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

3

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26 **ABSTRACT**

27 The fertility of dairy cows has decreased dramatically worldwide over the last few decades, and  
28 several causes of this trend have been reported. Several studies have associated compromised udder  
29 health with deteriorating reproduction performance. Subclinical ketosis (SCK) has also been reported  
30 to be a risk factor for decreased conception. The objective of the present study was to describe how  
31 SCK might interact with the reported association between udder health and conception in dairy cows.  
32 Data from the French Milk Control Program and data on 8,549,667 instances of artificial insemination  
33 (AI) and their corresponding preceding and subsequent test-days from 5,979,701 Holstein cows were  
34 examined over a 5-year period (2008-2012). The effect of udder health was evaluated through a low  
35 (L) or high (H) somatic cell count (SCC) before and after AI using a threshold of 200,000 cells/mL,  
36 and transformed into 4 groups (LL, LH, HL, and HH). Three proxies for defining SCK were proposed  
37 based on the milk fat and protein content (or their ratio) before AI. Statistical analysis first included a  
38 generalized additive model to help defining the optimal threshold values. Next, a logistic regression  
39 with a Poisson correction was performed. On average, the risk of conception at first AI was reduced  
40 by 14% for LH or HH cows (relative risk [and 95% CI] = 0.86 [0.85-0.87]) when the SCC increased or  
41 remained high within 40 days before and after AI, relative to LL group. The reduction of conception  
42 success associated with SCK (fat and protein contents changes) varied from 3 to 17% depending on  
43 the used SCK proxy. Including the interaction term  $SCC^*SCK$  clearly showed that the association of  
44 increased SCC around AI with conception success was modified by the presence of SCK. A cow that  
45 already has SCK and experiences an increase in SCC around or after AI exhibits up to 2 times further  
46 decrease in conception success compared to a cow with a high SCC without SCK. In conclusion, this  
47 study reinforces the previously described association between intramammary inflammation around or  
48 after AI and a decreased rate of conception. These findings highlight how SCK interacts with the  
49 above-mentioned relationship by strengthening the negative association between mastitis and  
50 conception success. Additionally, the present work supports the theory that local inflammation may  
51 affect the whole-body response and alter the functions of other organs, such as the reproductive tract.

52

53 **Key words:** dairy cow, subclinical ketosis, conception, mastitis, interaction.

54

## 55 **1. Introduction**

56 Reproductive performance has always been considered a major determinant of dairy herd  
57 profitability because of its associations with the amount of milk produced, the culling rate, the cost of  
58 breeding and the value of calves. Over the last fifteen years, reproductive performance has been  
59 declining worldwide [1]. Genetic selection for high milk yield has likely exerted negative effects on  
60 reproduction and health, and the demand for high milk production can contribute to a decrease in  
61 reproduction ability and reduce the likelihood of pregnancy establishment in high-producing cows [1–  
62 3]. Dairy cow reproduction is also highly dependent on peripartum infectious [4,5] or nutrition-based  
63 disorders [1,6]. Many studies have shown that most of these disorders are risk factors for other  
64 disorders. Depending on the studied disorder, the descriptions of epidemiological relationships have  
65 varied in comprehensiveness, and the strength of the associations remains imprecise in some cases. It  
66 is of great interest to clearly understand the relationships and interactions among different peripartum  
67 disorders of dairy cows, particularly for decision-making, prioritization of risk factors in the field, and,  
68 more broadly, resource allocation [7]. The indirect positive or negative consequences of these  
69 disorders must be accounted for during decision-making.

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

77 conception success has been reported to be at the time of [11,13] or within the month after service  
78 [8,10].

79 In addition, most transition dairy cows experience a negative energy balance (NEB) as a  
80 consequence of increased energy demands around parturition and decreased dry matter intake shortly  
81 before calving. Negative energy balance may be associated with an increased incidence of metabolic  
82 disorders, impaired fertility and other health problems [15]. Both udder health and conception have  
83 been shown to deteriorate in cases of subclinical ketosis (SCK), although clear epidemiologic evidence  
84 supporting these relationships is scarce [16]. Increased concentrations of both  $\beta$ -hydroxybutyrate  
85 (BHBA) and non-esterified fatty acids (NEFA) in blood are often used as markers of SCK, and the  
86 most common definition of SCK is a blood BHBA level greater than 1.4 mM during early lactation  
87 [17]. Changes in the milk fat and protein contents are additional markers used to identify SCK because  
88 lipolysis results in an increased fat content in milk and because a lack of energy in the rumen results in  
89 low protein synthesis by ruminal bacteria and a consequent decrease in the protein content [18,19].

