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Association between clinical and subclinical mastitis and reproductive performance of cows at Nottingham dairy centre

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Article information	Abstract
<i>Article history:</i> Received March 27, 2020 Accepted April 28, 2020 Available online March 15, 2021	Clinical and subclinical mastitis regarded as important diseases causes a reduction in reproductive performance in dairy cows in the UK during the last decade. This study was aimed to assess the association between clinical mastitis, subclinical mastitis and the reproductive performance of cows. Data from 184 multiparous Holstein dairy cows were
<i>Keywords</i> : Clinical Mastitis Somatic cells count Reproductive performance Dairy cows	collected. Binomial logistic regression used to determine the incidence rate of clinical mastitis and subclinical mastitis between parity, calving year and seasons of the year. Linear mixed model used to determine the effect of clinical and subclinical mastitis on reproductive
Daily cows	a cow to get pregnant at 1st, 2nd or 3rd service was evaluated using binomial logistic
Correspondence:	regression. The same model used to illustrate the probability of a cow getting pregnant from
Z.A. Mohammed	30-60 days postpartum or 61-90 days postpartum. The incidence of clinical mastitis was
zeravantovi@gmail.com	different between calving years (P<0.01). Cow with clinical mastitis or subclinical mastitis had longer calving to first service interval, calving to conception interval (P<0.05). Cows
	clinical mastitis had a lower rate to get pregnant within 20-30 days postpartum compared to
	healthy cows (P<0.05). Cows with higher somatic cells count, (especially cows with greater
	than 399,000 cells/mL of milk), had a higher number of services compared to cows with a
	lower number of individual cow somatic cell counts. The study concluded that both clinical
	and subclinical mastitis have a relationship with a reduction in reproductive performance in
	high yielding dairy cows.

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Introduction

Fertility in dairy herds has been declined in the UK and over the world during the last decade and regarded as a major negative factor of herd profitability (1-3). There are numerous factors that influence the reproductive performance of highly yielding dairy cows including uterine inflammation (4,5) and lameness (6,7). Clinical mastitis (CM) regarded as an important disease event in dairy cattle, with the recent data estimate that incidence rate of clinical mastitis in the UK ranged from 50 to 70 cases per 100 cow/years (8). The economic impact of clinical mastitis on dairy herds is associated with decreased production of milk, increased the costs of milk due to the treatment and discarded milk, decreased milk quality, with increases in somatic cell count (SCC), and increased culling rate (9). In addition to clinical mastitis, detection of subclinical mastitis in dairy herds is very problematic. Cows with subclinical mastitis look healthy, without systemic reaction, milk looks normal. It has been reported that subclinical mastitis causes a great decline in milk yield and the undetected cows would be the main causes for spreading the infection in the herd especially when the causes of mastitis is bacteria. Identification of somatic cells count in milk regarded as an accurate and indirect method for predicting the incidence of mastitis in dairy cattle. It has been documented that the level of SCC in milk does not reach 100x10³cells/ml in healthy cows. However, cows considered to be sick with subclinical

mastitis when SCC in the milk was $\geq 200 \times 10^3$ cells/ml. It has been reported that milk yield loss due to SCC was found to vary considerably depending on the level of SCC (10-12). Many previous studies have been carried out about the effect of CM and subclinical mastitis (SCC in milk) in high professional yielding dairy herds in the world, but the results of that studies were unclear how clinical mastitis and somatic cells count in milk have negative impact on reproductive performance in dairy cows. Recently, Hertl et al. (13) and Hudson et al. (14) have estimated the importance of the timing of clinical mastitis and its associated with a lower probability on conception. More recent studies have been tended to concentrate on the relationship between clinical mastitis and subclinical mastitis and the odds of cows become pregnant after artificial insemination. Therefore, this study aimed to evaluate the association between clinical and subclinical mastitis (somatic cells count in milk, SCC) and reproductive performance in high yielding dairy cows at Nottingham dairy centre.

Material and methods

Animals

Ethical Review Committee from The University of Nottingham approved this study, which was achieved in agreement with the UK Home Office Animal (Scientific Procedures) Act 1986 under Project Licence PPL40/1621. This study conducted on 184 multiparous Holstein dairy cows from the University of Nottingham dairy centre in the East Midlands, UK. Data of cows that calved between 2009 and 2014 were analysed. Animals were kept in herd and managed according to normal farm practice and fed with a total mixed ration. In addition, cows were fed on concentrate fed during milking according to yield. Cows were milked by robot (Lely Astronaut A3, Adoption of automatic milking systems (AMS). All cows with udder disorders were treated according to routine farm practice with no experimental intervention.

