Abstract: The objective of this study was to evaluate the effect of subclinical intramammary infections (IMI) on calving-to-first-service interval (CFS), calving-to-conception interval (CC), and the number of services per conception (S/C) in grazing Holstein and Normande cows. Primiparous (n = 43) and multiparous (n = 165) cows were selected from five dairy herds. Two composite milk samples were aseptically collected from each cow at drying-off, and then every week during the first postpartum month. One sample was used for somatic cell count (SCC), and the other one for bacteriological analysis. Cows were followed up to 300 d after calving. Non-parametric and parametric survival models, and negative binomial regression were used to assess the association between IMIs and reproductive indices. Staphylococcus spp. (CNS), Staph. aureus and Streptococcus uberis were the most frequent isolated pathogens. The SCC was higher at drying-off compared to the average in the first month postpartum. Subclinical mastitis in the first month of lactation was not associated with CFS; however, the CC interval was longer in cows with high SCC compared to healthy cows, and also had a higher number of S/C.
Dr. Risco has experience in Dairy Production Medicine, his focus has been on reproductive management of dairy cattle. Therefore, he is a reliable reviewer of our manuscript.

Jantijn Swinkels Ph.D
MSD
jantijn.swinkels@merck.com
Dr. Swinkels has been working recently on mastitis and udder health programs in dairy herds. He has also experience with data analysis from DHIA records and how to apply them to make decisions on udder health programs. He is also a good option as reviewer for our manuscript.
Manizales, Colombia, March 23 of 2016

Doctor:
M. D. SALMAN
Editor-in-chief
Preventive Veterinary Medicine
Elsevier B.V.

REF: Manuscript submission.

Dear Dr. Salman:

I am pleased to submit the manuscript titled: The association between subclinical mastitis and reproductive performance in dairy cows in Colombia, which has been prepared for a group of veterinarians that participated in a project funded by the Vice Chancellor’s Office of Research and Postgraduate Studies of Universidad de Caldas in Colombia.

This has been the result of tight collaboration between Universidad de Caldas, University of Prince Edward Island and University of West Indies.

We would appreciate your attention to send this manuscript for review and further publication in the Preventive Veterinary Medicine journal, if accepted by the peer reviewers.

Sincerely,

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Universidad de Caldas
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The association between subclinical mastitis and reproductive performance in dairy cows in Colombia


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1 Project 0182912 funded by the Vice Chancellor’s Office of Research and Postgraduate Studies of Universidad de Caldas.
Abstract

The objective of this study was to evaluate the effect of subclinical intramammary infections (IMI) on calving-to-first-service interval (CFS), calving-to-conception interval (CC), and the number of services per conception (S/C) in grazing Holstein and Normande cows. Primiparous (n = 43) and multiparous (n = 165) cows were selected from five dairy herds. Two composite milk samples were aseptically collected from each cow at drying-off, and then every week during the first postpartum month. One sample was used for somatic cell count (SCC), and the other one for bacteriological analysis. Cows were followed up to 300 d after calving. Non-parametric and parametric survival models, and negative binomial regression were used to assess the association between IMIs and reproductive indices. *Staphylococcus* spp. (CNS), *Staph. aureus* and *Streptococcus uberis* were the most frequent isolated pathogens. The SCC was higher at drying-off compared to the average in the first month postpartum. Subclinical mastitis in the first month of lactation was not associated with CFS; however, the CC interval was longer in cows with high SCC compared to healthy cows, and also had a higher number of S/C.

**Keywords:** Intramammary infection, reproductive index, calving-to-conception interval, survival analysis
1. Introduction

Bovine mastitis is a costly disease that affects the profitability of the milk industry due to high discarded milk, early culling of cows, and treatments. The cost of the udder health program has been estimated in one third of the total money invested for preventing diseases in dairy herds (Fourichon et al., 2001; Bar et al., 2008). The reproductive performance of the herd has also an impact on the economic results of the dairy business, as low reproductive efficiency may cause a decrease in milk production, and in the number of calves born per year (Maizon et al., 2004).

