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# An Interconnection between Elevated SCC Levels in Milk around the First Artificial Insemination and Pregnancy Rate in Dairy Cows

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## Abstract

The aim of this study was to evaluate the impact of the somatic cell count (SCC) levels measured at 28-test-day intervals or pregnancy rate at the first artificial insemination of Holstein Friesian cows. All necessary information was taken from test day and farm records. Levels of SCC in milk, 30 days before, and 30 days after the first artificial insemination (FAI) date were divided into 4 categories and their interconnection with pregnancy rate (PR) at FAI was evaluated by applying logistic models. A SCC threshold of 150.000 cells/ml of milk was used to differentiate uninfected udders and infected udders of Holstein Friesian cows. A level of SCC > 150.000 cells/ml in milk, and a level of SCC < 150.000 cells/ml before and after FAI respectively were considered udders with new infections. A level of SCC > 150.000 cells/ml in milk before FAI as well as after FAI, was considered chronically infected udders or subclinical intramammary infections (IMI). Data from 792 FAIs from farm and test day records over a 4-year period (2019-2022) were evaluated. The outcome of this study revealed that the risk for low PR at the first AI was increased in cows with infected udders (OR = 1.33, CI 0.99 - 1.78), compared with uninfected udders. Increased levels of SCC after FAI had a negative effect on PR, while before FAI was not affected. To achieve a good PR, mastitis control measures must be fully adopted to a large extent around the first AI.

## Keywords

Somatic Cell Count, Fertility, Pregnant Rate, Test-Day-Milk Data

## 1. Introduction

The incidence of intramammary infections is associated with negative effects on production and reproduction performance (Halasa *et al.*, 2007; Santos *et al.*, 2004;

Lavon *et al.*, 2011) [1] [2] [3].

Somatic cell count (SCC) in milk is a measure of the activity of the cellular immune defense system of the mammary gland of dairy cows. Milk from uninfected udders has a "healthy" cell count from 18.000 to 200.0000 cells/ml of milk. Milk from infected udders has a cell count higher than 200.000 cells/ml and varies with the bacterial species involved [4] [5].

Clinical mastitis is an acute short-term event caused by Gram-negative bacteria and it is characterized by systemic signs and a rise in SCC in the milk.

Subclinical mastitis is a long-term event caused by gram-positive bacteria and is more common than clinical mastitis [6].

Previous studies have demonstrated that an increased SCC before and after the first Artificial Insemination (FAI) negatively affects conception rate (CR), days open (DO) and pregnancy loss (PL) in dairy cows [3] [7]. Timing regarding before or after the FAI is still controversial. Some studies suggest no effect on CR [8]. Others observed that high SCC before AI has some effect on the non-return rate [9] or higher number of DO. Sanots *et al.*, (2004) [2] observed a CR lowered by clinical mastitis occurring before AI. In the previous studies, the authors showed that reduction in PR could be related to acute-phase response to IMI that includes inflammatory proteins cytokines and prostaglandins. Mastitis-induced increase in PGF2$\alpha$ and possibly TNF$\alpha$ has been correlated to corpus luteum (CL) regression [10]. Herzog *et al.*, (2012) [11] showed that E. coli lipopolysaccharide (LPS) causes a decline in CL size and a lower plasma progesterone level. In contrast, Lavon *et al.*, (2008) [12] showed that intramammary administration of LPS at the onset of estrus did not cause early luteal regression and did not induce lower progesterone concentrations.

Some studies present a large diversity in follicular responses to the infection among individual cows [3] [13] and [14].

Most of the above-mentioned studies show some relationship between elevated SCC levels in milk and the fertility of dairy cows. Considering this and the fact that there is little literature about this subject in the South West of Romania, the need for research regarding this issue is indubitable. The aim of this study was to evaluate the impact of the SCC levels measured at 28 test day intervals on pregnancy rate at first AI in dairy cows.

## 2. Materials and Methods

A retrospective research study from 2019 to 2022 was conducted at the dairy farm of Agricultural Research and Development Station Șimnic-Craiova, located in the South-West of Romania (182 m above sea level, 44˚19'N, 23˚48'E). The herd size is 280 animals with 115 - 120 lactating cows. All lactating cows are tested at 28-day intervals, as part of the Romania Dairy herd improvement (DHI) program. The farm uses a milking routine that includes fore-stripping quarters for detection of mastitis, and uses antimicrobials to treat affected cows.