Milk samples

Nottingham dairy centre is equipped with Lely Astronaut, AMS. Milk samples were collected from all milking per cow during a period of 24 hours on all AMS. The manufacturer's automatic sampling device was used to sample each cow for somatic cell count. Lely Astronaut A3, Adoption of automatic milking systems AMS equipped with milk quality Control-C (MQC-C) sensors were programmed to measure somatic cell count of the milk regularly. Any abnormalities in the milk which could be of concern are exposed immediately in the Lely T4C management programme.

Data collection

Data from each lactation were collected from Lely T4C management programme and stored in Microsoft Excel 2010 (Microsoft Corporation, 2010). Basic statistics describing the herd is provided in Table 1.

Table1: Shows descriptive statistics for the herd used for this study

Parameter	Mean	Min.	Percentile				Mar
			10%	25%	75%	90%	- Max.
305-day in milk (kg)	10678	1540	6357	9261	12719	14454	18730
Calving to first service interval (CFSI, d)	57.82	19	31	37.25	71	95	170
Open days (d)	112.2	29	40.2	56.5	158	205.8	291
Calving interval (CI, d)	390.5	307	320	338.3	430.8	495.1	560
ICSCC ($x10^3$ /ml)	277	11	20	45	358	464	4020

ICSCC=Individual cows somatic cell counted from milk-recording data which recorded form bulk milk.

For binary descriptive variables such as pregnancy outcome at 1st, 2nd or 3rd insemination dichotomous values were used. Cases of clinical mastitis were diagnosed according to the normal practice of the herd. Incidence of mastitis was coded "1" for each cow and or was coded into "0" when cows were free from clinical mastitis. Calving to first service interval were categorised into: 20-30d; 31-60d; 61-90d and \geq 91d. Individual cow somatic cells (ICSCC) variables were classified into 3 categories, as presented in Table 2, to identify the detrimental effect of ICSCC on reproductive performances. Other descriptive variables were calculated in this study Table 3. Variables such as the parity variable were recorded. Cows with parity ≥ 3 were grouped into a single category due to low number of data.

Table 2: Individual cow somatic cell, ICSCC (cells/mL) categories

Category	Description
1	0-200,000
2	200,000-399,000
3	>399,000

Table 3: Variables (with variable type) calculated at each level of data

Variable	Variable type			
Served	Dichotomous (served or not served)			
Become pregnant	Dichotomous (served or not served)			
Season of year	Category (Jan-March, Apr-Jun, Jul-Sep, or Oct-Dec)			
Parity of cow	Categorical (1, 2, or 3)			
CM 20-30dpp	Dichotomous (case of CM recorded or not)			
CM 31-60dpp	Dichotomous (case of CM recorded or not)			
CM 61-90dpp	Dichotomous (case of CM recorded or not)			
CM≥91dpp	Dichotomous (case of CM recorded or not)			
Milk yield (× 1,000 kg) at 305-d	Continuous			
ICSCC 20-30dpp	Categorical-see Table 2			
ICSCC31-60dpp	Categorical-see Table 2			
ICSCC 61-90dpp	Categorical-see Table 2			
ICSCC and >91dpp	Categorical-see Table 2			
Calving year	Categorical (2009, 2010, 2011, 2012, 2013 or 2014)			
M- Clinical mastiris: ICSCC-Individual cow sometic calls count: dnn- day post partum period				

CM= Clinical mastitis; ICSCC=Individual cow somatic cells count; dpp= day post-partum period.

Statistical analysis

The prevalence of Clinical mastitis (CM)

Binomial logistic regression (Generalized logistic regression) used to determine the prevalence of CM between calving years and lactation number. All statistical analysis was performed using GenStat 12th edition (VSN International Ltd, Hemel Hempstead, UK).