Fertility in dairy herds has declined in the past five decades while milk yield has increased (Walsh et al., 2011). Nevertheless, how much variation in cow fertility is explained by high milk yield remains unclear, as fertility is affected by many other factors not only milk yield (e.g. heat detection, nutrition, environment, stress, and cow’s genetics and health status) (Dohoo et al., 2001; LeBlanc, 2010). In recent years, studies on the physiology of reproduction have looked for different explanations on how physiological, immunological and pathological processes have an impact on fertility in different species (Pate et al., 2010; Bromfield and Sheldon, 2011). For example, in cows with intramammary infections (IMI) there is an activation of inflammatory and immune responses outside the reproductive organs that can lead to embryonic death (Soto et al., 2003).

Recent studies found that cows with subclinical or clinical IMI had a significant decrease in their first service conception rate (Fuenzalida et al., 2015). Likewise, cows with clinical IMI have a higher number of services per conception (S/C), and longer interval from calving-to-first-service (CFS) or calving-to-conception (CC) than that of healthy cows. It has been reported that
the number of S/C was higher in cows with clinical cases after their first service than in cows with clinical mastitis before the first service (Gunay and Gunay, 2008).

Dairy cows under grazing conditions suffering a subclinical IMI needed more services than their healthy counterparts cows without IMI (Gómez-Cifuentes et al., 2014). Meta-analytic studies suggested that reproductive pathologies occurring around calving, such as retained placenta and ovarian cysts, might negatively affect the reproductive performance (i.e. increasing of intervals from calving-to-first-service; CFS, and CC); however, clinical mastitis did not show a significant effect on the reproductive performance (Fourichon et al., 2000). Nevertheless, an increased production of interleukins and mediators of inflammation (i.e. prostaglandins and cortisol) may mediate the effect of mastitis, either subclinical or clinical, on the reproductive performance. These compounds may have a negative effect on the development and quality of oocytes and corpus luteum, affecting the length of the estrous cycle in cows (Risco et al., 1999; Schurick et al., 2001).

In Colombia, results from several studies suggested that mastitis-causing pathogens belong to Gram positives (Ramírez et al., 2014; Reyes et al., 2015), while in other countries where dairy cows are confined, Gram negative microorganisms are more prevalent (Zadoks et al., 2011). Although the most prevalent type of mastitis-causing pathogens in Colombian dairy herds has been described, there are no studies conducted to evaluate what is the effect of these infections on the reproductive performance of dairy cows. Therefore, the main objective of this study was to test the hypothesis that subclinical IMIs occurring in the first month after calving,
defined according to somatic cell count (SCC) and milk culture, have an affect on the intervals from CFS and CC, and the number of S/C in dairy cows maintained on grazing dairy systems.

2. Materials and Methods

A prospective longitudinal study was designed, and conducted between August 2013 and September 2014. Five dairy herds located in Manizales and Villamaria, Colombia were enrolled. Herds were selected due to the willingness of the owner to participate in the study, and if the farmers had: permanent veterinary advisory service, records from each cow of the herd, and a health management plan.

Cows (n = 208) were selected by convenience according to their expected calving date, without clinical cases of mastitis at drying-off, and having no blind quarters. Cows were assigned to one of two groups according to their breed: Holstein, n = 151 or Normande, n = 57. A total of 165 cows were multiparous and 43 were primiparous. The general characteristics of the herds were described elsewhere (Villa et al., 2016). Briefly, herds were managed under rotational grazing systems, and cows received supplementation with concentrates according to milk yield. The predominant pasture was a mixed of Kikuyu grass (*Pennisetum clandestinum*), Orchardgrass (*Dactylis glomerata* L.), and Yorkshire fog grass (*Holcus lanatus*). Concentrates used were commercial mixes of cereals, containing 14% to 16% of crude protein, and approximately 2.9 Mcal of metabolizable energy/kg of dry matter. Concentrates were fed since three wk before calving (2 kg/cow/d), and at a rate of 1 kg per 4 kg of milk yield after calving. Mineral supplements and water were available ad-libitum.
2.1. Sampling

Two samples of composite milk from the four quarters were aseptically collected one week prior to drying-off, for SCC and microbiological analysis, respectively. Then every cow received an intramammary antibiotic dry cow therapy. Once again, two samples of milk were collected from each cow approximately three d after calving, and then once a week until the first postpartum month. Samples were cooled, and submitted for SCC and, depending on the SCC result, the second sample was used for microbiological analysis. The SCC cut-off point for deciding the need of bacteriological diagnose was established at 100,000 and 200,000 cells/mL for primiparous and multiparous cows, respectively (Ceballos et al., 2011). SCC was performed using a Fossomatic electronic somatic cell counter (Foss Electric Hillerød, Denmark). The SCC was transformed to its natural log (LnSCC, in thousands/mL) to normalize the distribution (Ali and Shook, 1980).