A total of 640 Holstein Friesian cows were enrolled, some of them with 2 or 3 consecutive calvings (AI). The dairy cows' parity was from 1 to 6, and artificial insemination was used in the breeding program.

Records from 792 AI, of 640 primiparous and multiparous cows, were evaluated. Data have been gathered from the test days and farm records by the research team, to control the productive, reproductive and health status of the lactating cows. For this study, the pertinent data were: data of calvings, parity number, and somatic cell count (SCC), data of first AI and health status regarding postpartum uterine diseases (metritis or pyometra) at least 40 days from calving. SCC was used to assess the udder's health status. A threshold value of 150.0000 cells/ml of milk was used to identify uninfected udders (<150.000 cells/ml) or infected udders (>150.000 cells/ml). The threshold used in this study was based on previously published studies [4] [5], proving that in infected udders with Coagulase Negative Staphylococci (CNS), the average SCC is 155.000 cells/ml.

All 792 first AI were divided into 4 categories according to SCC level on 2 milk-test-28 days around the first AI date: one (27 days) before AI data, and one (27 days) after the AI date. Category 1: (n = 326 AI) of cows with uninfected udders with the SCC level <150.000 cells/ml milk on all 2 milk test-28 days (steady low SCC pattern). Category 2: (n = 118 AI) of cows with infected udders before the AI date (SCC level ≥150.000 cells/ml) and with uninfected udders after the AI date (SCC level <150.000 cells/ml, decline SCC pattern). Category 3: (n = 178 AI) of cows with uninfected udders before the AI date (SCC level <150.000 cells/ml), and with infected udders after the AI date (SCC level ≥150.000 cells/ml, increasing SCC pattern). Category 4: (n = 170 AI) of cows with infected udders before and after the FAI date (>150.000 cells/ml; steady high pattern of SCC).

Category 2 represented healed infected udders, category 3 represented new infections of udders and category 4 represented chronic infections of udders. A subcategory was formed combining category 2 and 3 as IMI before and after the first AI (FAI). The category was divided into 3 subcategories: modest (150,000 to 350,000 cells/ml of milk), medium (450,000 to 750,000 cells/ml) and high (>750,000 cells/ml).

### Data Analysis

The data were entered into Microsoft Excel computer program 2007. Stata version 14 was used to summarize the data and descriptive statistics were used to express the results. Multivariate statistical analysis used for the assessment of the relationship between various factors was the tool for interpreting the phenomenon in udder study. Differences were considered significant at $p<0.05$.

## 3. Results

Overall SCC categories, the mean pregnancy rate for the first AI was 37.5%. The pregnancy rate of cows with uninfected udders was higher (41.41%) than that of cows with infected udders (34.76%).

Regarding each SCC category, the pregnancy rate was 41.41%, 37.29%, 35.39%, and 32.35% in SCC category 1, 2, 3 and 4 respectively.

The SCC categories 2, 3 and 4 represented the type of intramammary infection (healed, new infections and chronic infections) (Figure 1).

Multiple Logistic Regression Analysis (Table 1) showed that the risk for low PR at first AI was increased in cows with infected udders (OR = 1.33; CI 0.99 - 1.78). REGARDING SCC categories the risk for low PR at first AI was highest in cows with chronic infections (OR = 1.48 CI 1 - 2.18) compared with cows with new infections (OR = 1.29 CI 0.88 - 1.88) or with cows with healed infections (OR = 1.19, CI 0.77 - 1.83). SCC category 1 (uninfected cows) was used as a reference (control; SCC category 1) (Table 1).

The analysis regarding the association of SCC level with the probability of pregnancy at first AI in chronic infections showed that pregnancy rate (PR) varied with SCC level (Figure 2).

Pregnancy rates in cows with chronic infections were 36.00%, 33.96%, and 28.36% relative to elevated SCC subcategories: 150,000 – 450,000, 450,000 – 750,000 and >750,000 respectively (Figure 2). The pregnancy rate in uninfected cows was 41.41%.