The association between CM and subsequent reproductive performance

Linear mixed model (Wald test) used to determine the association between CM status (included as the factor) and reproductive performance such as CFSI, CCI, SPC, CI and 305d Milk yield (included as variables). Binomial logistic regression was performed to determine the incidence rate of CM between lactation numbers and to evaluate the association between CM and the probability of a cow to get pregnant during 20-30d; 31-60d; 61-90d; or \geq 91d. Moreover, binomial logistic regression used to evaluate probability of a cow becoming pregnant at 1st, 2nd or 3rd service.

The association between ICSCC categories and the reproductive performance

Linear mixed model (Wald test) used to determine the association between ISCC categories 1, 2 or 3 status (included as the factor) and reproductive performance such as CFSI, CCI, SPC, CI and 305d Milk yield (included as the variables). Individual cow somatic cell count (ICSCC) number was recorded per each cow. Binomial logistic regression used to evaluate the association between ICSCC categories and the probability of a cow to get pregnant during 20-30d; 31-60d; 61-90d and \geq 91d. Furthermore, binomial logistic regression used to evaluate the probability of a cow to get pregnant at 1st, 2nd or 3rd service. Binomial regression was performed for determining the odds ratio of ICSCC

incidence rate between parity, calving year and seasons of the year.

Results

Prevalence of clinical mastitis (CM)

The results demonstrated that the prevalence of CM was significantly difference among calving year (P<0.01; Fig.1). The mean prevalence of CM across the whole study was 25.0%. The highest incidence was observed in 2009 and the lowest rate was seen in 2014 at 39 % and 10%, respectively. It should be noted that this study found that the season of the year had no effect on the prevalence of CM (P>0.05, Fig.2). Moreover, this study found that no effect of lactation number on the prevalence the prevalence on CM (P>0.05; Fig. 3).



Figure 1: The prevalence of CM between years 2009 and 2014 at Nottingham dairy herds (P<0.001). The prevalence of CM varied from 10-39%.



Figure 2: The prevalence of CM between seasons of the year. A) The prevalence of CM between twelve month of the year from Jan-Dec. (P>0.05). B) The prevalence of CM between four seasons (P>0.05).

The effect of clinical mastitis (CM) on reproductive performance

The results showed that CM had impact on the reproductive performance in dairy cows. Cows with CM had longer calving to first service interval about 9 days (P<0.05, Table 4). In addition, cows with CM had longer calving to conception interval about 21 days (P<0.05, table 4). However, this study found no significant effect of CM on number of service per-conception and calving interval as well (P>0.05, Table 4). It should be noted that this study found that CM had no effect on the pregnancy rate at 1^{st} , 2^{nd} or 3^{rd} services (P>0.05, Table 4). Cows with CM had the largest reduction in the probability of pregnancy about 13% in the period from 20-30dpp, when the case of CM being evaluated (P<0.01, Table 4). However, this study found that

there was no such relationship was identified for the probability of pregnancy during 31-60dpp; 61-90dpp and >90dpp relating to CM cases (P>0.05, Table 4).



Figure 3: The Incidence rate of CM between parity. No significant difference of CM incidence seen between parity (P>0.05).

Logistic regression showed that year of calving, the season of the year, did not affect the incidence of subclinical mastitis in high yielding dairy cows at Nottingham dairy centre (P >0.05), Table 5. This study found that parity 1 and 3 did not affect the incidence of subclinical mastitis. However, this study showed that as the odds ratio of parity 2 decreased the incidence rate of subclinical mastitis decreased significantly (P <0.05), Table 5. It should be noted that subclinical mastitis did not affect on 305d milk yield (P >0.05) (Figure 4).

Reproductive parameter	No.CM (Mean ± SE)	CM (Mean ± SE)	P value
Calving to first service interval (d)	51.8 ± 3.0	60.8 ± 2.6	0.02
Calving to conception interval (d)	98.8 ± 7.5	121.3 ± 6.4	0.024
Number of service per conception (n)	2.51 ± 0.2	2.66 ± 0.2	0.536
Calving interval (d)	383.6 ± 11.7	393.3 ± 9.0	0.51
1 st service pregnancy rate (%)	31.6%	26.2%	0.435
2 nd service pregnancy rate (%)	21.1%	21.4%	0.961
3 rd service pregnancy rate (%)	18.4%	17.5%	0.871
Cows become pregnant in 20-30 dpp	15.8%	2.9%	0.002
Cows become pregnant in 31-60 dpp	54.0%	62.4%	0.274
Cows become pregnant in 61-90 dpp	21.1%	25.2%	0.516
Cows become pregnant in >90 dpp	9.2%	9.7%	0.911