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

92

## 93 **2. Materials and Methods**

### 94 ***2.1. Data and variables***

95 The records from herds in the Milk Control Program (MCP) in France from 2008 to 2012  
96 (inclusive) were provided by France Génétique Elevage (<http://www.francegenetique-elevage.fr/>).  
97 These records included the lactation number, calving date, all test-day milk results and lactation data  
98 (length and production) for all lactations. The French MCP represented 61, 57 and 85% of the herds,  
99 cows and milk produced, respectively. The representation of dairy cows included in the MCP varies  
100 among dairy productions areas and ranges from 40 to 67%. Measurements of milk urea is optional for  
101 farmers registered in the MCP and is conducted on an average of 57.7% of test-days. The records for

102 milk urea during the same time period were provided by the France Conseil Elevage  
103 (<http://www.france-conseil-elevage.fr/>). The French Livestock Institute (<http://idele.fr/>) provided data  
104 on artificial insemination (AI), including the identities of the dams and sires and the dates of all AIs.  
105 Data were collected using MySQL software (MySQL, version 5.0, Oracle Corp., Redwood City, CA).  
106 Restrictions were implemented so that only Holstein cows and instances of AI prior to 200 days in  
107 milk (DIM) were included in the study. The milk yield was adjusted to correspond to a reference  
108 lactation period of 305 days (305-day MY). A brief description of the contents of the dataset is shown  
109 in Table 1.

110 For each AI, conception was considered as a binary trait and defined as successful if the  
111 instance of AI was followed by a calving 265-295 days later. This duration was defined as the average  
112 period of gestation for Holstein cows  $\pm 15$  days, as recommended by the French Livestock Institute.  
113 Udder health around AI was investigated through the SCC on the test-day. The SCC has been  
114 classified as low (L) or high (H) based on various thresholds but only results for a threshold of  
115 200,000 cells/mL will be detailed in this study. For each AI, cows were categorized into four groups  
116 according to the dynamics of the SCC as follows: cows with a low SCC both before and after AI (LL,  
117 control group); cows with a low level before and a high level after AI (LH); cows with a high SCC  
118 before and a low SCC after AI (HL); and cows with a high SCC both before and after AI (HH). In  
119 accordance with the average of 1 test-day per month in France, the test-days within the 40 days before  
120 and 40 days after AI were primarily included in the analysis. Test-days from other period, including  
121 from 40 to 80 days before and after AI, were also assessed. The SCC status at the time of AI was  
122 defined by the milk fat and protein contents of the same test-days in which the SCC was quantified  
123 [18,19]. A descriptive schematic of the experimental time periods and test-days included in this study  
124 is presented in Fig. 1.

## 125 **2.2. Statistical analysis**

126 Data were analyzed using R (version 2.10.1, 2009–12–14, The R Foundation for Statistical  
127 Computing, Vienna, Austria). A two-step statistical approach was used. The optimal thresholds for

128 stratifying the categorical variables included in the final logistic regression (SCC, SCK, DIM and 305-  
129 day MY) were first obtained using a generalized additive model (GAM, the gam package in R). The  
130 use of GAMs allowed the nature of the relationship between the response and the set of the  
131 explanatory variables to be determined rather than assuming some form of parametric relationship  
132 [20]. All continuous explanatory variables were introduced into the model as smooth without any *a*  
133 *priori* assumptions. This approach allowed to determine the most efficient thresholds for stratifying  
134 the explanatory variables, such as SCC, DIM, 305-day MY, urea concentration, fat and protein  
135 contents and the fat/protein ratio. Conception was explained with explanatory variables maintained in  
136 their continuous distribution. Univariate models were separately generated for all of the above-  
137 mentioned explanatory variables, and the multivariate models were constructed by integrating the  
138 significant variables ( $p < 0.05$ ) into the same model one by one. No interaction was tested because this  
139 was outside the scope of this first step.

140 The final logistic regression with a Poisson correction was then performed using the nlme package of  
141 R. A log link model with a Poisson distribution was proposed as a method to calculate relative risks  
142 instead of odd ratios and to simultaneously address the issue of the lack of convergence occasionally  
143 observed when performing log binomial regressions [21–23]. The same step-by-step procedure was  
144 used to include explanatory variables as that described above for the GAM. This model included the  
145 SCC as a categorical variable and SCK as a binary variable, as well as a term representing interaction  
146 between these variables. Additionally, this model was adjusted for the lactation stage, milk yield and  
147 parity. The urea concentration was not retained because of the consistent lack of statistical significance  
148 based on the univariate analysis. This model was applied for the different proxies of SCK created by  
149 the GAM. The logistic model, which included herd as a random variable, was applied to the entire  
150 dataset from the period 2008-2012 or from each year either to the first AI (AI1) following each  
151 calving or to all AIs. Although only the results for conception at AI1 are discussed in this article, the  
152 results were similar for all AIs.

153

### 154 3. Results

#### 155 3.1. GAM and definition of thresholds

156 The thresholds defined by the smoothing function did not differ between the univariate and  
157 multivariate models, and only graphs from the univariate model are shown here (Figures 2 and 3). The  
158 results showed high consistency between years unrelated to the explanatory variable. A linear but  
159 small decrease in conception was associated with an increased SCC before AI (Figure 2). The  
160 conception rate was strongly associated with SCC after AI, with a linear decrease in conception rate  
161 up to 400,000 to 500,000 cells/mL followed by an overall very gradual decrease in conception rate as  
162 the SCC further increased. Conception success was more closely associated with changes in milk fat  
163 content after AI than before AI (Figure 3). The association between milk protein and conception  
164 (Figure 3) was present both before and after AI, although it was stronger after AI. The results of the  
165 GAM led to the retention of 200,000 cells/mL as the main threshold of the SCC on which to base the  
166 associated categorical variable. A threshold of 7,000 kg of 305-day MY was used to define highly  
167 productive cows. Three proxies of SCK (Table 2) were implemented according to the associations of  
168 fat and protein contents with conception success, as shown in Fig. 3.