Bacteriological analysis were performed according to protocols established by the National Mastitis Council (Hogan et al., 1999). Milk samples were cultured with a sterile cotton swab on blood agar enriched with esculin at 0.1%. Morphology and Gram staining were used for initial identification. The catalase test was used to differentiate staphylococci from streptococci. Coagulase test was performed on catalase positive cocci to confirm *Staphylococcus aureus*. Gram positive and catalase negative cocci were differentiated by their reaction to the hydrolysis of esculin under ultraviolet light. Christie, Atkins, Munch-Petersen test and esculin reaction were used to differentiate *Streptococcus agalactiae* and *Strep. dysgalactiae*, and esculin reaction and culture in enterococcusel agar were used for the identification *Strep. uberis*. *Corynebacterium* spp. was identified by their morphology and Gram stain. Gram negative bacteria were identified
by their morphology and growth in MacConkey agar. Biochemical tests, such as citrate, indole, motility and oxidase, were used to differentiate Gram negative bacteria.

2.2. Definition of intramammary infection

A cow was considered having a subclinical IMI at drying-off when SCC was greater than 200,000 cells/mL, and at least one colony of a mastitis-causing pathogen was isolated. This criterion was not applied to the primiparous cows, as milk samples in this group were only collected after calving. Cows were samples at drying-off to have a gross idea about the infection status of the mammary gland; however, this information was not used for analytical purposes. An IMI was declared at the beginning of lactation when the mean SCC for the four weeks after calving was greater than 100,000 or 200,000 cells/mL in primiparous and multiparous cows, respectively, and at least one colony of any mammary pathogen was isolated in two out of three consecutive samples (i.e. between weeks 1 and 3 or between weeks 2 and 4). Milk samples containing more than two bacterial species were considered contaminated and not informative of IMI.

2.3. Reproductive parameters

Cows were followed up for 300 d after calving to record their reproductive indices. The interval from CFS was defined as the number of days between calving and the first insemination date. The first service in each herd did occur after the voluntary waiting period, which was 40 d after calving. The interval from CC was defined as the number of days between calving and the conception date. The number of S/C was defined as the number of services that result in a conception and confirmed by veterinary examination.
Artificial insemination was used in all herds. Experienced veterinarians made the diagnosis of gestation approximately 45 d after the last service. In one of the dairies (n = 80 cows) the diagnosis was made by transrectal ultrasound (Tringa Linear, Vet Pie Medical, Maastricht, The Netherlands). Once the cows were confirmed as pregnant, they were not reconfirmed later. Additionally, body condition was evaluated from 1 to 5 according to Schröder and Staufenbiel (2006).

2.4. Statistical analysis

All data were initially introduced in an Excel spreadsheet (Microsoft Corp., Redmond, WA), and then exported to Stata version 14.0 (StataCorp, College Station, TX, USA) for data cleaning, coding and analysis.

2.5. Interval from calving-to-first-service and calving-to-conception

As mentioned above, cows were followed up for 300 d, cows that did not get pregnant during this period were considered censored. Given that the interest was on the time from calving to either first service or conception, the associations between IMI and the intervals from CFS and CC were analyzed by survival analysis. Preliminary evaluations were made using Kaplan-Meier survival graphs according to the absence or presence of IMI. Any eventual differences between the survival functions in cows with no IMIs and those having an IMI were analyzed by the Log-rank, Wilcoxon, and Tarone-Ware tests (Dohoo et al., 2009). Significance level was set at P <0.05.
The effect of IMIs at the beginning of lactation on the intervals to CFS and to CC was then analyzed using parametric survival models. These models assume a specific form of hazard across time. For example, the exponential model assumes a constant hazard, while in the Weibull model hazard is monotonic (i.e. may increase or decrease over time) (Dohoo et al., 2009). The data obtained in this study were assessed using both survival parametric models. The model that fitted better the data was selected by its lowest Akaike’s information criterion. The coefficients of the model were presented in the metric form of hazard. This means that each unit change in the predictor represents a unit of change on the frequency of the outcome, which in this case was measured as a hazard, assuming a linear relationship between the regressors and the hazard (Dohoo et al., 2009). In general the model can be described as a semi-parametric model following the equation:

\[ h(t) = h_0(t)e^{\beta X} \]  

where \( h_0(t) \) corresponds to the baseline hazard, dependent on time and is assumed to have a specific functional form. The term \( e^{\beta X} \) corresponds to a non-negative specific function of the subject of the \( X \) covariate, not time \( t \) dependent and scales the baseline hazard, which is common to all individuals enrolled in the study (Dohoo et al., 2009).

An evaluation of the effect of the independent variables: IMI at drying-off and IMI around calving (negative, positive), herd (categorized 1 to 5), breed (Holstein, Normande), number of lactations (categorized into 1, 2 and 3 or more lactations), average milk production in the first month of lactation, and days (time; d) to the occurrence of the event on a continuous scale were included in the model. An evaluation of the interaction between IMI and time was also carried out. The model fit was assessed by calculating the Cox-Snell residuals and deviance,
where the latter are also useful for the identification of unusual values (outliers) having a high influence on the model. Once the influential points were identified, they were removed and sensitivity analyzes were made to evaluate their effect on the final estimates (Dohoo et al., 2009). The estimates of the final model were presented.

2.6. Services per conception (S/C)

To fit a model of the effect of IMIs around calving on the S/C, only the information of pregnant cows was included. The dependent variable S/C was analyzed using a negative binomial model, being necessary to subtract one from each value of S/C for fitting the model. The effect of the independent variables described above was also included in the model (e.g. herd, IMI at calving, parity, breed, and average milk yield for the first month of lactation). The general model was as follows:

$$\log(S/C) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n$$  \[2\]

where the number of S/C is expressed on log scale; $\beta_0$ corresponds to the intercept, and $\beta_n$ corresponds to the coefficient of the effect when the independent variable $x_n$ was present. Obtaining Anscombe residuals and Cook’s distance was used to assess the fit of the model and detect influential points. The significance level was set at $P < 0.05$.

3. Results

The average number of cows per herd was 41.6 (range: 24 and 80). The average time between drying-off and calving was 71 ± 18 d. The average milk yield for the first month of
lactation was 30.5 ± 6.3 kg/d in Holsteins and 14.2 ± 4.0 kg/d in Normande cows (P = 0.03), after controlling for the effect of herd.

3.1 Intramammary infections

Seventy-four cows out of 165 were classified as IMI positive at drying-off. However, 12 cows had a low SCC, and their milk culture was positive, while 42 cows showed a high SCC with negative culture results. Twelve cows (16.2%) remained infected with the same pathogen, having a high SCC after calving. The most frequent mammary pathogens isolated were Staph. spp. (CNS), Staph. aureus, and Strep. uberis. The information about the infection status at drying-off was only collected in multiparous but further analyses only included the data collected after calving, as primiparous cows did not have any record about udder health before calving. On average, SCC at drying-off were 0.43 ± 0.09 (LnSCC, thousands/mL) higher compared to the average after calving (P < 0.01). No differences were found in the mean SCC according to breed (P = 0.20).

3.2. Intervals from calving-to-first-service and calving-to-conception

The Kaplan-Meier survival function for cows with and without IMI is showed in Fig. 1. These functions were compared using different tests that did not yield significant differences for the interval from CFS (Fig. 1a; P > 0.05), while for the interval from CC the three tests (i.e. Log-rank, Wilcoxon, and Tarone-Ware) indicated that cows with IMI appeared to have a lower risk of conception during the follow-up period (Fig. 2b; P < 0.05).
In both parametric models, exponential and Weibull, the effect of some of the independent variables, such as breed, parity, average milk yield, and the interaction between IMI around calving and time, did not significantly affect the CC interval ($P > 0.10$). Subsequently, the single effect of IMIs and time was evaluated (Table 1). While both models showed a significant effect of IMI in the first month after calving on the risk of conception in the follow-up, the Weibull model had a lower AIC compared to the exponential model (Table 1). At any time after calving, cows with an IMI had a lower hazard of conception compared to non infected cows, the hazard ration was $0.67 = \exp(-0.403)$.

