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SHORT COMMUNICATION

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Effect of subclinical mastitis on reproductive performance of Holstein dairy cows in the Northwest of Spain

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Abstract

Aim of study: To investigate the effect of subclinical mastitis (SCM) before and after first artificial insemination (AI), characterized by a somatic cell count (SCC) higher than 200×10^3 cell/mL, on reproductive performance including first service conception rate (FSCR) and pregnancy loss (PL) in Holstein dairy cows.

Area of study: The central area of Lugo, Galicia, Spain.

Material and methods: This retrospective study was conducted on herd database of a population of 80 commercial Holstein dairy cow farms. A total number of 2053 lactations were included in this study. A binary logistic regression was carried out to analyse all data.

Main results: The results of this study indicated that cows that registered a SCC lower than 200×10^3 cell/mL within 30 days after first AI were more likely to conceive pregnancy than cows with a higher SCC (31.2% and 25.1% FSCR, respectively; OR=1.285, 95% CI=1.000-1.653). Additionally, an increased SCC neither 30 days before nor 30 days after first AI had a negative effect on prevalence of PL in dairy cows.

Research highlights: These findings revealed that SCM within 30 days after first AI negatively affected FSCR, whilst 30 days before first AI did not affect it. Therefore, it could be suggested that preventing subclinical mastitis after first AI, during a critical period of 30 days, is important to maximize the reproductive performance of dairy cows.

Additional key words: dairy cattle; somatic cell count; milk yield; fertility; conception; pregnancy loss.

Abbreviations used: AI (Artificial Insemination); CI (Confidence Interval); CM (Clinical Mastitis); CR (Conception Rate); DFS (Days to First Service); DO (Days Open); EEL (Early Embryonic Loss); FSCR (First Service Conception Rate); GDCP (Galician dairy control program); IMI (Intramammary Infections); LIGAL (Laboratorio Interprofesional Galego de Análise do Leite); LNSCC (Linear Somatic Cell Counts); OR (Odds Ratio); PL (Pregnancy Loss); SCC (Somatic Cell Count); SCM (Subclinical Mastitis); VWP (Voluntary Waiting Period)

Authors' contributions: Study design: HS, UY, AIDP, and LAQ. Acquisition of data: AIDP and LAQ. Analysis of data: HS, UY, and LAQ. Drafting of the manuscript: HS, UY, JJB, PGH, and AIP. Critical revision: JJB, PGH, AIP, LAQ, and FG.

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Introduction

Mastitis and infertility are the two main problems in the dairy cow industry. Mastitis is a worldwide-spread issue that negatively affects the farms' economy by reducing milk production and increasing both the use of drugs and culling rates (Halasa *et al.*, 2007; Pérez-Cabal *et al.*, 2008). Moreover, according to the literature, the incidence of mastitis is also associated with a negative effect on reproductive performance such as conception rate (CR), days open (DO), and number of services per conception (Schrick *et al.*, 2001; Santos *et al.*, 2004; Lavon *et al.*, 2011). Unlike clinical mastitis (CM), subclinical mastitis (SCM) is not characterized by visible symptoms of inflammation. In fact, in the SCM-affected cows just an increased somatic cell count (SCC) and a decreased milk production are observed (Hansen *et al.*, 2004). Thus, SCC is a useful and valuable diagnostic approach to detection of SCM (Ruegg & Erskine, 2014). To differentiate healthy cows from subclinical cows, a threshold of 200,000 to 250,000 cell/mL has been repeatedly considered as a cut-off point (Schepers *et al.*, 1997; Schukken *et al.*, 2003).

Previous studies have demonstrated that an increased SCC before and after artificial insemination (AI) negatively affects CR and pregnancy loss (PL) (Schrick *et al.*, 2001; Pinedo *et al.*, 2009; Lavon *et al.*, 2011), although the timing regarding before or after AI is still controversial. Lavon *et al.* (2011) reported a considerable reduction in CR in SCM-affected cows when SCC rose 10 days before to 30 days after AI; moreover, these researchers observed that such reduction was related to the degree of SCC elevation, considering the CR plummeted as SCC soared. However, some studies claim that there is no effect on CR and DO (Klaas *et al.*, 2004), whereas others declare that a high SCC before AI has some effect on the nonreturn rate (Miller *et al.*, 2001).