#### 169 3.2. The relationship between SCC and conception is strengthened by the presence of SCK

170 The logistic regression model clearly showed that an increase in the SCC (from low SCC  
171 within 40 days before AI1 to a high SCC within 40 days after AI1; LH group) was associated with a  
172 12 to 17% decrease in conception success at AI1 (Table 3). Similar associations were observed for  
173 cows with a high SCC both before and after AI1 (HH group) compared to those with a low SCC  
174 before and after AI1 (LL group). Including all AIs in the model, a similar reduction in conception  
175 success was observed (results not shown). For alternative thresholds of the SCC, the average decreases  
176 in conception success in the LH or HH groups compared to the LL group were 10, 15, 21 and 26%  
177 using SCC thresholds of 100,000, 200,000, 400,000 and 800,000 cells/mL, respectively. The  
178 conception success at AI1 was significantly higher when performed between 50 and 100 DIM than

179 when performed outside that period, for primiparous cows than for multiparous cows and for low-  
180 producing cows compared to high-producing cows (Tables 3 to 5). No interaction of the above-  
181 mentioned variables was significant.

182 Depending on the SCK proxy, the decrease in the risk of conception at AI1 associated with  
183 SCK was between 3 and 17% for AI1 (Table 3). The coefficients of the interactions of SCC with SCK  
184 were systematically negative ( $RR < 1$ ). A cow that already had SCK and experiencing an increase in  
185 the SCC around or after AI1 (LH and HH groups; Tables 3 to 5) exhibited a decrease in the relative  
186 risk of conception compared to a cow with high SCC without SCK due to the multiplication of the  
187 coefficients of these two events (multiplicative effect), in addition to the inclusion of an interaction  
188 term with a coefficient of 0.97 to 0.93. Consequently, the relative risk of conception could be  
189 decreased up to 0.66 in cases of SCK concomitant with a high SCC. The effect sizes of the LH\*SCK  
190 and HH\*SCK interactions were greater for certain years, particularly for SCK2, in which the  
191 interaction term had a coefficient of 0.90 and even 0.86 (data not shown). Similar results were  
192 obtained via analysis of all AIs instead of only AI1.

193 Using the periods of 40-80 days before AI1 to define SCK and 0-40 days before and after AI  
194 to define SCC or 40-80 days before and after AI1 to define all indicators instead of the period 0-40  
195 days led to similar results (Tables 4 and 5).

196

#### 197 4. Discussion

198 The relationship between changes in SCC and conception described in the present study is in  
199 accordance with previous studies that reported a negative association between udder inflammation  
200 around or after AI and deteriorated reproduction performance [12–14,24]. The comparison of the  
201 present results with the literature is limited by the inconsistency of studies on (i) the outcome variables  
202 used to qualify reproduction performance; (ii) the definitions of udder health status and SCC  
203 variations; (iii) the adjustment of regressions on co-variables; (iv) the timing of the events, particularly

204 the period of udder inflammation around AI; and (v) the manner in which the results were reported  
205 (odd ratios, relative risks or mean values among groups). Despite these limitations, previous studies  
206 showed a correlation between more severe mastitis (i.e., a large SCC increase compared to a small  
207 SCC increase or clinical mastitis compared to only a SCC increase) and a greater decrease in  
208 conception rates. Specifically, the percent decreases in conception were approximately 15-18% when  
209 SCC increased above the threshold of 280,000 cells/mL [12]; 5-12% when various SCC thresholds  
210 and various periods of increased SCC were considered [13]; and up to 60% when SCC increased  
211 above the threshold of 200,000 cells/mL during the conception period [14]. This last result represents  
212 an exception among the above-mentioned studies. Previous studies reported that the percent decrease  
213 in the conception rate in cases of clinical mastitis was higher, at approximately 13-32% depending on  
214 the time of the clinical mastitis relative to AI [13] and at 33-44% in cases of clinical mastitis occurring  
215 after an SCC increase, respectively [24]. Those results are in accordance with the observed decrease in  
216 conception success with increased SCC in the present study and with the greater decrease in  
217 conception success when the higher thresholds of SCC were tested. Together, these findings reinforce  
218 previous but limited studies showing that more severe udder inflammation leads to greater decrease in  
219 the conception success [24].

220 The GAM allows the relationship between two variables to be described without any *a priori*  
221 assumptions. The results of the GAM showed a higher impact of SCC change on conception rate after  
222 AI than before AI, and this finding is in accordance with the results of logistic regression analysis.  
223 These results are also in accordance with the popular finding that conception is more strongly affected  
224 by clinical mastitis or increased SCC when these events occur during or only after AI compared to  
225 before AI [8,9,11,13,25]. One exceptional result was obtained in a study focusing on SCC only before  
226 AI [26], and another exceptional result was produced in the context of a definition of mastitis based on  
227 culture findings, among other factors [11]. The similar relative risks of an increased SCC at the time of  
228 AI (LH group) and a high SCC both before and after AI (HH group) observed in the present study  
229 confirm that conception is dramatically impacted by udder health status following AI.