Table 4: Association between CM and the reproductive performance in high yielding dairy cows during postpartum period

Note: The data presented in this table were compared between healthy cows and cows with clinical mastitis

Cows with subclinical mastitis ($\geq 200,000$ cells/ml) had 703L more milk but statistically not different. This study found that ICSCC categories 2 and 3 were associated with

longer CFSI compared to category 1 about 16, 26 days, respectively (P<0.01, Table 6). Furthermore, ICSCC categories 2 and 3 were associated with longer open days

compared to category 1 about 9, 33 days, respectively (P<0.05, Table 6). In addition, this study found that ICSCC categories 2 and 3 had a higher number of services compared with category 1 about 0.3, 1.1 respectively (P<0.01, Table 5). However, ICSCC categories did not have effect on pregnancy rate at 1, 2 and 3 with 1^{st} , 2^{nd} and 3^{rd} service (P>0.05, Table 6). Individual SCC categories 1, 2 and/or 3 had no effect on the probability of cows to become pregnancy during 30-60dpp and 61-90 dpp (P>0.05, Table 6).



Figure 4: The effect of subclinical mastitis on 305d milk yield.

Table 5: Parameters estimate the effects of different factors on the incidence of subclinical mastitis ICSCC (≥200,000 cells/ml)

F eature	Parameter estimate \pm SE		D .1 .		
Factors		odds ration	Lower	Upper	- P-value
Parity					
1		1	Reference		0.17
2	-0.83±0.36	0.4364	0.2154	0.8842	0.02
3	-0.40±0.36	0.6734	0.3314	1.368	0.27
Year of calving					
2010		1 Reference			
2011	-0.25±0.59	0.78	0.24	2.50	0.68
2012	-0.03±0.55	0.97	0.33	2.83	0.96
2013	-0.48 ± 0.54	0.62	0.21	1.80	0.38
2014	-0.93±0.58	0.39	0.13	1.22	0.11
Season of year					
1 (Jan-Mar)		1 Reference			
2 (Ape-Jun)	0.04 ± 0.52	1.04	0.38	2.86	0.93
3 (Jul-Sep)	-0.67 ± 0.42	0.51	0.23	1.16	0.11
4 (Oct-Dec)	-0.25 ± 0.48	0.78	0.31	1.97	0.59

Table 6: Reproductive performance among different ICSCC categories

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Reproductive performance —	1	2	3	r-value	
Calving to first service interval (d)	48.4±2.59 ^a	64.19±3.44 ^b	73.6±4.3°	< 0.001	
Calving to conception interval (d)	103.4±6.64 ^a	112.8 ± 8.80^{ab}	136.2±11.20 ^b	0.045	
Number of service per conception	2.3±0.16 ^a	2.6 ± 0.2^{ab}	3.4±0.3°	0.001	
Calving interval (d)	387.5±10.0	386.2±12.3	404.2±15.1	0.598	
1st service pregnancy rate	28.4%	31.5%	25.7%	0.838	
2nd service pregnancy rate	25.3	16.7%	20.0%	0.46	
3rd service pregnancy rate	14.7%	14.8%	31.4%	0.069	
Cows become pregnant in 31-60 dpp	14.5%	16.0%	15.3%	0.597	
Cows become pregnant in 61-90 dpp	19.6%	22.0%	22.0%	0.596	

Different letters a,b,c at the same rows were significant (P<0.05), ab were not significant (P>0.05).

Discussion

The present study agrees with the previous works in the same filed reporting that both CM and ICSCC could have substantial effect on the reproductive performance in high yielding dairy cows during the post-partum period at Nottingham dairy centre. The incidence rate of clinical mastitis in this study during 305-day was about 25.0%, which has impact on the reproductive performance during postpartum period. This incidence rate of CM is nearly about the same rate 26.9% reported by Hertl *et al.* (13) in Turkey and 23% recoded by Hudson *et al.* (14) in France. However, it is lower than 40% that estimated in England during 1994-1996 (15) and 37.5% was estimated in Salahaldeen province (16). Furthermore, this incidence is much lower that reported in than reported in England and wales 2007 (8). The low incidence of CM in this study may be due to inadequate data used in this study compared to previous studies carried out in England and Wales.

