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Original article

Association of subclinical endometritis with acyclia and their additive effect on fertility performance in dairy cows

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Abstract

The aim of this study was to evaluate the relationship between acyclia and subclinical endometritis (SE) and their combined effect on fertility performance in dairy cows. The study was performed on 449 Holstein Friesian cows. The cows were examined in the 4th week postpartum by ultrasound and endometrial samples were collected by cytobrush. Acyclia was defined as the absence of the corpus luteum and follicles > 5 mm on the ovaries. The threshold for SE was set at ≥ 5% of polymorphonuclear neutrophils in endometrial cytology. Intervals calving to estrus and calving to conception, first artificial insemination (AI) pregnancy rate, pregnancy rate 200 days postpartum, pregnancy loss and culling rate were calculated. Acyclia was found in 144 (32.1%) of 449 examined cows. The incidence of SE in acyclic cows was significantly ($p \leq 0.05$) higher than in cyclic cows (52.8% vs 36.7%). The intervals calving to estrus and calving to conception were significantly ($p \leq 0.05$) longer in acyclic cows with SE than in cyclic cows with SE (99.1 ± 28.9 days vs 77.4 ± 24.3 days and 160.2 ± 60.9 days vs 131.6 ± 46.6 days, respectively). The first AI conception rate was significantly ($p \leq 0.05$) lower in acyclic cows with SE than in cyclic cows with SE (17.1% vs 34.8%). The pregnancy loss was significantly ($p \leq 0.05$) higher in acyclic cows with SE than in cyclic cows with SE (11.8% vs 5.3%). In conclusion, the study showed that acyclia and SE were associated and had an additive negative effect on reproductive performance in dairy cows.

Keywords: cattle, cyclicity, postpartum, uterine disease

Introduction

Postpartum uterine diseases are common in dairy cows. The main inflammatory diseases are metritis, pyometra, clinical and subclinical endometritis. Subclinical endometritis (SE) is the superficial inflammation of the endometrium, defined by the presence of > 5% of polymorphonuclear neutrophils (PMNs) after 21 days after parturition in endometrial samples obtained using cytobrush, low-volume flushing of the uterus or cytotape in the absence of clinical signs (Madoz et al. 2013, Pascottini at al. 2017, Wagener et al. 2017). SE is also referred to as cytological endometritis (Dubuc et al. 2010, McDougall et al. 2011). The pathomechanism of SE is not clear. It seems that SE is more associated with unspecific infections than with common uterine pathogens (Wegener et al. 2017). A study using sequencing of the 16S rRNA gene showed that the uterine microbiota in healthy and SE cows did not differ (Pascottini et al. 2020). Thus, it is hypothesized that SE is the result of transition period maladaptation including excessive metabolic stress, which results in immune dysregulation (LeBlanc 2023, Pascottini et al. 2023). The prevalence of SE depends on the time of examination in the postpartum period, the threshold for PMNs and herd specific factors, and ranges from 15% to 70% (Kasimanickam et al. 2004, Gilbert et al. 2005, Barański et al. 2012, Madoz et al. 2013). Several factors such as BCS, dystocia, retained placenta, metritis, clinical endometritis, ketosis and housing conditions are risk factors for SE (Cheong et al. 2011, Ribeiro et al. 2013, Prunner et al. 2014). The majority of studies have shown a negative effect of SE on the reproductive performance of dairy cows (Kasimanickam et al. 2004, Gilbert et al. 2005, Barański et al. 2013, Madoz et al. 2013).

An early resumption of normal ovarian cyclicity after calving is associated with improved uterine health and fertility. In lactating dairy cows, the interval from calving to first ovulation typically lasts 2 to 4 weeks (Darwash et al. 1997, McCoy et al. 2006, Crowe et al. 2014). However, 20-40 % of cows show delayed resumption of ovarian activity (acyclia) (Opsomer et al. 1998, Walsh et al. 2007, Santos et al. 2009, Galvão et al. 2010). The major cause of delayed ovulation in postpartum cows is an infrequent LH pulse frequency (Crowe et al. 2014). Risk factors for delayed resumption of cyclicity include BCS loss associated with severe negative energy balance, parity, periparturient disorders, season of calving, management, mastitis, lameness and heat stress (Walsh et al. 2007, Dubuc et al. 2012, Crowe et al. 2014). An extended interval from calving to first ovulation is associated with impaired reproductive performance (Shrestha et al. 2004, Galvão et al. 2010, Walsh et al. 2011).