230 Subclinical ketosis is a risk factor for deteriorated reproductive performance, including a  
231 reduced first service conception rate [16], as well as for mastitis [27,28]. To the authors' knowledge,  
232 no peer-reviewed quantification of the link between SCK and increased SCC or clinical mastitis is  
233 available [16]. Despite the proxy used in place of a consensus definition of SCK (based on BHBA or  
234 NEFA), the present study reported a decrease in conception success ranging between 3 and 17% in  
235 cows exhibiting SCK. The proxies of SCK used in this study were defined by the GAM and are in  
236 accordance with the criteria generally used in the field and reported in the literature [18,19]. The  
237 sensitivity and specificity of these indicators remain moderate [18]. The fact that the effect of SCK  
238 remained similar between the period of 0-40 days before AI and the period of 40-80 days before AI is  
239 in accordance with previous results showing a significant decrease in conception associated with the  
240 NEB that occurs 80 to 60 days before AI [29].

241 No previous study has reported an interaction between SCK and SCC that affects conception.  
242 This is the first report demonstrating that SCK may interact with the relationship between SCC and  
243 conception. For all the definitions of SCK proxies used here, the presence of SCK magnified the  
244 negative association between high or increased SCC and conception success. Further work is needed  
245 using a definition of SCK that better aligns with the consensus definition.

246 The variability in the coefficients of the interaction term among the different years (in  
247 particular for SCK2) observed in the present study may originate from the changes in the prevalence  
248 of SCK between the years of the study. The validity of an interaction between the SCC and SCK is  
249 strengthened by the difference in the coefficient for the SCC variable when the model without  
250 interaction was alternatively used to analyze the populations with and without SCK (results not  
251 shown).

252 The mechanism underlying the relationship described in the present work remains unclear.  
253 However, a positive genetic correlation between udder health and conception has been reported in  
254 several studies [30,31]. The mechanisms by which clinical or subclinical mastitis potentially interacts  
255 with conception may include alterations in endocrine profiles and follicular development, inhibition of

256 LH and FSH via cytokine release, and intrauterine embryonic survival [32]. Whether the link between  
257 mammary inflammation and reproduction failure may involve changes in the cytokine network is also  
258 uncertain. The well-described changes in immune function during SCK [33,34] are also in accordance  
259 with the SCC\*SCK interactions that explain conception success in the present work. This is also in  
260 accordance with a recent study showing that ewes from high-SCC genetic lines have increased NEFA  
261 and BHBA concentrations after energy restriction, which reinforces the hypothesis of a genetic  
262 association between mastitis susceptibility and energy metabolism [35].

263 From a practical perspective, the present results clearly support the concept of loser cows  
264 [36,37]. As a result of the strong associations among disorders, an ill animal is more likely to be  
265 affected by other disorders, thereby creating a vicious cycle. This characteristic is of importance in  
266 allocating resources and calculating the indirect costs of disorders [7]. However, the present results  
267 should not be used as a criterion to select cows to be served at this point. First, cows with mastitis  
268 before AI and no mastitis within 1 or 2 months after AI were not highly affected (HL cows), and there  
269 is no justification for excluding cows with mastitis before estrous from being served unless the  
270 mastitis is chronic. More broadly, excluding cows from being served because they were predicted to  
271 have a low rate of conception success (e.g., with SCK) improves the indicator “number of AIs per  
272 conception”, but may simultaneously reduce the calving interval or increase culling and turnover. The  
273 cost of AI (induced labor) remains the key parameter for deciding whether to serve a given cow in this  
274 situation.

275

## 276 **5. Conclusion**

277 The present work is in accordance with previous studies that demonstrated the negative impact  
278 of mastitis at the time of AI on conception success. These results also reinforce previous but limited  
279 studies showing that the more severe udder health infection or inflammation exacerbates the decrease  
280 in conception success. A high SCC after AI has a much greater negative association with decreased

281 conception success than a high SCC before AI. An increase in the SCC was clearly associated with a 12  
282 to 17% decrease in the conception success. A cow that already had SCK and experiences an increase  
283 in SCC at the time of or after AI exhibits a multiplicative decrease in the conception success compared  
284 to a cow with a high SCC without SCK. However, further research is needed to improve the  
285 quantification of these interactions using more accurate definitions of SCK.

286

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390 **Table 1.**

391 Data summary for the study population by year.

Item	Year				
	2008	2009	2010	2011	2012
Number of herds	44,124	42,169	39,648	37,905	36,683
Number of cows	1,325,171	1,228,949	1,180,592	1,171,971	1,073,018
% in 1 <sup>st</sup> lactation	36.4	35.3	36.7	38.1	38.9
% in 2 <sup>nd</sup> lactation	26.5	27.8	26.5	26.7	27.7
% in 3 <sup>rd</sup> lactation	17.8	17.7	18.1	17.1	16.5
% in >3 <sup>rd</sup> lactation	19.3	19.2	18.7	18.2	16.9
Mean of DIM <sup>1</sup> (days)±SD	91.0±32.6	92.5±33.1	93.0±33.2	94.4±33.4	95.6±33.7
Mean of 305-day MY <sup>2</sup> (kg)	8187	8187	8383	8608	8476
Number of all AIs <sup>3</sup>	1,909,776	1,761,726	1,686,025	1,674,491	1,517,649
Success rate (%)	36.7	36.5	37.3	36.3	38.1
Number of first AI	1,050,762	983,333	950,582	946,876	867,175
Success rate (%)	39.6	39.6	40.5	39.4	41.5

392 <sup>1</sup>Days in milk at the time of the first artificial insemination.393 <sup>2</sup>Milk yield adjusted to a reference lactation period of 305 days.394 <sup>3</sup>Artificial insemination.