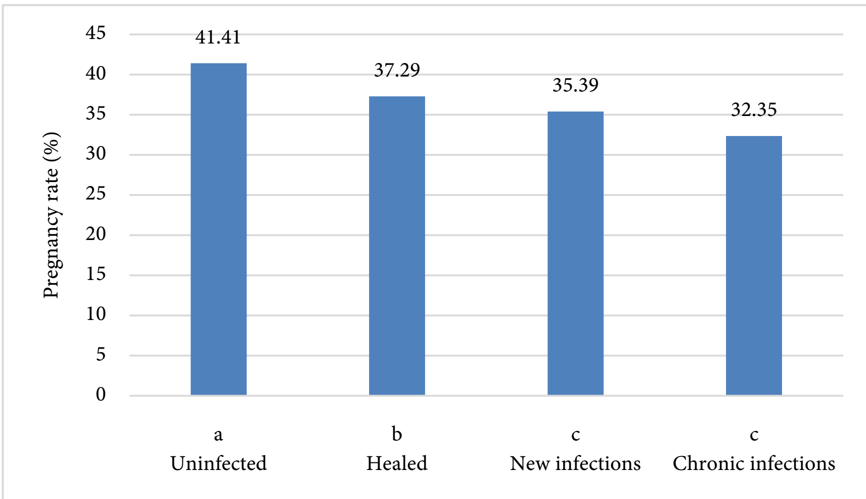


Figure 1. Type of IMI and pregnancy rates at the first AI. Different letters indicate significant differences ($p < 0.05$).

Table 1. Odds ratio for PR at the first AI in Holstein Friesian cows.

| SCC categories | SCC level (×1000 cells/ml) | Odds-ratio (OR) | 95% confidence interval of the OR |
|---|---|---|---|
| 1. Uninfected | <150 | Reference | - |
| 2. Healed | >150 before AI date<br><150 after AI date | 1.19 | 0.77 - 1.83 |
| 3. New infection | <150 before AI date<br>>150 after AI date | 1.29 | 0.88 - 1.88 |
| 4. Chronic infection | >150,000 before and after AI date | 1.48 | 1.00 - 2.18 |
| Total infected (2 + 3 + 4) | <150 | 1.33 | 0.99 - 1.78 |

The risk for low PR at first AI was highest in cows with SCC levels over 750.000 cells/ml (odds ratio 1.79, CI 1.00 - 3.17) compared with uninfected cows (<150.000 cells/ml). The odds ratio for cows with SCC level between 450.000 cells/ml and 750.000 cells/ml was 137 CI 0.75 - 2.53 and for cows with SCC level between 150.000 cells/ml and 450.000 cells/ml was 1.26, CI 0.68 - 2.33 (Table 2).

A raised SCC levels (>750.000 cells/ml) before or after FAI are mostly associated with clinical cases of IMI, being in time with the clinical event, than lower SCC levels (450.000 - 750.000 cells/ml or 150.000 - 450.000 cells/ml) which could represent the later past-event time. In this study, the pregnancy rate before FAI (−27 days) was 40.0% and after FAI (+27 days) was 35.48% (Figure 3).

Our analysis shows that the risk for low PR at FAI was increased in cows after FAI (35.48%) compared with cows before FAI (40%) or with uninfected cows (41.41%) (Figure 3).

The odds ratio (OR) for PR at FAI in clinical IMI was 1.06, CI 0.6 - 1.86 before FAI and 1.29, CI 0.8 - 2.07 after FAI. The risk for low PR at FAI in clinical cases of IMI was higher in cows after FAI than in cows before FAI (Table 3), compared with uninfected cows.