The occurrence of SE in cows with clinical diseases, such as dystocia, retained placenta, metritis, clinical endometritis, clinical hypocalcaemia, ketosis, clinical mastitis and severe lameness had an additive negative effect on fertility (Valdamann et al. 2022). However, there were only few studies on the relationship between SE and delayed ovarian function in postpartum dairy cows and the results were inconsistent (Galvao et al. 2010, Dourey et al. 2011, Dubuc et al. 2012, Vieira-Neto et al. 2014). Thus, the aim of this study was to evaluate the occurrence of SE in cows with acyclia in the 4th week postpartum and their combined effect on fertility in dairy cows.

Materials and Methods

The study was conducted on 449 Polish Holstein Friesian cows from three dairy herds in North-East Poland. The herds were under a veterinary herd-health management program and the cows were patients of the clinic and therefore an Ethic Committee Agreement was not required. The cows were housed in loose housing barns and fed total mixed ration based on grass silage, maize silage and concentrate. The average milk yield was about 9000 L per year. Cows with retained placenta, metritis, pyometra, acute mastitis, clinical ketosis or severe lameness were not included into this study. The clinically healthy cows were examined in the 4th week postpartum by ultrasound using a Honda 1500 scanner with a 5 MHz linear transducer and endometrial samples were collected by cytobrush (Cervical Rambrush type IC, Shanghai International Holding Corp. GmbH, Germany). Anovulation was defined as the absence of the corpus luteum and follicles > 5 mm on the ovaries. To diagnose subclinical endometritis cytological examination was used as previously described (Barański et al. 2012). The threshold for subclinical endometritis was set as equal to or over 5% of PMNs.

After detection of estrus the cows were artificially inseminated (AI). Pregnancy was diagnosed by ultrasonography 30 days after AI. Cows diagnosed as pregnant were re-examined on day 200 after AI. The following reproductive performances were calculated for acyclic and cyclic cows with SE: intervals calving to estrus and calving to conception, first AI pregnancy rate, pregnancy rate 200 days after AI, pregnancy loss and culling rate. Pregnancy loss was defined as the percentage of non-pregnant cows 200 days after AI diagnosed 30 days after AI as pregnant.

Statistical analysis was performed using GraphPad Prism version 9.00 (GraphPad Software, San Diego, CA, USA). The data were analysed using the Mann-Whitney test and chi-square test with Yates correction. Statistical significance was set at $p<0.05$.

Table 1. Incidence of subclinical endometritis (SE) in acyclic and cyclic dairy cows in the 4th week postpartum.

Group	No	%	SE	
			No	%
Acyclic cows	144	32.1	76	52.8[a]
Cyclic cows	305	67.9	112	36.7[b]
Total	449	100.0	188	41.9

a, b – difference between groups statistically significant at p≤0.05.

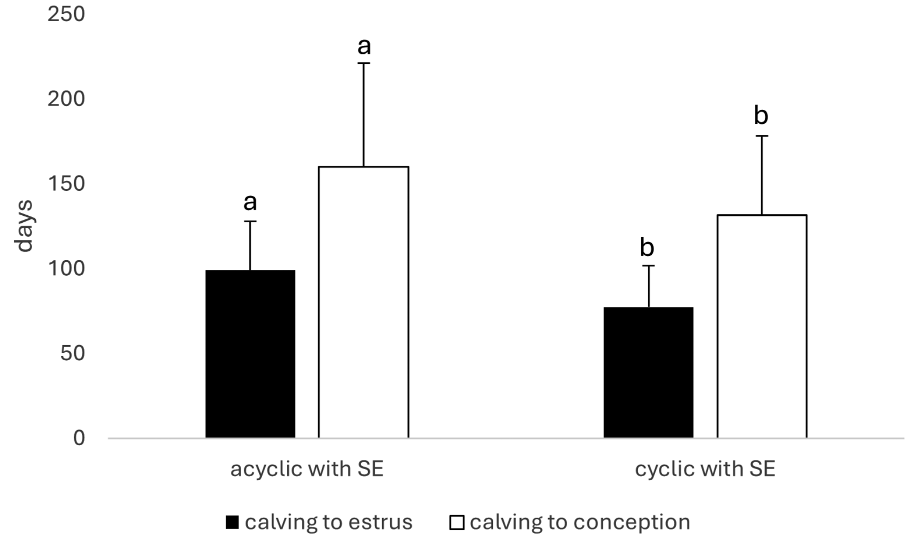

Fig. 1. Intervals calving to estrus and calving to conception (mean ± SD) in acyclic and cyclic cows with SE. Different superscript letters indicate statistical significance at p≤0.05.