395 **Table 2.**

396 Definition of subclinical ketosis (SCK) proxies based on milk fat and protein contents (mg/kg) or their  
 397 ratio and prevalence of SCK according to each corresponding definition.

Proxy code	Definition		Prevalence of SCK
	Presence of SCK	Absence of SCK	
SCK1	Protein before AI <sup>1</sup> <30	Protein before AI ≥30	35%
SCK2	Fat before AI >45 and protein before AI <28	Fat before AI between 35 and 45 and protein before AI ≥32	5%
SCK3	Fat/protein ratio before AI >1.5	Fat/protein ratio before AI between 1.1 and 1.4	10%

398 <sup>1</sup>Artificial insemination.

399 **Table 3.**

400 Relative risk (95% CI) of conception associated with the different explanatory variables for the first  
 401 artificial insemination (AI1) and for all subclinical ketosis (SCK) proxies considering records from the  
 402 period of 0-40 days around AI1.

Variable		SCK proxy		
		SCK1	SCK2	SCK3
Class of SCC	LL	Reference	Reference	Reference
	LH	0.86(0.85-0.87) <sup>***</sup>	0.87(0.86-0.88) <sup>***</sup>	0.84(0.83-0.84) <sup>***</sup>
	HL	0.96(0.95-0.98) <sup>***</sup>	0.96(0.95-0.97) <sup>***</sup>	0.98(0.97-0.99) <sup>**</sup>
	HH	0.86(0.85-0.87) <sup>***</sup>	0.86(0.86-0.87) <sup>***</sup>	0.83(0.82-0.84) <sup>***</sup>
SCK	Without	Reference	Reference	Reference
	With	0.93(0.92-0.93) <sup>***</sup>	0.83(0.82-0.85) <sup>***</sup>	0.97(0.95-0.99) <sup>***</sup>
Interaction	LH * SCK	0.99(0.97-1.01)	0.95(0.90-1.00) <sup>†</sup>	0.99(0.98-1.01)
	HL * SCK	0.99(0.97-1.01)	1.00(0.95-1.05)	0.96(0.94-0.98) <sup>***</sup>
	HH * SCK	0.97(0.96-0.99) <sup>***</sup>	0.93(0.89-0.98) <sup>**</sup>	0.94(0.93-0.94) <sup>***</sup>
DIM <sup>1</sup> (days)	50 - 100	Reference	Reference	Reference
	<50	0.82(0.81-0.83) <sup>***</sup>	0.84(0.82-0.86) <sup>***</sup>	0.83(0.80-0.85) <sup>***</sup>
	100 - 200	1.00(0.99-1.00)	0.98(0.97-0.99) <sup>***</sup>	1.00(1.00-1.01)
305-day MY <sup>2</sup> (kg)	<7000	Reference	Reference	Reference
	>7000	0.96(0.95-0.96) <sup>***</sup>	0.97(0.96-0.98) <sup>***</sup>	0.95(0.94-0.95) <sup>***</sup>
Parity	1 <sup>st</sup>	Reference	Reference	Reference
	2 <sup>nd</sup>	0.89(0.88-0.89) <sup>***</sup>	0.91(0.90-0.92) <sup>***</sup>	0.90(0.90-0.91) <sup>***</sup>
	3 <sup>rd</sup>	0.83(0.82-0.83) <sup>***</sup>	0.85(0.84-0.86) <sup>***</sup>	0.84(0.84-0.85) <sup>***</sup>

> 3<sup>rd</sup>      0.71(0.70-0.71)<sup>\*\*\*</sup>      0.73(0.72-0.74)<sup>\*\*\*</sup>      0.72(0.72-0.73)<sup>\*\*\*</sup>

403 <sup>\*\*\*</sup>*P*<0.001; <sup>\*\*</sup>*P*<0.01; <sup>\*</sup>*P*<0.05; <sup>†</sup>*P*<0.1.

404 <sup>1</sup>Days in milk at the time of the first artificial insemination.

405 <sup>2</sup>Milk yield adjusted to a reference lactation period of 305 days.

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406 **Table 4.**

407 Relative risk (95% CI) of conception associated with the different explanatory variables for the first  
 408 artificial insemination (AI1) and for all subclinical ketosis (SCK) proxies considering SCC records  
 409 from the period of 0-40 days around AI1 and considering records of SCK from the period of 40-80  
 410 days before AI1.