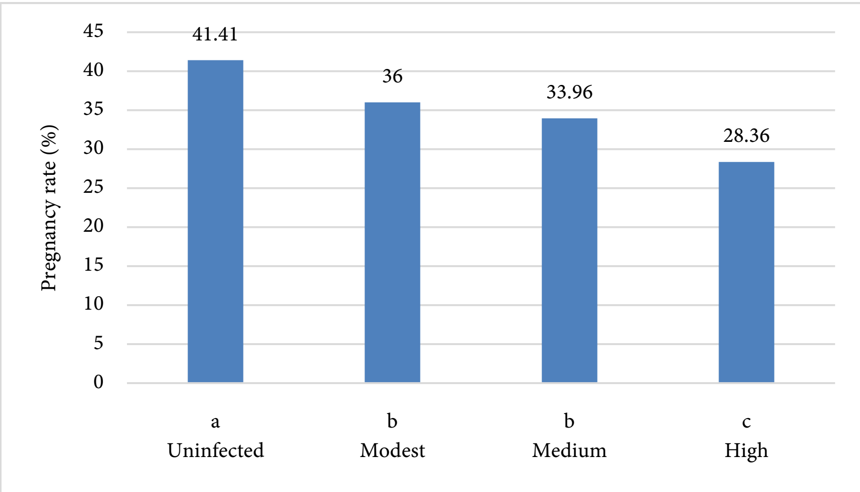


Figure 2. Effects of SCC levels on pregnancy rates (uninfected udders n = 326) vs. chronic infections (n = 170). Different letters indicate significant difference ($p < 0.05$).

Table 2. Odds ratio for PR at the first AI in chronic infections.

| SCC level (cells/ml) | Odds ratio (OR) | 95% Confidence interval of the OR |
|---|---|---|
| <150.000 (uninfected) | References | - |
| 150.000 - 450.000 (modest) | 1.26 | 0.68 - 2.33 |
| 450.000 - 750.000 (medium) | 1.37 | 0.75 - 2.53 |
| >750.000 (high) | 1.79 | 1.00 - 3.17 |

Table 3. Odds ratios for PR at FAI in clinical IMI.

| SCC level relative to FAI | Odds ratio (OR) | 95% confidence interval of the OR |
|---|---|---|
| Before first artificial insemination | 1.06 | 0.6 - 1.86 |
| After first artificial insemination | 1.29 | 0.8 - 2.07 |

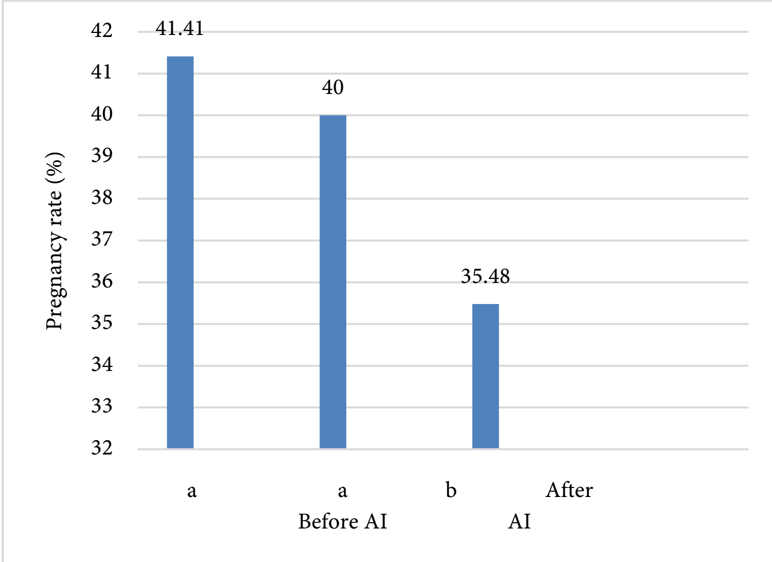


**Figure 3.** Effects of SCC levels on pregnancy rates (uninfected udders n = 326) vs. chronic infections (n = 170). Different letters indicate significant difference ($p < 0.05$).

## 4. Discussion

In this retrospective study, we evaluated an interconnection between elevated SCC levels in milk measured at 28 days intervals with pregnancy rate in dairy cows. Our results show that an increased level of SCC in milk has a detrimental effect on the pregnancy rate of dairy cows at first AI (FAI).

To date, an association between decreased fertility of dairy cows and clinical and subclinical intramammary infections (IMI) has been recognized [2] [3] and [15]. In an epidemiological study, Lavon *et al.*, 2011 [3] showed a significant decrease in the probability of conception in cows with elevated SCC (>150,000 cells/ml) in milk. Similar to our results have been also reported by Hudson *et al.*, (2012). In our study, the cows recorded with less than 150,000 cells/ml milk before and after FAI were designed uninfected (category 1 control). This cut-off point is important because IMI with skin flora opportunists as CNS, have been found that induce light SCC levels in the milk of affected cows [4] [16] [17].