The current study investigated that variables such as parity, seasons of the years did not effect on the incidence of the CM. This is parallel to Lescourret et al. (17) reported that lactations number and seasons of the years were associated with the incidence rate of CM. It should be noted this study reported that the year of calving had associated with the incidence rate of CM. Disagreement with the previous study carried out by Lescourret et al. (17), who used data from the large number of dairy herds across UK, they found that there was no relation between calving years and the incidence of CM. Regarding to overall reproductive performance, CM had substantial association to reduced reproductive performance of high yielding dairy at Nottingham dairy centre. Cows with CM had longer calving to first service interval, calving to conception interval. Furthermore, CM had association with a lower probability of cows becoming pregnant from 20-30 dpp. This is parallel to the previous studies that cows with CM had longer days to first insemination interval; longer open days (18-22). It has been reported that cows producing milk more 21 litter/days had significantly longer calving interval and had more services per conception (23).

The mechanism by which CM effect on delay day to first service interval and longer calving to conception interval is might be due to affect hypothalamic-pituitary-ovary hormonal axis (24) and resulting delay onset of ovarian activity (25,26). Mohammed (5) investigated that metritis delay onset of post-partum ovarian cyclicity in high yielding dairy cow at Nottingham dairy centre. Metritis increased abnormal progesterone profile particularly and consequently delayed onset of post-partum ovarian cyclicity. It is interesting to note that cows with metritis had smaller corpus luteum and produced low amount of P4 (27). It has been reported that cows with CM experienced to suppression of LH surge which cause delay ovulation or increased PGF2a which cause decreased P4 production this may cause the follicles failure to develop (28). Lescourret et al. (17) demonstrated that cows with CM caused by S. uberis cause the uterus to be sensitive to release PGF2 α during luteal phase and this lead to decrease reproductive efficiency through reducing the luteal phase by decreasing embryo development.

Lescourret *et al.* (17) reported that CM was associated with reduced pregnancy rate during 70 days after service. The causes of cows with CM to get pregnant during this time is might be related to the management decisions not to serve

a cow with CM, or might be related to suppress of ovulation or failure a cow to return to oestrus cycle. This study found that the rate of subclinical mastitis (\geq 200,000 cells/ml) was not different among calving year, season of the year and even parity 1 and 3. However, this study demonstrated that cows with parity 2 had lower incidence rate of subclinical mastitis. The mechanism behind this phenomenon is fully understood, but this is could be due to the low number of data or may be associated with the functions of the leukocytes with increasing the age of the cow.

In accordance with previous studies (17,29-31) they reported that parity increased the incidence of subclinical mastitis in high yielding dairy cows. The present study reported that cows with subclinical mastitis (≥200,000 cells/ml) produced more 305d milk, but the effect was moderate and was not statistically significant. The relationship between subclinical mastitis (ICSCC ≥200,000 cells/ml) and overall reproductive performance in high yielding dairy cows are generally supported by previous works and provide new information in terms the timing of subclinical mastitis. It should be noted that the present study used ICSCC as a proxy for subclinical mastitis, and data for cows with CM are also included in the study. Therefore, this study will represent the results as the association between subclinical mastitis (ICSCC ≥200,000 cells/ml) and reproductive performance.

The present study demonstrated that cows with ICSCC category 3 had significantly longer interval for calving to first service interval and calving to conception interval by about 10 and 25 days compared to ICSCC categories 2 and 1, respectively. In accordance with Santons et al. (22) that cows with subclinical mastitis (as diagnosed by bacteriological sampling) had longer calving-to-conception interval. However, the same study did not find significant effect of subclinical mastitis on either first service pregnancy rate diagnosis or later in lactation (22). The present study reported that cows with subclinical mastitis (ICSCC \geq 200.000 cells/ml) have effect on the number of service per conception. This indicates that subclinical mastitis has a substantial relationship with reproductive performance in high yielding dairy cows. Cows with ICSCC category 3 (ICSCC ≥399.000 cells/ml) had 1.1 more service per conception. Similarly to the previous study Lescourret et al. (17) demonstrated the odds of service leading to pregnancy decreased 18% with increased ICSCC from 200,000 and 399,000 cells/mL, however, the odds were decreased significantly by about 26% in cows with ICSCC >399,000 cells/ml. There are many different mechanisms have been reviewed to clarify the effect of udder health on reproductive outcomes in high yielding dairy cows. These mechanisms are widely studied by Schrick et al. (24), but mostly involve the negative influence of inflammatory mediators on ovarian follicular function (32,33), ovarian activity (5,34) and luteal function (25), embryonic survival in the uterus (35), and the balance between PGF2 α and PGE2 α after conception (36).

