO – subgroup of healthy non-ovulated cows, MO – subgroup of metritic ovulated cows, MNO – subgroup of metritic an-ovulated cows, P/AI – pregnancy per artificial insemination; data are presented as mean value ± SEM (standard error of the mean); # – means within a row with different superscripts are significantly different at $P < 0.05$

Group H and Group M at the time of G2

The study showed that follicle size at G2 was significantly larger in Subgroup MO compared to Subgroup HO ($P < 0.05$). Comparatively, there was no significant difference between follicle size in Subgroup MNO and Subgroup HNO during G2. All cows injected with G2 showed complete luteolysis and P4 levels below 0.4 ng/ml (Table 4).

Pregnancy rate on day 33 and 63 after AI

Comparing Subgroups HO and MO with Subgroups HNO and MNO, respectively, no differences were found in pregnancy rates after the synchronisation protocol ($P > 0.05$). However, a similar embryo mortality rate was observed in both Groups M and H between days 33 and 63 (12.0 and 11.3% respectively, $P > 0.05$) (Table 4).

Discussion

Metritis is a postpartum disease that negatively affects reproductive parameters in dairy farm animals (Esposito et al., 2014). Metritis, depending on the farm, can be found in 2 to 37% of dairy cows after parturition (Knudsen et al., 2015). In addition, dairy cows with uterine inflammation have poorer reproductive performance, which affects pregnancy rates and causes higher pregnancy losses (Vieira-Neto et al., 2014). There is very limited information on how cows that suffer from metritis respond to hormonal treatment. On the other hand, hormone therapy is well characterized in healthy cows (Barletta et al., 2018; Carvalho et al., 2019). The response to G1 treatment in the Ovsynch protocol is highly dependent on the stage of the oestrous cycle and varies from 32 to 47.2% in various studies (Colazo et al., 2013; Bisinotto et al., 2015; Barleta

et al., 2018). In the current work, the ovulation rate after G1 treatment was similar to the values determined in previous studies only for healthy cows, while metritic cows tended to show lower ovulation rates. This could be explained by the lower number of cows that were between days 5 and 9 of the oestrous cycle (Group H – 100% and Group M – 75%), when there was the highest risk of ovulation after G1 treatment (Bello et al., 2006; Bello and Pursley, 2007). It is therefore not possible to speculate that metritic cows are less likely to ovulate during the ovulation synchronisation protocol.

Moreover, it was known that most of the cows that did not ovulate were in late dioestrus, which explained higher P4 levels at G1. Additionally, the dominant follicles were larger in diameter, especially in Subgroup MNO compared to Subgroups HO and MO. Thus, it was more likely that these follicles were older and in atresia, and thus unable to ovulate.

Metritis was shown to have a negative effect on luteal size and P4 concentration after calving (Strüve et al., 2013). It was found that CL size was smaller in metritic cows, but only in the first oestrous cycle. Other studies by Sheldon et al. (2009b) and Williams (2013) also demonstrated that cows after metritis had smaller CLs and lower P4 levels in the first oestrous cycle postpartum. We applied the synchronisation protocol in the second month of lactation, which was a later period than those investigated in previous studies. The present study found that at the time of synchronisation, P4 concentration and luteal volume were lower in metritic cows compared to healthy herdmates. Based on these results, it could be concluded that the negative effect of metritis lasted longer than reported in previous studies (Sheldon et al., 2009b; Strüve at al., 2013).

However, the response to each hormonal treatment played a key role in successful synchronisation. P4 levels were higher in Subgroups HO and MO compared to Subgroups HNO and MNO. Cows with higher P4 concentrations before insemination showed higher pregnancy rates per artificial insemination (P/AI) compared to herdmates with low P4 concentrations (Cunha et al., 2008; Wiltbank et al., 2011; Bisinotto et al., 2013); this phenomenon was observed even in Subgroup MO.

Furthermore, suboptimal plasma P4 concentrations during the growth of ovulatory follicle were associated with increased levels of luteinizing hormone (LH) (Endo et al., 2012), which affected the growth rate of this ovulatory follicle and reduced follicular fluid IGF-1 levels (Cerri et al., 2011). This finding was consistent with the different results obtained in Subgroup HO and Subgroup MO in the current study, as lower P4 concentrations and larger dominant follicles were observed in Subgroup MO when PGF$_2\alpha$ was administered.

Moreover, new CLs were observed in cows that responded to G1 on the day of PGF$_2\alpha$ administration, as well as higher P4 levels and lower possibility to spontaneous luteolysis before the end of the AI protocol (Galvão et al., 2007). In the present study, P4 concentrations > 1 ng/ml were observed in all cows from Subgroups HO and MO, which could explain why luteolysis had not started before PGF$_2\alpha$. In contrast, P4 concentrations in cows that did not react to G1 (Subgroup HNO – 34.8%, Subgroup MNO – 38.5% cows), were below 1 ng/ml. It indicated that these cows were in the later stages of the oestrous cycle and underwent luteal regression before PGF$_2\alpha$ administration (Giordano et al., 2012).