Besides, the influence of mastitis on PL has also been investigated. Chebel *et al.* (2004) found an increased incidence of PL from day 31 to 45 after AI if CM occurred between AI and pregnancy reconfirmation (performed by palpation per rectum 45 days after AI and 14 days after pregnancy diagnosis). Furthermore, Pinedo *et al.* (2009) indicated that a high SCC during the first 90 days of gestation also increases the probability of abortion (loss of conceptus between days 45 and 270 of gestation).

Altogether, most of the abovementioned studies show a clear relationship between the level of SCC before and after AI and the fertility of cows. However, there are noticeable differences in location and factors taken into account among them. Considering this, and the fact that there is little literature about this subject in the Northwest of Spain (Pérez-Méndez *et al.*, 2020), the need for further research regarding this issue is certain. Accordingly, the aim of the current study was to investigate the effect of SCM before and after first AI, characterized by SCC higher than 200×10^3 cell/mL, on reproductive performance including first service conception rate (FSCR) and prevalence of PL (between ~28 and 60 days after AI) of Holstein dairy cows in the Galicia region of Spain. As all the farms included in the study were affiliated with the Galician Dairy Control Program (GDCP), monthly information about SCC was provided. Consequently, an interval of 30 days before and after the first AI was chosen to include as much data as possible. Moreover, a second objective was to verify if the records of the GDCP were a reliable source of information to predict fertility based on the SCC levels.

Material and methods

Study design

Data from November 2009 to November 2010 were provided by a collaborator veterinarian, who collected all the information on the software ReproGTV. This retrospective, observational study included a total number of 2,053 lactations and was conducted on a population of 80 commercial Holstein dairy farms located in the central area of Lugo, Galicia, Spain.

Farms and management

To carry out this study, we selected all farms within the collaborator veterinarian's client list. These had an average of 35 cows in milk per farm. All farms had a conventional milking parlour, and cows were milked twice a day. Composite milk SCC was determined monthly by the GDCP in LIGAL (Laboratorio Interprofesional Galego de Análise do Leite). On test day, composite milk samples were collected from each lactating cow in 50 mL plastic containers with the preservative bronopol (2-bromo-2nitro-1,3-propanediol) previously added, and SCC (cell/mL) values were then analysed by using cell counter FOSSOMATIC™ (MilkoScan, Foss, Hillerød, Denmark) according to the manufacturer's instructions.

Regarding the reproductive management, routine AI was performed following detection of oestrus and oestrus synchronization program, and pregnancy diagnosis was performed by the veterinarian using ultrasonography, the first on ~ day 28 after AI (ranging from 26 to 35), and the second on ~ day 60 after AI (ranging from 55 to 65) in all farms. The voluntary waiting period (VWP) varied from 50 to 60 days in milk among herds.

Statistical analysis

The date of calving, parity, date of first AI, date of the first conception, incidence of pregnancy loss, milk yield, and SCC were collected for each animal from the software ReproGTV. For this study, reports of milk production and SCC of the GDCP records were used. All SCC data were collected monthly, except for August. Of these SCC records, the one before and after the first AI were used if they had been performed between 1 and 30 days before or after AI. The SCC records older than 30 days or carried out on the insemination day were not included.

