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# High somatic cell counts and changes in milk fat and protein contents around insemination are negatively associated with conception in dairy cows

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## ABSTRACT

The fertility of dairy cows has decreased dramatically worldwide over the last few decades, and several causes of this trend have been reported. Several studies have associated compromised udder health with deteriorating reproduction performance. Subclinical ketosis (SCK) has also been reported to be a risk factor for decreased conception. The objective of the present study was to describe how SCK might interact with the reported association between udder health and conception in dairy cows. Data from the French Milk Control Program and data on 8,549,667 instances of artificial insemination (AI) and their corresponding preceding and subsequent test-days from 5,979,701 Holstein cows were examined over a 5-year period (2008–2012). The effect of udder health was evaluated through a low (L) or high (H) somatic cell count (SCC) before and after AI using a threshold of 200,000 cells/mL, and transformed into four groups (LL, LH, HL, and HH). Three proxies for defining SCK were proposed based on the milk fat and protein content (or their ratio) before AI. Statistical analysis first included a generalized additive model to help define the optimal threshold values. Next, a logistic regression with a Poisson correction was performed. On average, the risk of conception at first AI was reduced by 14% for LH or HH cows (relative risk [and 95% CI] = 0.86 [0.85–0.87]) when the SCC increased or remained high within 40 days before and after AI, relative to LL group. The reduction of conception success associated with SCK (fat and protein contents changes) varied from 3% to 17% depending on the used SCK proxy. Including the interaction term SCC\*SCK clearly showed that the association of increased SCC around AI with conception success was modified by the presence of SCK. A cow that already has SCK and experiences an increase in SCC around or after AI exhibits up to 2 times further decrease in conception success compared with a cow with a high SCC and no SCK. In conclusion, this study reinforces the previously described association between intramammary inflammation around or after AI and a decreased rate of conception. These findings highlight how SCK interacts with the above-mentioned relationship by strengthening the negative association between mastitis and conception success. In addition, the present work supports the theory that local inflammation may affect the whole-body response and alter the functions of other organs, such as the reproductive tract.

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## 1. Introduction

Reproductive performance has always been considered a major determinant of dairy herd profitability because of its associations with the amount of milk produced, the

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culling rate, the cost of breeding, and the value of calves. Over the last 15 years, reproductive performance has been declining worldwide [1]. Genetic selection for high milk yield has likely exerted negative effects on reproduction and health, and the demand for high milk production can contribute to a decrease in reproduction ability and reduce the likelihood of pregnancy establishment in high-producing cows [1–3]. Dairy cow reproduction is also highly dependent on peripartum infections [4,5] or nutrition-based disorders [1,6]. Many studies have shown that most of these disorders are risk factors for other disorders. Depending on the studied disorder, the descriptions of epidemiological relationships have varied in comprehensiveness, and the strength of the associations remains imprecise in some cases. It is of great interest to clearly understand the relationships and interactions among different peripartum disorders of dairy cows, particularly for decision-making, prioritization of risk factors in the field, and, more broadly, resource allocation [7]. The indirect positive or negative consequences of these disorders must be accounted for during decision-making.

Recent studies have clearly shown that both the clinical and subclinical mastitis are associated with deteriorated fertility parameters, such as longer intervals from calving to conception, increased services per conception, and increased days open [8–10]. The odds ratio between clinical mastitis or high somatic cell count (SCC) and successful conception has varied from 0.40 to 0.85 [11–14]. The relationship between the severity of mastitis and conception rate appears to have a linear trend. The impact of mastitis on conception appears to be higher for clinical than for subclinical mastitis and for a large SCC increase than for a small increase. The most critical risk period in which mastitis can reduce conception success has been reported to be at the time of [11,13] or within the month after service [8,10].

In addition, most transition dairy cows experience a negative energy balance (NEB) as a consequence of increased energy demands around parturition and decreased dry matter intake shortly before calving. The NEB may be associated with an increased incidence of metabolic disorders, impaired fertility, and other health problems [15]. Both udder health and conception have been shown to deteriorate in cases of subclinical ketosis (SCK), although clear epidemiologic evidence supporting these relationships is scarce [16]. Increased concentrations of both  $\beta$ -hydroxybutyrate (BHBA) and nonesterified fatty acids (NEFA) in blood are often used as markers of SCK, and the most common definition of SCK is a blood BHBA level greater than 1.4 mM during early lactation [17]. Changes in the milk fat and protein contents are additional markers used to identify SCK because lipolysis results in an increased fat content in milk and because a lack of energy in the rumen results in low protein synthesis by ruminal bacteria and a consequent decrease in the protein content [18,19].

The present study aimed to investigate the complex relationship among conception, SCC changes, and metabolic disorders using milk components as indicators of SCK.

## 2. Materials and methods

### 2.1. Data and variables

The records from herds in the Milk Control Program (MCP) in France from 2008 to 2012 (inclusive) were provided by France Génétique Elevage (<http://www.francegenetique-elevage.fr/>). These records included the lactation number, calving date, all test-day milk results, and lactation data (length and production) for all lactations. The French MCP represented 61, 57 and 85% of the herds, cows, and milk produced, respectively. The representation of dairy cows included in the MCP varies among dairy production areas and ranges from 40% to 67%. Measurements of milk urea are optional for farmers registered in the MCP and are conducted on an average of 57.7% of test-days. The records for milk urea during the same time period were provided by the France Conseil Elevage (<http://www.france-conseil-elevage.fr/>). The French Livestock Institute (<http://idelle.fr/>) provided data on artificial insemination (AI), including the identities of the dams and sires and the dates of all AIs. Data were collected using MySQL software (MySQL, version 5.0, Oracle Corp., Redwood City, CA, USA). Restrictions were implemented so that only Holstein cows and instances of AI prior to 200 days in milk (DIM) were included in the study. The milk yield was adjusted to correspond to a reference lactation period of 305 days (305-day MY). A brief description of the contents of the dataset is shown in Table 1.

For each AI, conception was considered as a binary trait and defined as successful if the instance of AI was followed by a calving 265 to 295 days later. This duration was defined as the average period of gestation for Holstein cows  $\pm 15$  days, as recommended by the French Livestock Institute. Udder health around AI was investigated through the SCC on the test-day. The SCC has been classified as low (L) or high (H) based on various thresholds but only results for a threshold of 200,000 cells/mL will be detailed in this study. For each AI, cows were categorized into four groups according to the dynamics of the SCC as follows: cows with a low SCC both before and after AI (LL, control group); cows with a low level before and a high level after AI (LH); cows with a high SCC before and a low SCC after AI (HL); and cows with a high SCC both before and after AI (HH). In accordance with the average of 1 test-day per month in France, the test-days within the 40 days before and 40 days after AI were primarily included in the analysis. Test-days from other period, including from 40 to 80 days before and after AI, were also assessed. The SCK status at the time of AI was defined by the milk fat and protein contents of the same test-days in which the SCC was quantified [18,19]. A descriptive schematic of the experimental time periods and test-days included in this study is presented in Figure 1.

### 2.2. Statistical analysis

Data were analyzed using R (version 2.10.1, 2009–12–14, The R Foundation for Statistical Computing, Vienna, Austria). A two-step statistical approach was used. The optimal thresholds for stratifying the categorical variables included in the final logistic regression (SCC, SCK, DIM, and

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