According to Bisinotto et al. (2015), the size of preovulatory follicle at the time of G2 was 17.5 mm in cows that ovulated after G1 administration. Follicles of similar size were found in Subgroup MO, but preovulatory follicles in Subgroup HO were smaller. The results obtained in this work were consistent with the findings published in the aforementioned studies that cows after metritis produced less P4 and these suboptimal P4 concentrations were related to increased LH levels that promoted dominant follicle growth at its final stage, and that preovulatory follicles were larger in cows with postpartum uterine inflammation (Cerri et al., 2011; Endo et al., 2012; Bisinotto et al., 2013). Preovulatory follicles in this study were larger in diameter in Subgroup MO than in Subgroup HO.

Nevertheless, the results of the present study did not indicate that follicle size was associated with conception rates, as values of this parameter were appropriate in both groups. It is possible that good conception rates are more related to changes in the intrafollicular environment and oocyte quality than follicle size and long-term effects of uterine infection on the endometrium (Denicol et al., 2012; Ribeiro et al., 2016).

It is obvious that healthy uterus and good reaction to hormone therapy during oestrous synchronisation leads to high pregnancy rates. Ribeiro et al. (2016) suggested that uterine infection could also have a long-term effect on the endometrium and could be responsible for lower pregnancy rates in dairy cows. There is evidence that uterine infection induces prolonged inflammatory response that also damages uterine tissue. Inflammatory processes in the uterus result in a delayed time to the first insemination and more days open (Toni et al., 2015). The increase in days open is caused by endometritis or subclinical endometritis and has a long-term effect on reproductive performance (Sheldon et al., 2009a). According to Heppelmann et al. (2016), cows with endometritis had a higher incidence of degenerative vascular lesions in the uterine lumen than healthy cows, which could affect uterine functions. Clinical endometritis can be easily diagnosed due to purulent or mucopurulent vaginal discharge, but subclinical endometritis does not present clinical signs of illness and its prevalence may reach 34% during the first seven weeks (Kasimanickam et al., 2004). Considering that subclinical endometritis is not rare and that the negative effect of this disease plays an important role in the fertility of dairy cows (Pascottini and LeBlanc, 2020), it is very important to treat this disease before the insemination, especially on dairy farms that have a short voluntary waiting period and use ovulation synchronisation protocols for first insemination.

Surprisingly, pregnancy rates for cows with puerperal metritis postpartum were comparable to healthy herdmates. This can be explained by a good recovery rate after puerperal metritis treatment with ceftiofur, which, according to a number of studies, is an effective clinical solution in 74–77% of treated animals (Chenault et al., 2004; McLaughlin et al., 2012). Kasimanickam et al. (2006) also did not find significant differences in pregnancy rates at the first service between cows after uterine inflammation and healthy counterparts, which was consistent with the results of this study. According to Chebel et al. (2006), Keskin et al. (2010) and Bisinotto et al. (2015), cows that ovulated after G1 injection had a higher number of pregnancies per AI compared to those that did not ovulate at the beginning of the Ovsynch protocol. The same tendency was observed in Subgroups HO and MO in the current work.

The present research demonstrated that embryo losses were similar between post-metritis and healthy cows. However, these results differed from other studies that claimed that fertility in cows that were suffering from postpartum metritis or other diseases was decreased (Chebel et al., 2004). This implies that the main effect of metritis is that uterine environment is not conducive to sperm or embryo survival, as well as failure in embryo survival after AI due to oocyte defects (Spencer 2013). Although postpartum diseases reduce pregnancy rates, almost all previous studies have examined embryo mortality in dairy cows without distinguishing between cows with postpartum diseases and healthy ones. Contrary to Barletta et al. (2018) and Nowicki et al. (2018), this study revealed that pregnancy losses were higher in both H and M groups on second pregnancy check. Often, the main reason of high embryo mortality is incomplete luteolysis (Nowicki et al., 2019). In this study, low P4 levels were observed at the time of insemination in all groups. The high embryo mortality observed in this trial could be caused by infectious diseases such as *bovine* viral diarrhea. This requires further research.

Conclusions

The current worked demonstrated that at the time of synchronisation, P4 concentration and luteal volume size were lower in metritic cows compared to healthy herdmates. However, pregnancy and embryo mortality rates were similar in metritic and healthy cows.

Acknowledgments

Conflict of interest

The Authors declare that there is no conflict of interest.

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