The predicted median from CC time was 71 d and 95 d for healthy and infected cows around calving, respectively (Fig. 2). The shape parameter ($p$) of the Weibull time distribution was greater than one (Table 1), indicating that the hazard of conception in both groups of cows was not constant and increased with time after calving (Fig. 3).

Two cows were identified with deviance residuals greater than three. These cows did not show an IMI around calving and were pregnant during the first week of the follow-up period. On the other hand, there was a cow with a deviance residual less than three, this cow did not show an IMI around calving and was not pregnant after 300 d. The latter cow was considered censored. The Weibull model was fitted again after removing the influential cows, there were no substantial changes either in the magnitude or direction of the coefficients, therefore the full dataset was retained.

3.3. Services per conception (S/C)
The overall mean for the number of S/C in pregnant cows was $2.1 \pm 1.5$. Cows with no IMI had, on average, $1.9 \pm 1.3$ S/C, while positive cows had $2.5 \pm 1.8$ S/C. The negative binomial model showed a tendency for higher S/C in cows positive to IMI around calving after controlling for herd effect (Table 2; $P = 0.11$). Other independent variables such as breed, parity, and average milk yield in the first month of lactation were not significant. These variables were further removed from the final model. Three cows were highly influential on the model. Those cows were not having an IMI and had, on average, five S/C. The subsequent sensitivity analysis showed a significant effect of IMIs around calving on the number of S/C, as infected cows required more services per conception compared to negative cows (Table 2). Cows with IMI had on average 1.83 more services per conception as compared to IMI negative cows.

4. Discussion

The most frequent isolated bacteria in this study were *Staph. aureus*, *Staph. spp.* (CNS) and *Strep. agalactiae*, These results coincide with other studies conducted in grazing dairy systems (Compton et al., 2007; Pinedo et al., 2012). The frequency of *Staph. aureus* isolations was higher than that observed by Dufour et al. (2012), who found a quarter-level prevalence up to 18.3%, and an increase in the likelihood of being *Staph. aureus* positive when the cow was infected with CNS.

Somatic cell count at drying-off was high, which may be due to an effect of dilution at the end of lactation (Green et al., 2006). Similarly, an increase in SCC in the first week after calving might be related to a dilution effect, once milk yield increases the SCC decreases in cows with no IMIs (Dohoo, 1993; Barkema et al., 1999). Regarding to the association between low SCC
(i.e. < 200,000 cells/mL) after calving and reproductive performance, logistic regression analysis indicated that cows having low SCC after calving are 2.5 times more likely to become pregnant (Gómez-Cifuentes et al., 2014). Our results were consistent with some other studies. Thus, IMIs after calving were associated with longer intervals from CFS and from CC, and also with a higher number of S/C (Maizon et al., 2004). Studies conducted in dual purpose cows in Venezuela and Argentina, have indicated that clinical or subclinical IMIs were associated with an increase interval from CFS and CC, and higher S/C (Nava et al., 2010; Gómez-Cifuentes et al., 2014).

Indices of reproductive performance can be related among them; however, under the conditions of this study, subclinical IMIs did not appear to affect the resumption of ovarian function after calving but did affect the hazard of conception. Non-detected embryonic deaths, factors related to the blood progesterone concentration at the time of first service, the presence of chemical mediators that would affect the viability of the embryo might explain the effect of IMIs on the reproductive performance of the cow, in particular on the CC interval (Soto et al., 2003) (Risco et al., 1999; Huszenicza et al., 2004).

Proinflammatory cytokines might trigger febrile reactions that which induce changes in the pattern of hormone secretion, characterized by inhibiting the gonadotropin release necessary for steroidogenesis (Huszenicza et al., 2004; Pate et al., 2010). As a consequence, inflammatory states around calving, not necessarily caused by infections, would have a negative effect on reproduction (Bertoni et al., 2008). Although the immune response to an infection will not trigger necessarily a severe response that affects substantially the reproductive performance, an
IMI around calving might affect the reproductive performance of the cow. Moreover, the severity of the effect on reproduction will depend on the response degree to inflammation (Fuenzalida et al., 2015).