The variables measured for this study were: SCC before or after the first AI categorizing as $>200\times10^3$ cell/mL (SCM, n=430 before AI and n=442 after AI) or $\leq200\times10^3$ cell/mL (non-SCM, n=1329 before AI and n=1315 after AI); days to first service (DFS), the interval from calving to first service, defined in two groups: before (n=1110) or after (n=943) postpartum day 70; parity categorizing as primiparous (n=555) or multiparous (n=1498); the 305-d milk yield divided, according to the median distribution, into two groups: >8780 L (n=1025) or ≤8780 L (n=1024); season of AI (autumn, winter, spring, and summer); first service conception rate (FSCR) defined as the proportion of cows diagnosed pregnant following first insemination postpartum; and pregnancy loss (PL) defined as a cow that was diagnosed as pregnant on day ~28 after AI (first pregnancy diagnosis) and had lost her pregnancy by day ~60 after AI (second pregnancy diagnosis).

The obtained data were analysed using Pearson's chi-squared test, in order to preselect the significant variables, using FSCR and PL as dependent variables and SCC before or after the first AI, DFS, 305-d milk yield, parity and season as independent factors. Secondly, a binary logistic regression by using the backward conditional stepwise method was performed. Interactions between SCC and the other factors were included in the analysis. To include the maximum number of animals, two binary logistic regressions were carried out for each dependent variable: one selecting all cases with a SCC record within 30 days before AI, and the other including only animals with a SCC record within 30 days after AI. All analysis were conducted in SPSS version 20.0 for Windows (SPSS Inc, Chicago, IL, USA). Differences were considered significant at $p < 0.05$, and values between 0.05 and 0.1 were considered trends.

Results and discussion

FSCR and PL proportion considering SCC before first AI, SCC after first AI, 305-d milk yield, DFS, parity, and season are presented in Table 1. Regarding both FSCR and PL, no significant interactions between factors were found in this study.

First service conception rate

Overall, the average FSCR for all the cows involved in this study was 28.9%. FSCR did not differ between non-SCM and SCM groups considering SCC before first AI (29.6% and 25.8%, respectively; p=0.134). After first AI, the SCM group had lower FSCR than the non-SCM group (25.1% and 31.2%, respectively; p=0.016). In relation to 305-d milk yield, FSCR was lower for cows in the >8780 L group than for cows in the ≤8780 L group (22.7% and 35.1%, respectively; p=0.00). The FSCR for primiparous cows was higher than for multiparous cows (35.5% and 26.5%, respectively; p=0.00). Finally, the FSCR was 30.1% and 27.6% for cows in <71 DFS and

Table 1. First service conception rate and pregnancy loss proportion considering several variables. Blank variables in the last 3 columns were not included in the final model of the logistic regression. The binary logistic regression model for first service conception rate (FSCR) includes the results for animals with a somatic cell count (SCC) record within 30 days after artificial insemination (AI).

		Pearson's chi^2				Binary logistic regression model for FSCR[a, b]		
Variables	**Level**	**FSCR**	***p*-value**	**PL**	***p*-value**	**AOR**	**95% CI**	***p*-value**
SCC before AI	<200×10^3 cell/mL	29.6% (393/1329)	0.134	8.4% (31/370)	0.585	-	-	-
	>200×10^3 cell/mL	25.8% (111/430)		6.7% (7/104)				
SCC after AI	<200×10^3 cell/mL	31.2% (410/1315)	0.016	7.8% (30/386)	0.616	1.285	1.000-1.653	0.050
	>200×10^3 cell/mL	25.1% (111/442)		9.3% (10/108)		1.000	-	
305-d milk yield	<8780 L	35.1% (359/1024)	0.000	5.6% (19/341)	0.006	1.820	1.469-2.257	0.000
	>8780 L	22.7% (233/1025)		12.0% (26/216)		1.000	-	
DFS	<71 d	30.1% (334/1110)	0.210	8.6% (27/313)	0.572	-	-	-
	>70 d	27.6% (231/943)		7.3% (18/246)				
Parity	Primiparous	35.5% (197/555)	0.000	4.9% (9/184)	0.055	-	-	-
	Multiparous	26.5% (397/1498)		9.6% (36/375)				
Season	Winter	30.8% (132/429)	0.401	4.8% (6/124)	0.232	-	-	-
	Spring	26% (108/415)		11.8% (12/102)				
	Summer	28.5% (200/701)		9.2% (17/185)				
	Autumn	30.3% (154/508)		6.8% (10/148)				

DFS: days to first service. PL: pregnancy loss. AOR: adjusted odds ratio. CI: confidence interval. [a] Hosmer & Lemeshow test: p=0.863. [b] Cox & Snell R^2=0.026

>70 DFS groups, respectively (p=0.210), and was not influenced by season (30.8%, 26% 28.5%, and 30.3% for winter, spring, summer, and autumn, respectively, p=0.401).