Results

Acyclia was found in 144 (32.1%) of 449 examined cows. The incidence of SE in acyclic cows was significantly (p≤0.05) higher than in cyclic cows (52.8% vs 36.7%) (Table 1). The interval calving to estrus was significantly (p≤0.05) longer in acyclic cows with SE than in cyclic cows with SE (99.1 ± 28.9 days vs 77.4 ± 24.3 days). Compared to cyclic cows with SE, acyclic cows with SE showed a longer interval of calving to conception (160.2 ± 60.9 days vs 131.6 ± 46.6 days; p≤0.05) (Fig. 1). The first AI conception rate was significantly (p≤0.05) lower in acyclic cows with SE than in cyclic cows with SE (17.1% vs 34.8%). Compared to cyclic cows with SE, acyclic cows with SE showed a tendency towards a lower conception rate 200 days after AI (61.8% vs 73.2%) (Fig. 2). There was no statistical difference in the culling rate between acyclic and cyclic cows with SE (34.2% vs 26.8%; p>0.05). The pregnancy loss was significantly (p≤0.05) higher in acyclic cows with SE than in cyclic cows with SE (11.8% vs 5.3%) (Fig. 3).

Discussion

Follicular growth generally resumes within 7 to 10 days postpartum in the majority of cows (Crowe et al. 2014). In dairy cows with a normal puerperium the first postpartum ovulation occurs between 15 and 21 days after calving, but the standard deviation is large (Darwash et al. 1997, Beam and Butler 1999, Crowe et al. 2014). Approximately 40-50% of Holstein-Friesian cows ovulate the dominant follicle of the first follicular wave within 21 days (Beam and Butler 1999). In our study, acyclia was found in the 4th week postpartum in 32.1% of the cows. The proportion of acyclic cows was within reported ranges (Opsomer et al. 1998, Kawashima et al. 2006, Galvão et al. 2010, Shrestha et al. 2014).

In our study the proportion of cows with SE was greater for acyclic cows than cyclic cows (52.8% vs. 36.7%). However, only limited data are available on the relationship between SE and delayed ovarian function in postpartum dairy cows, and the results are conflicting. Gilbert et al. (2005) reported that SE significantly delayed the days to first service. Cows acyclic 21 days postpartum had increased prevalence of subclinical endometritis compared with cyclic cows (Galvão et al.

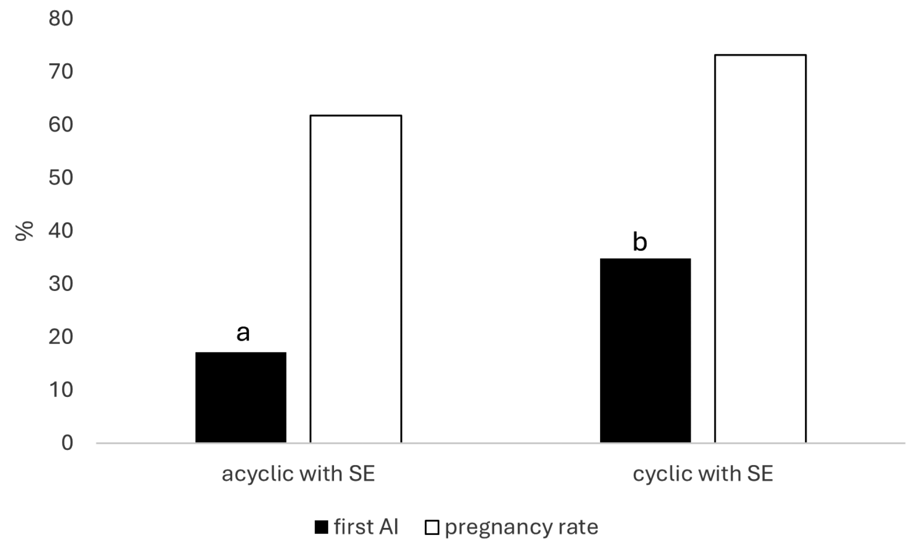

Fig. 2. Pregnancy rates after 1st artificial insemination (AI) and 260 days after AI (%) in acyclic and cyclic cows with SE. Different superscript letters indicate statistical significance at p≤0.05.