The herd effect cannot be ignored; we found a significant effect of this variable on the reproductive indices (Tables 1 and 2). Herd-level factors that were not evaluated in our study, such as nutrition and methods used for heat detection can affect the intervals from CFS and from CC. However, cow-level factors, such as time of calving, milk yield, metabolic diseases occurring around calving, and the interval between calving regarding to the previous lactation might also have an impact on the intervals from CFS and from CC (Dohoo et al., 2001). Among the cow-level factors, breed and potential milk yield were the most important factors that may affect the interval from CFS, which is an indication that genetics influences the reproductive performance (Dohoo et al., 2001). Same authors concluded that the reproductive performance of individual cows affect the overall performance of the herd. Nevertheless, we did not find evidence to support that cow-level variables, such as breed, average milk yield around calving or parity, affected the overall reproductive indices of the herds in this study.

Mastitis is associated with anovulatory estrus, failures in fertilization and embryonic death, which causes an increase in the interval from CC and in the number of S/C (Huszenicza et al., 2005; Hansen et al., 2004). A study about the effect of IMIs caused by Strep. uberis on the expression of estrous after synchronization, indicated that 33% of the cows showed estrous, while a high percent of the cows did not. Those cows not showing estrous had lower levels of estrogens, and less frequency of LH pulses (Hockett et al., 2005). Other studies have also
indicated that cows with IMI showed lower estrogen levels when they are in estrous, which would favor a delay in ovulation (Lavon et al., 2010). This might affect the intervals we studied, since delayed estrous detection increases the time that cow needs to get a new conception after calving.

The effect of mastitis-causing pathogens on reproductive performance may be explained by the effect of mediators of inflammation released during the infections, such as lipopolysaccharides (LPS) and lipoteichoic acid (LTA), and their effects on the pituitary and gonadal hormones (Hertl et al., 2010). Gram negative bacteria would have a greater impact on reproductive performance than Gram positive bacteria. This effect will be more significant when IMIs occur close to the time of artificial insemination (Hertl et al., 2010; Lavon et al., 2011). The net effect of Gram negative IMIs was a larger number of S/C in positive cows, and their probability of conception was lower compared to negative cows (Hertl et al., 2014). In Colombia the most frequent isolations are for Gram positives, probably due to differences in dairy production systems. Independently of the isolated pathogen, proinflammatory cytokines such as TNFα, proinflammatory interleukins such as IL6, and chemokines such as IL8 have receptors at the granulosa cells level that interfere with the synthesis of estrogens in the dominant follicle and on recruited follicles (Sakumoto et al., 2003; Herath et al., 2007; Bromfield and Sheldon, 2011). Since granulosa and theca cells have TNFα receptors that modulate steroidogenesis and protein production from gonadotropins, changes in ovarian physiology might be explaining the delays in the interval from CC associated with IMIs (Sakumoto et al., 2003).

The LTA produced by *Staph. aureus* had a higher impact on reproductive physiology
compared to the LTA produced by streptococci. This effect was found in a study where labor in mice was prematurely induced by LTA-producing bacteria, such as *Staph. aureus* (Kajikawa et al., 1998). Although the objective of our study was not to evaluate the specific effect of LTA on calving, it might be a cause of low reproductive performance in cows infected with *Staph. aureus*, as this bacteria was highly prevalent in our study. Also, endotoxins produced by other bacteria increase body temperature and induce the release of prostaglandins and cortisol, which would have a negative effect on the development and quality of the oocyte and the corpus luteum, besides causing a disruption of the estrous cycle (Risco et al., 1999; Schurick et al., 2001; Hockett et al., 2005). This would confirm the effect of IMIs on reproduction and would indicate that this effect is mediated by multiple mechanisms, not only at the pituitary level but also at the gonadal level.

Under the conditions of our study, the effect of clinical mastitis on reproduction was not evaluated, as there were only 12 cases of clinical mastitis. A negative effect of clinical mastitis on conception rates and pregnancy has been reported (Santos et al., 2004). This effect has been observed when mastitis, no matter the causing pathogen, occurred between the first service date and pregnancy confirmation. In addition, IMIs affect embryo development (Hansen et al., 2004). Clinical IMIs induce embryonic death before the gestation was confirmed, where this embryonic loss occurred due to the action of proinflammatory cytokines (Chebel et al., 2004; Hansen et al., 2004). Therefore, cows with IMI would need a larger number of S/C, which in turns increases the interval from CC.