Variable		SCK proxy		
		SCK1	SCK2	SCK3
Class of SCC	LL	Reference	Reference	Reference
	LH	0.87(0.86-0.88) <sup>***</sup>	0.87(0.85-0.88) <sup>***</sup>	0.87(0.86-0.88) <sup>***</sup>
	HL	0.96(0.94-0.97) <sup>***</sup>	0.97(0.96-0.99) <sup>***</sup>	0.96(0.95-0.97) <sup>***</sup>
	HH	0.87(0.86-0.88) <sup>***</sup>	0.87(0.86-0.88) <sup>***</sup>	0.87(0.86-0.87) <sup>***</sup>
SCK	Without	Reference	Reference	Reference
	With	0.95(0.95-0.96) <sup>***</sup>	0.87(0.86-0.88) <sup>***</sup>	0.93(0.92-0.94) <sup>***</sup>
Interaction	LH * SCK	1.00(0.98-1.01)	0.97(0.94-1.01)	1.00(0.97-1.02)
	HL * SCK	1.00(0.98-1.02)	0.98(0.93-1.02)	0.99(0.97-1.02)
	HH * SCK	0.98(0.96-0.99) <sup>**</sup>	0.96(0.93-0.99) <sup>*</sup>	0.96(0.94-0.98) <sup>***</sup>
DIM <sup>1</sup> (days)	50 - 100	Reference	Reference	Reference
	<50	0.81(0.79-0.84) <sup>***</sup>	0.83(0.80-0.86) <sup>***</sup>	0.82(0.80-0.85) <sup>***</sup>
	100 - 200	1.00(1.00-1.01)	1.00(0.99-1.01)	1.00(0.99-1.00)
305-day MY <sup>2</sup> (kg)	<7000	Reference	Reference	Reference
	>7000	0.96(0.95-0.96) <sup>***</sup>	0.93(0.93-0.94) <sup>***</sup>	0.94(0.94-0.95) <sup>***</sup>
Parity	1 <sup>st</sup>	Reference	Reference	Reference
	2 <sup>nd</sup>	0.89(0.89-0.90) <sup>***</sup>	0.91(0.90-0.92) <sup>***</sup>	0.90(0.89-0.90) <sup>***</sup>

3 <sup>rd</sup>	0.83(0.83-0.84)***	0.85(0.84-0.86)***	0.84(0.83-0.84)***
> 3 <sup>rd</sup>	0.71(0.70-0.71)***	0.72(0.71-0.73)***	0.72(0.71-0.72)***

411 \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; † $P < 0.1$ .

412 <sup>1</sup>Days in milk at the time of the first artificial insemination.

413 <sup>2</sup>Milk yield adjusted to a reference lactation period of 305 days.

414 **Table 5.**

415 Relative risk (95% CI) of conception associated with the different explanatory variables for the first  
 416 artificial insemination (AI1) and for all subclinical ketosis (SCK) proxies considering records from the  
 417 period of 40-80 days around AI1.

Variable		SCK proxy		
		SCK1	SCK2	SCK3
Class of SCC	LL	Reference	Reference	Reference
	LH	0.84(0.83-0.85)***	0.84(0.83-0.85)***	0.84(0.83-0.84)***
	HL	0.98(0.97-0.99)***	0.98(0.97-0.99)***	0.98(0.97-0.99)**
	HH	0.83(0.82-0.84)***	0.84(0.83-0.85)***	0.83(0.82-0.84)***
SCK	Without	Reference	Reference	Reference
	With	0.96(0.95-0.96)***	0.87(0.86-0.89)***	0.94(0.93-0.94)***
Interaction	LH * SCK	0.99(0.97-1.00) <sup>†</sup>	0.94(0.91-0.97)***	0.97(0.95-0.99)***
	HL * SCK	0.98(0.96-1.00)*	1.00(0.97-1.03)	0.99(0.98-1.01)
	HH * SCK	0.97(0.96-0.99)***	0.93(0.90-0.96)***	0.96(0.94-0.98)***
DIM <sup>1</sup> (days)	50 - 100	Reference	Reference	Reference
	<50	0.82(0.79-0.84)***	0.83(0.80-0.85)***	0.83(0.80-0.85)***
	100 - 200	1.01(1.00-1.01)***	1.00(1.00-1.01)	1.00(1.00-1.01)
305-day MY <sup>2</sup> (kg)	<7000	Reference	Reference	Reference
	>7000	0.95(0.95-0.96)***	0.93(0.93-0.94)***	0.95(0.94-0.95)***
Parity	1 <sup>st</sup>	Reference	Reference	Reference
	2 <sup>nd</sup>	0.90(0.89-0.90)***	0.92(0.91-0.92)***	0.90(0.90-0.91)***
	3 <sup>rd</sup>	0.84(0.83-0.84)***	0.86(0.85-0.87)***	0.84(0.84-0.85)***