The results of the logistic regression for FSCR, including animals with a SCC record within 30 days after AI, are depicted in Table 1. Regarding SCC after first AI, cows in the non-SCM group were more likely to conceive pregnancy than cows in the SCM group (OR=1.285; 95% CI: 1.000-1.653; p=0.050). Moreover, cows with milk productions ≤8780 L/305-d were 1.820 (95% CI: 1.469-2.257) times more likely to become pregnant than cows that produced >8780 L/305-d (p=0.00). Concerning cows with a SCC record within 30 days before AI, our results show that SCC factor was not included in the final model. In addition, primiparous cows, animals inseminated during spring or summer and <70 d after parturition were more likely to become pregnant (OR=1.332, 95% CI: 1.052-1.688, p=0.011; OR=1.407, 95% CI: 1.026-1.929, p=0.034; OR=1.351, 95% CI: 1.003-1.819, p=0.044; OR=1.250, 95% CI: 1.011-1.546, p=0.033, respectively). Furthermore, cows with milk productions ≤8780 L/305-d had a higher probability of conceiving pregnancy (OR=1.803, 95% CI: 1.448-2.245, p=0.00).

According to our analysis, an increased SCC level within 30 days after first AI, higher than 200×10^3 cell/mL, significantly reduced FSCR. This result is in accordance with previous studies suggesting that an elevation in SCC within the first month after AI was associated with a decrease in pregnancy rate (Pinedo *et al.*, 2009; Lavon *et al.*, 2011; Hudson *et al.*, 2012). Considering that mastitis after AI may interfere with corpus luteum formation and regression, progesterone secretion, endometrial functions, and embryonic development (Gilbert *et al.*, 1990; Mann & Lamming, 2001; Spencer *et al.*, 2004), the negative effect of a sudden increase in the SCC level after first AI on FSCR in our study could have resulted from those disruptive effects. Hence, it could be suggested that, to achieve the best CR and diminish economic loss, farmers need to recognize the consequences that subclinical mastitis can have on FSCR and focus on preventing this disease within 30 days after first AI, which is one of the critical periods concerning the success of pregnancy.

It should be noted that, in our study, an increased SCC before first AI had no influence on FSCR. According to the literature, the effect of SCC before AI on FSCR is controversial. Two studies stated that an increase in SCC 30 days before AI (Pinedo *et al.*, 2009) and 10 days before AI (Lavon *et al.*, 2011) diminished the odds of conception. Hudson *et al.* (2012) also showed that clinical and subclinical intramammary infections (IMI) occurring before or after AI reduce reproductive performance. Furthermore, Schrick *et al.* (2001) indicated that clinical and subclinical mastitis before AI increase days open and services per conception, irrespective of the pathogen type. Additionally, McDougall *et al.* (2016) observed a slower conception in cows diagnosed with mastitis before rather than after the first AI. Besides, Miller *et al.* (2001) have reported that high SCC before AI has a minimal effect on the non-return rate. These results may be explained by the production of pro-inflammatory cytokines triggered by mastitis (SCC >400,000 cell/mL), which reduce the concentrations of steroid and gonadotrophins, leading to an impairment of follicular development and lower oocyte quality (Santos *et al.*, 2018). In contrast, there are some studies that support our findings that SCC before AI has no effect on conception. In this regard, Klaas *et al.* (2004) claimed that there is no effect on CR or DO. Moreover, Fernandes *et al.* (2021) reported that SCM before first AI did not affect the likelihood of pregnancy. Reasons for the differences among studies could result from differences in cut-off values of SCC between healthy and infected cows, or timing of IMI relative to AI (Wolfenson *et al.*, 2019).