According to our analysis, an increased SCC levels recorded in categories 3 and 4, significantly reduced pregnancy rate at FAI. The SCC levels in category 2 represented cows with healed infected udders. Cytological cure, a lesser discussed form of the healing process, can be considered the point at which the SCC level in the mammary gland has returned to "healthy" SCC level or below 150,000 cells/ml of milk. The complex acute-phase responses are decreased and the secretion of inflammatory proteins, cytokines and prostaglandins is reduced. The categories 3 and 4 included cows with new and chronic infections, respectively. The risk for low PR at FAI was higher in cows with chronic infections (OR 148, CI 1.00 - 2.18) compared with new infection (OR = 1.29, CI 0.88 - 1.88), or with healed category (OR = 1.19, CI 0.77 - 1.83) using uninfected category as reference. Chronic infections represented subclinical IMI with steady SCC > 150,000 cells/ml of milk.

In this report, all chronic SCC levels were divided into chronically high SCC (>750,000 cells/ml), medium SCC (450,000 - 750,000 cells/ml), and moderate (150,000 - 450,000 cells/ml of milk). Subclinical mastitis can persist for several months, and its long-term effects decrease fertility [6] [7] and [10].

In our study, a high elevation of SCC (>750.000 cells/ml) before and after FAI decreased the probability of pregnancy (OR 1.79; CI 1.00 - 3.17) further down than moderate (OR 1.26; CI 0.68 - 2.33) and medium (OR 1.37; CI 0.75 - 2.53) chronic elevation SCC. Lavon *et al.*, 2011 [3] reported the same results. Konig *et al.*, 2006 [18] showed a low pregnancy rate in cows with medium chronic elevation of SCC (>400,000 cells/ml), but not in cows with moderate chronic elevation of SCC (150,000 to 400,000 cells/ml).

Moderate and medium chronic elevation of SCC is associated with CNS-induced long-term, chronic IMI [4] [16].

Combining the categories 2 and 3 we evaluated the association between short-term elevated SCC levels before and after FAI with pregnancy rate. Short-term elevated SCC levels in milk are specific for acute clinical IMI. The clinical IMI activates an increased secretion of inflammatory proteins, cytokine and prostaglandin, which can be detected in plasma and milk [19] [20].

The effect of IMI before FAI on reproduction is controversial.

Our study shows no effect on pregnancy rate, and our findings support studies of Loeffler *et al.*, (1999) [21], Klaas *et al.*, (2004) [8], Hudson (2012) [22], Sadeghi *et al.*, (2021) [7] suggesting that an elevation in SCC within the first month after AI is associated with a low pregnancy rate.

It is considered that IMI after AI may interfere with corpus luteum formation and regression, progesterone secretion, endometrial functions and embryonic development [23] [24] [25]. Walfenson *et al.*, (2015) [6] showed that in naturally occurring mastitis the small, almost non-existent effect of clinical events on premature luteal regression suggests that the cause of low fertility associated with clinical mastitis after AI must be positioned at the embryo level and not at corpus luteum. To see the effect of IMI on follicular function distinguishing between clinical and subclinical IMI is important. Induction of clinical IMI before AI during the follicular phase significantly reduced the pulsatile luteinizing hormone (LH) secretion and subsequently induced low secretion of estradiol close to estrus and delayed LH surge and ovulation [12] [26].

Changes in pulsatile LH secretion were not observed in subclinical events [27]. Asaf *et al.*, (2014) [28] using follicular fluid from Gram- or Gram + toxin induced mastitis as oocyte maturation medium reported reduced rates of cleavage and blastocyst formation. Roth *et al.*, (2013) [29] observed impairment of oocyte competence in the pool of oocytes at the germinal vesicle stage in naturally occurring mastitis.

In consequence, to achieve a good PR, mastitis control measures must be fully adopted to a large extent around the first AI.

## 5. Conclusions

Intramammary infections are associated with a significant reduction in the probability of pregnancy. The degree of lowering the pregnancy rate is interconnected with the pattern and level of SCC elevation around the first AI. The use of form and test-day reports is good sources of information to predict pregnancy rates based on SCC levels. Further research is needed regarding early embryonic loss and pregnancy loss and their interconnections with pattern and level of SCC elevation.

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## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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