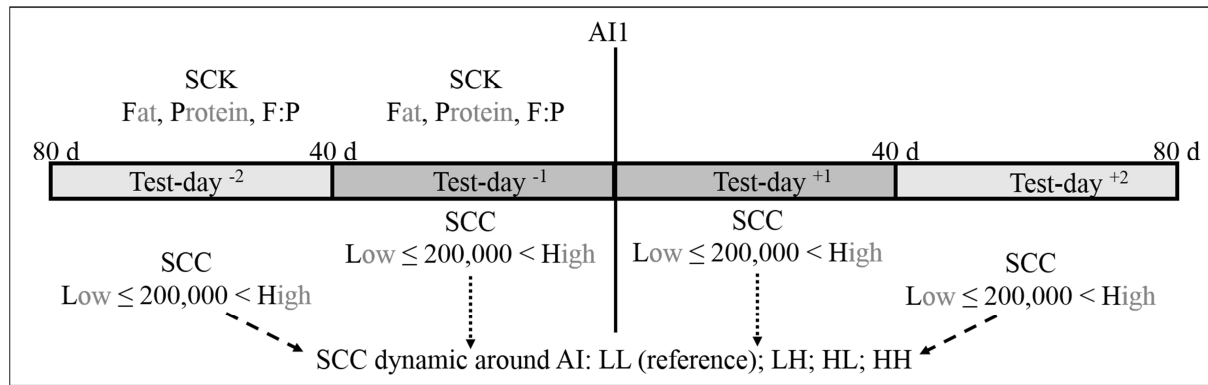
> 3<sup>rd</sup>      0.72(0.72-0.73)<sup>\*\*\*</sup>      0.73(0.72-0.74)<sup>\*\*\*</sup>      0.72(0.72-0.73)<sup>\*\*\*</sup>

418 <sup>\*\*\*</sup>*P*<0.001; <sup>\*\*</sup>*P*<0.01; <sup>\*</sup>*P*<0.05; <sup>†</sup>*P*<0.1.

419 <sup>1</sup>Days in milk at the time of the first artificial insemination.

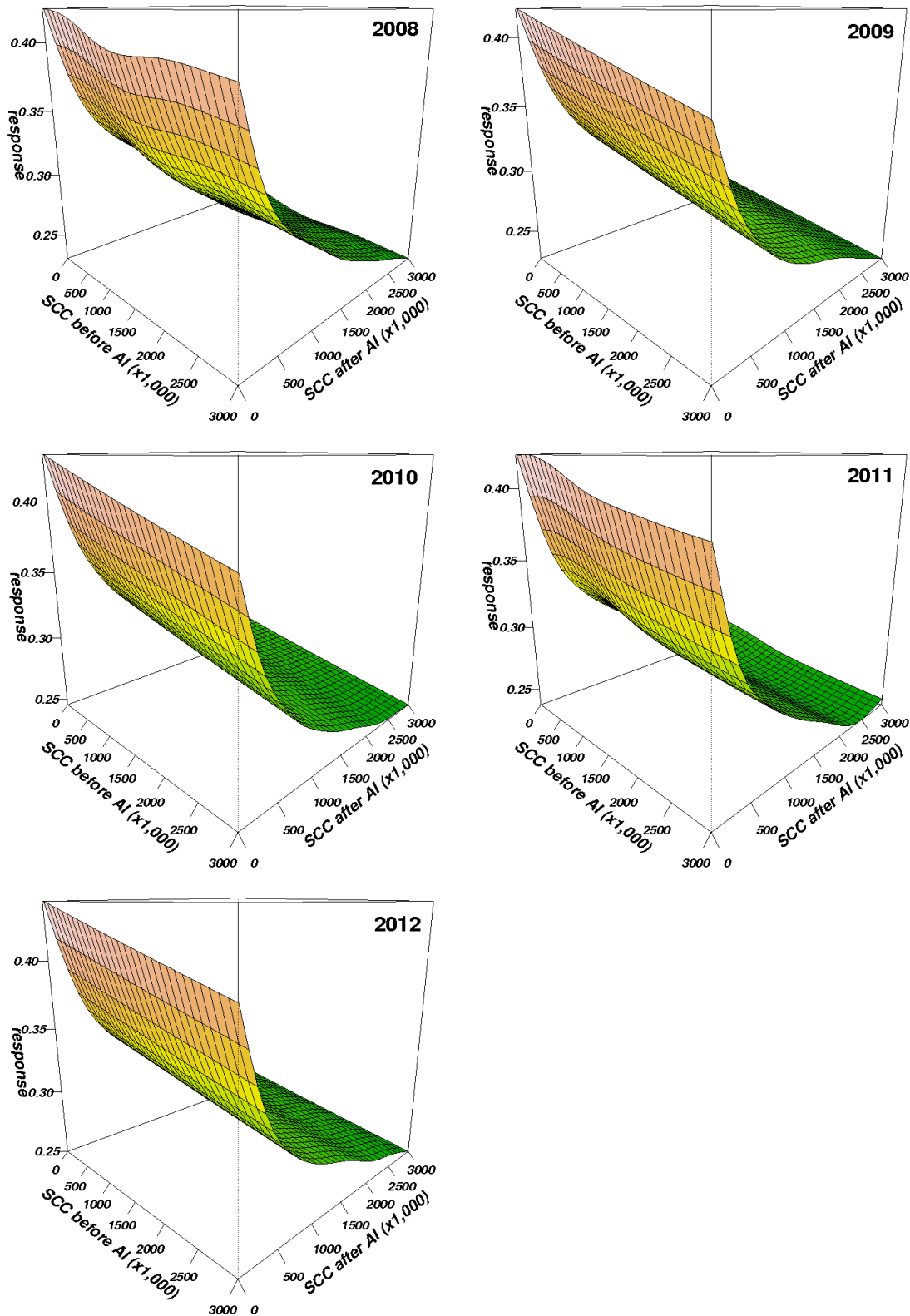
420 <sup>2</sup>Milk yield adjusted to a reference lactation period of 305 days.