We found that 305-d milk yields >8780 L and parity negatively affected CR. This is in accordance with several previous studies. Buckley *et al.* (2003) reported that the hastily increased milk production diminishes FSCR due to the physiological stress. Other researchers claimed that genetics plays a key role regarding this issue (Pryce & Veerkamp, 2001; Grimard *et al.*, 2006), in combination with the disruptive effects of negative energy balance in high producing cows (Grimard *et al.*, 2006). In addition, it is noteworthy that not only does 305-d milk yield increase with increasing parity, but also does the occurrence of periparturient disorders such as retained placenta, endometritis, and metabolic disorders (Lee & Kim, 2006; Pinedo *et al.*, 2020). Consequently, several authors reported a significant effect of parity on pregnancy rate and success at first AI (Tenhagen *et al.*, 2003; Windig *et al.*, 2005; Balendran *et al.*, 2008; Inchaisri *et al.*, 2010; Pinedo *et al.*, 2020). In spite of this, Lee & Kim (2006) claimed that it is difficult to determine the relationship between parity and fertility due to the confusing effect of culling under farm conditions. In a similar way, Lucy (2001), Rocha *et al.* (2001), Melendez & Pinedo (2007) and Yehia *et al.* (2020) suggested that primiparous cows show lesser conception rates at first insemination, which may be due to a more severe negative energy balance caused by a greater energy requirement for growth, the impact of the first lactation, and the stress of calving.

As far as season and calving-first AI interval are concerned, different results of the logistic regressions could be due to the exclusion of the SCC factor of the final model when filtering by 30 days before AI, therefore including these factors that were not significant when selecting cases by 30 days after AI. Our results disagree with those obtained by other researchers, who stated that heat stress during summer, either by reducing feed intake or negatively affecting the activity of the hipotalamus-pituitary-ovary axis, has a negative influence on reproductive performance (De Rensis *et al.*, 2017). One possible explanation could be that the area where this study was carried out is not characterised by remarkable high temperatures neither during summer nor throughout the year. In fact, Lopez-Gatius (2003) declared that a cool environment could reduce the risk of reproductive disorders. Regarding calving-first AI interval, our results may be explained by the fact that, given the VWP established in all farms, animals in which the first AI was performed >70 d after calving were probably undergoing certain issues that not only led to a delayed first AI, but may also interfere with fertility. Nevertheless, Kim & Jeong (2019) stated that this factor was not associated with FSCR; however, it should be noted that the VWPs differ between our study and the one performed by these authors (50-60 *vs* 45 d).

Pregnancy loss

The prevalence of PL during the second month of gestation was 7.6%, and it was not influenced by SCC before first AI, SCC after first AI, DFS, and season (p=0.585, p=0.616, p=0.572 and p=0.232, respectively, Table1). However, cows belonging to the >8780 L group had a doubled proportion of PL than cows with lower milk production (12.0% and 5.6%, p=0.006). In addition, multiparous cows were more likely to tend towards PL than primiparous cows (9.6% and 4.9%, p=0.055). According to the logistic regressions, cows with milk productions >8780 L/305-d were more likely to undergo PL (OR=2.074, 95% CI: 1.063-4.047, p=0.032; OR=2.027, 95% CI: 1.057-3.889, p=0.033 in cows with SCC within 30 days before and after AI, respectively).

The results of this study showed that a sudden increase in SCC neither before nor after AI affects the prevalence of PL between ~28-60 days after AI. On the contrary, Pinedo *et al.* (2009) reported that cows experiencing a linear somatic cell counts (LNSCC) ≥4.5 (≥300,000 cell/mL) during the first 90 days of gestation had an increased risk of abortion. Similarly, Moore *et al.* (2005) concluded that cows with a LNSCC >4.5 before breeding were twice as likely to lose their embryo by 35 to 41 days compared with cows with a score <4.5. We analysed the effect of SCC (higher than 200×10^3 cell/mL) within 30 days before and after first AI on PL by day between 28-60 of gestation, whereas other studies analysed the effect of LNSCC on PL during the first 90 days of gestation (Pinedo *et al.*, 2009) and before breeding (Moore *et al.*, 2005). Altogether, a different result from our study and other studies may be explained by differences in our design and other designs above-mentioned, as well as different environmental conditions and herd managements among studies.