5. Conclusion
Under the conditions of this study, the survival models used were suitable for evaluating the effect of IMIs on the interval from CFS and from CC. The IMI occurred at drying-off were highly prevalent in our study, keeping chronic at calving in one fourth of the cows. Nevertheless, the number of chronic infections was not enough to stratify the analysis by the duration of IMIs. However, the presence of IMIs in the first month after calving was associated with longer intervals from CC; therefore, a cow will need a higher number of S/C to become pregnant. Although cow-level factors did not affect the indices studied, there was an effect of the overall herd management that should not be dismissed as a potential explanation for the results. The reproductive performance is the result of the impact of multiple factors, not explained only by mammary health.

Acknowledgements

Authors want to express their gratitude to the Office of the Vice-president of Research and Postgraduate Studies of Universidad de Caldas for funding this study. We would like to express also our gratitude to the owners and employees of dairy herds enrolled in the study for their willingness to participate, and to all members of the Research Group on Milk Quality and Veterinary Epidemiology of Universidad de Caldas for their collaboration.
References

Uncategorized References


Table 1 Effect of intramammary infections (IMI) in the first month of lactation on the interval from calving-to-conception (CC) in grazing Holstein and Normande cows. The coefficient represents the effect of IMI on the interval (log scale) after controlling for herd effects. The results correspond to parametric survival models with Weibull distribution, time effect was not significant (P = 0.20) and not showed in the table.

<table>
<thead>
<tr>
<th>Days after calving</th>
<th>Coefficient</th>
<th>IC (95%)</th>
<th>Value of P</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows with IMI</td>
<td>-0.403</td>
<td>-0.741; -0.065</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Herd:</td>
<td></td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-1.248</td>
<td>-1.784; -0.712</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.772</td>
<td>-1.426; -0.118</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-0.356</td>
<td>-0.844; 0.132</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.162</td>
<td>-0.424; 0.749</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-6.645</td>
<td>-6.469; -4.821</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Shape parameter</td>
<td>1.387</td>
<td>1.236; 1.556</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Effect of intramammary infections (IMI) in the first month after calving on the number of services per conception (S/C) in Holstein and Normande cows. The results of negative binomial regression models are presented after removing three cows highly influential in the model.

<table>
<thead>
<tr>
<th>Number of S/C</th>
<th>Coefficient</th>
<th>IC (95%)</th>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows with IMI</td>
<td>0.605</td>
<td>0.172; 1.037</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Herd:</td>
<td></td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.705</td>
<td>-0.183; 1.593</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>0.989</td>
<td>0.058; 1.920</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>1.051</td>
<td>0.232; 1.870</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>-0.332</td>
<td>-1.434; 0.770</td>
<td>0.55</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.202</td>
<td>-1.990; -0.417</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
**Fig. 1.** Kaplan-Meier survival function curves for cows without (-) and with (---) intramammary infection (IMI) in the first four weeks after calving on the interval from calving-to-first service (a; CFS) and on the interval from calving-to-conception (b; CC). The survival function for CFS did not show any difference according to the IMI (a; P > 0.05). Negative cows to IMIs appeared to be at a greater risk of conception after calving than positive cows (b; P < 0.05). The follow-up period started once the voluntary waiting period was over (i.e. 40 d after calving).

**Fig. 2.** Predicted survival time for the interval from calving-to-conception (CC) in cows without (-) and with (---) intramammary infection (IMI) in the first four weeks after calving. The prediction is subsequent to a parametric model with Weibull distribution. The follow-up period started once the voluntary waiting period was over (i.e. 40 d after calving).

**Fig. 3.** Representation of the shape parameter (p) obtained in Weibull distribution model for the risk of conception in cows without (-) and with (---) intramammary infection (IMI) in the first four weeks after calving. The p parameter was greater than 1.0, indicating that the risk was not constant and was increased with time. The follow-up period started once the voluntary waiting period was over (i.e. 40 d after calving).
Fig. 1.
Fig. 2.
Fig. 3.