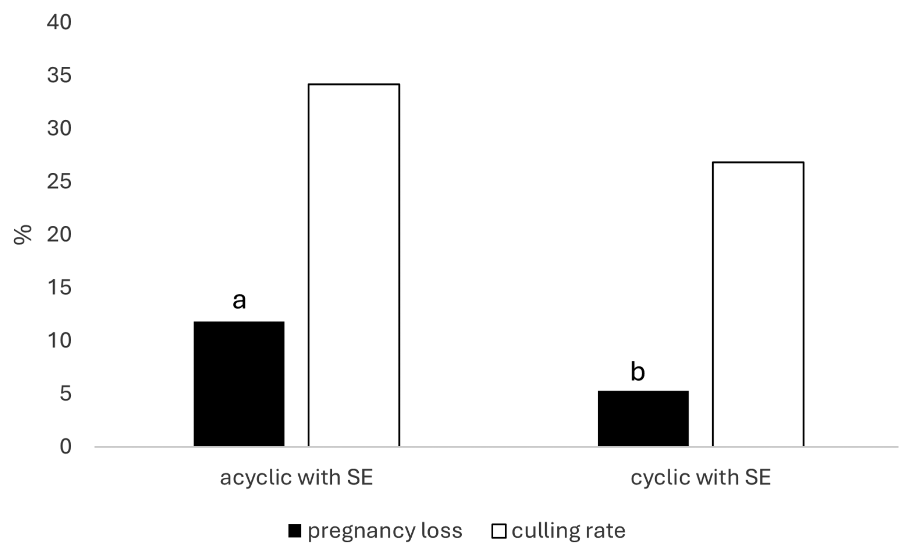

Fig. 3. Pregnancy loss (%) and culling rate (%) in acyclic and cyclic cows with SE. Different superscript letters indicate statistical significance at p≤0.05.

2010). The interval from calving to first ovulation was longer in cows with PMN > 8% on day 25 postpartum, than in low PMN cows (Dourey et al. 2011). Cytological endometritis in postpartum cows was associated with an increased likelihood of anovulation (Dubuc et al. 2012). In contrast, some studies showed that that the interval from calving to first ovulation was not affected by SE (Gobikrushanth et al. 2016, Valdmann et al. 2022).

The underlying cause for delayed ovarian function and subclinical endometritis is a negative energy balance postpartum (Beam and Butler 1999, Dubuc et al. 2010, Crowe et al. 2014, Pascottini et al. 2023). Early ovarian function postpartum is a marker of a low degree of energy deficiency and stimulates clearance of the uterus (Luginbühl and Küpfer 1980, Beam and Butler 1999, Galvão et al. 2010). On the other hand, an early postpartum first ovulation in the presence of uterine infection can lead to pyometra with persistence of a corpus luteum (Olson et al. 1984) and uterine infections are risk factors for ovarian dysfunction (Opsomer et al. 2000, Sheldon et al. 2002, Senosy et al. 2011). The delayed resumption of ovarian activity in cows with uterine diseases could be associated with the effects of the inflammatory mediators on the hypothalamus and pituitary (Sheldon et al. 2009).

Separately, delayed ovarian function (Shrestha et al. 2004, Galvão et al. 2010, Walsh et al. 2011) and subclinical endometritis (Kasimanickam et al. 2004, Gilbert et al. 2005, Barański et al. 2013, Madoz et al. 2013) are associated with impaired reproductive performance in dairy cows. It has been shown that clinical diseases in early postpartum and SE on day 40 after calving had a combined negative effect on the fertility of dairy cows (Valdmann et al. 2022). There is only one report on the combined effect of anovulation and cytological endometritis on the reproductive performance of dairy cows (Vieira-Neto et al. 2014). In this study it was found that both conditions had a combined negative effect on the fertility of cows. The pregnancy per AI was lower in cows having anovulation and SE compared with cyclic cows without SE. The effect was found in cows synchronized using a timed-AI program. The hazard of pregnancy up to 300 d in milk was decreased in cows having anovulation and SE together or alone, regardless of the AI programme.

In our study the effects of acyclia and SE on reproductive performance were additive as the fertility performance of acyclic cows with SE was lower than in cyclic cows with SE. The interval calving to estrus was longer, the first AI conception rate was lower, the interval calving to conception was longer and the pregnancy loss was higher. The extension of the interval calving to conception in cows with acyclia and SE was a result of the later occurrence of estrus, as well as the low pregnancy rate after first AI and the high pregnancy loss. The effect of acyclia is likely mediated by the lack of progesterone priming from earlier estrous cycles, resulting in low oocyte quality and inadequate preparation of the uterus for pregnancy (Santos et al. 2016). SE, on the other hand, is associated with local inflammatory reactions causing an unfavourable uterine environment for embryo development (Wagener et al. 2017). Culture of bovine embryos in media conditioned by exposure to an inflamed endometrium reduced their quality (Hill and Gilbert 2008). SE in recipient cows resulted in reduced pregnancy per embryo transfer (Barnes et al. 2023).

In conclusion, the study showed that acyclia and SE were associated and had an additive negative effect on the reproductive performance of dairy cows. Future studies with a larger number of animals are needed to confirm our findings and to further evaluate the combined effect of acyclia and SE on fertility performance in dairy cows.

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