Due to the divergence of results and to the fact that we defined PL based on the second pregnancy diagnosis, there is a possibility that SCM may have a short rather than a long-term effect on pregnancy loss. According to our method, we could only detect pregnancy losses that occurred between ~28 to 60 days of pregnancy (pivotal period 3, according to Wiltbank *et al.*, 2016). However, if SCM had a short-term negative effect on pregnancy loss, that is, by causing either early embryonic loss (EEL, before maternal recognition of pregnancy) or embryonic loss up to 28 days of gestation (pivotal periods 1 and 2; Wiltbank *et al.*, 2016), we would not be able to report it. As it was previously mentioned, mastitis may play a disruptive role on corpus luteum formation and progesterone secretion, which are essential to achieve a successful maintenance of pregnancy (Mann & Lamming, 2001). Moreover, Roth *et al.* (2013) observed that the proportion of blastocyst obtained 7-8 d after fertilization was significantly less in medium (>200×10^3 to <600×10^3 cell/mL) and high (>600×10^3 cell/mL) SCC groups, and they related this impairment to the oocyte quality.

Herein, it was observed that the season variable did not have any influence on PL. This result may be explained by the cool ambient temperature in Galicia region. Besides, it has been claimed that the embryo is susceptible to heat stress mainly during the peri-implantation period, as high temperatures especially affect pre-attachment stage embryos and the magnitude of this effect decreases as embryos develop (De Rensis & Scaramuzzi, 2003; De Rensis *et al.*, 2017). Consequently, this variable may have an influence on EEL, a period that was beyond the scope of this study. Moreover, PL did not differ between <71 and >70 DFS groups. This result is in agreement with the finding of a previous study indicating that calving to insemination interval had no significant influence on embryonic loss (Silke *et al.*, 2002). However, a threshold of 70 days was set in our study, which may be considered not early enough to detect any possible effect of DFS on PL.

On the other hand, among all the variables analysed, our results show that cows in the group with a higher milk yield were more prone to suffer PL, as previously indicated by other researchers (Michel *et al.*, 2003; Grimard *et al.*, 2006). Despite this, Silke *et al.* (2002) reported that there was no significant relationship between total lactation yield and embryonic loss. Nevertheless, differences between studies may be due to the divergence in milk production, as the mean milk yield used in our study noticeably differed from the one used by Silke *et al.* (2002) (8780 L and ~7035 L, respectively). It should be noted that not a significant effect, but a tendency, was observed between parity and PL in the current study. This subject is still controversial, as there are researchers who claimed that parity was positively linked to PL (Humblot, 2001; Lee & Kim, 2007; Fernandez-Novo *et al.*, 2020), and those who have reported no effect regarding these two variables (Labernia *et al.*, 1996; Moore *et al.*, 2005). One explanation for a possible relationship between both variables is that the high milk production at third parity in comparison to previous parities would mean a major mobilisation of body fat and a severe loss of body condition (Lee & Kim, 2006). Finally, other factors that were not considered in this study, such as fixed-time-AI and number of AI, were associated with PL (Fernandez-Novo *et al.*, 2020).

It can be concluded that an episode of SCM within 30 days after AI has a disruptive effect on FSCR. Consequently, the adoption of preventive measures should be considered to tackle this issue and diminish the economic loss. Moreover, it may be possible to use the reports of the GDCP as a source of information to predict fertility based on the SCC levels. In addition, although we did not observe any influence of SCM on PL between ~28-60 days after AI, further research should be carried out regarding SCM and both EEL and PL before the first pregnancy diagnosis.

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