In this study, both clinical and subclinical mastitis extended calving to conception interval were could be more associated with ovarian function. It should be noted that the date were used in the this study could be lower than been used in the previous studies for example, study performed by Lescourret *et al.* (17) they used data form the large scale herds in the UK. This study used date from a herd where could be managed more carefully compared with dairy herds of UK as a whole. The sizeable body of work examining the possible mechanisms through which CM and subclinical mastitis could affect fertility outcome in dairy cow already exists, and this could help researchers to determine the suitable methods such as therapy for minimizing the effect of this udder health on dairy cows.

Conclusions

The present study concluded that relationship between both CM and subclinical mastitis and decreased reproductive performance in high yielding dairy cows. Cows with CM and subclinical mastitis need longer time to return their ovarian cyclicity and had longer calving to conception interval with a higher number of services per-conception especially in subclinical mastitis cases. So this study concluded that it is essential to examine cows for both CM and subclinical mastitis during the first 30dpp to reduce the incidence rate mastitis and subclinical mastitis in the herd.

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Conflict of interest

The author declares no conflict of interest

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الارتباط بين التهاب الضرع السريري وعدد الخلايا الجسمية في الحليب والأداء التناسلي في أبقار الحليب العالية الإنتاج في مركز نوتنغهام للألبان

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الخلاصة

يعتبر التهاب الضرع السريري وتحت السريري من الأمراض الشائعة واللذان يسببان انخفاضاً ملحوظاً في الأداء التناسلي في أبقار الحليب في المملكة المتحدة خلال العقد الماضي. لذلك كان الهدف من هذه الدراسة هو تقييم العلاقة بين التهاب الضرع السريري وتحت السريري مع الأداء التناسلي للأبقار ألمنتجة للحليب. تم جمع البيانات من ١٨٤ بقرة حليب من نوع هولشتاين، وتم استخدام الانحدار اللوجستي ذي الحدين لتحديد تأثير كُل من معدل الو لادات، سنة الو لادة و موسم الو لادات على معدل الإصابة بالتهاب الضرع السريري وتحت السريري. كما أستخدم نموذج ألخطى المختلط لتحديد تأثير التهاب الضرع السريري وتحت السريري على الأداء التناسلي. باستخدام الانحدار اللوجستي ذي الحدين تم تقييم العلاقة بين التهاب الصرع السريري أو تحت السريري ونسبة احتمال حصول الحمل من التلقيح الأولى أو الثانية أو الثالثة بالإضافة إلى احتمال الحمل بين ٣٠-٦٠ يومًا بعد الولادة أو ٢١-٩٠ يومًا بعد الولادة. أظهرت النتائج بان حدوث الإصابة بالتهاب الضرع السريري تختلف باختلاف سنوات الولادة، كما بينت أن الأبقار المصابة بالتهاب الضرع السريري أو تحت السريري لها أطول فترة من الولادة إلى فترة التلقيح الأولى مع أطول فترة الحمل. كما بينت النتائج جود انخفاض معدل حصول الحمل في الأبقار التي كانت تعانى من التّهاب الضرع السريري خلال ٢٠-٣٠ يومًا بعد الولادة مقارنة بالأبقار السليمة. كما أظهرت النتائج بان الأبقار التي عندها عدد عالي من الخلايا الجسمية (خصوصاً تلك آلتي تكون فيها الخلايا أكثر من ٣٩٩٠٠٠ خلية/ مل منُ الحليب) عدد أكبر من التلقيحات مقارنة بالأبقار المصابة بالتهاب الضرع تحت السريري والتي لديها عدد أقل من تعداد تلك الخلايا. تستنتج من هذه الدراسة أن كلاً من التهاب الضرع السريري وتحت السريري لُهما علاقة مباشره بانخفاض الأداء التناسلي للأبقار الحلوب