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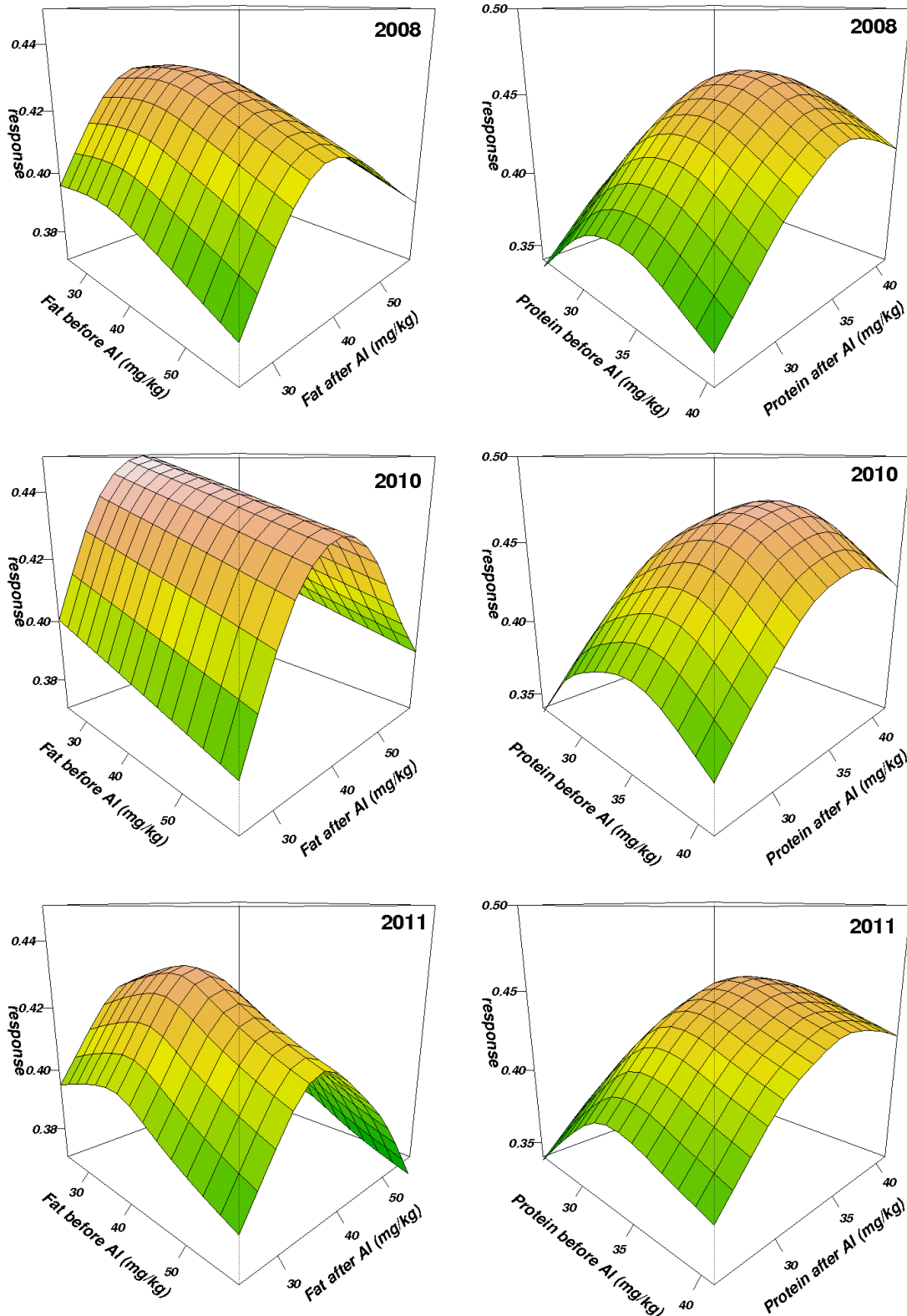


421

422 **Figure 1.** A Schematic representation of the time periods around the first artificial insemination (AI1)  
 423 and the measurements examined in this study. Several somatic cells counts (SCC) thresholds were  
 424 tested within 40 days before and after AI1, but only the results for the threshold of 200,000 cells/mL  
 425 were detailed in this article. A proxy of subclinical ketosis (SCK) was built using the fat and protein  
 426 contents (mg/kg) or their ratio within 0-40 days and within 40-80 days before AI1.



430 **Figure 2.** A 3-dimensional representation of the annual relationship between SCC before and  
 431 after the first AI and conception success (vertical axis).



432

433

434

435 **Figure 3.** A 3-dimensional representation of the annual relationship between milk fat (left) or milk  
 436 protein (right) contents before and after the first AI and conception success (vertical axis). Only 3  
 437 years of data are reported.

Worsened udder health is known to be negatively associated with reproduction. Early postpartum dairy cows often experience a negative energy balance that may lead to subclinical ketosis. Subclinical ketosis has also been reported to be deleterious to both conception and mastitis. However, the interactions between subclinical ketosis, udder health and conception are still unclear. The present paper is an attempt to identify and quantify these interactions.

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