Journal of Dairy Research (2007) **74** 174–179. © *Proprietors of Journal of Dairy Research 2007*
doi:10.1017/S002202990600238X *First published online 17 January 2007 Printed in the United Kingdom*

Cell function in the bovine mammary gland: a preliminary study on interdependence of healthy and infected udder quarters

Roswitha Merle[1,2]*, Anke Schröder[1] and Jörn Hamann[1]

[1] Department of Food Quality and Safety, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, D-30173 Hannover, Germany
[2] WHO Collaborating Centre for Research and Training in Veterinary Public Health, University of Veterinary Medicine Hannover, Foundation, Buenteweg 2, D-30559 Hannover, Germany

Received 26 June 2006 and accepted for publication 28 September 2006

Udder defence mechanisms are not completely explained by current mastitis research. The anatomical construction of the udder implies that infection of one udder quarter does not influence the immune status of neighbouring quarters. To test this hypothesis, we compared the immune reactions of individual udder quarters in response to microbial attacks. In the course of immune reactions, polymorphonuclear leucocytes (PMN) release oxygen radicals, which can be determined by chemiluminescence (CL). Milk from 140 udder quarters of 36 cows was analysed for somatic cell count (SCC), differential cell count, viability and CL activity. Quarters with an SCC <100 000 cells/ml and free of pathogens were defined as uninfected, all other quarters were categorized as infected. Three groups of cows were classified cytologically: group A (healthy, 11 animals, SCC limit <100 000 cells/ml); group B (moderate mastitis, 8 cows, SCC ⩾100 000 and <400 000 cells/ml in at least one quarter); and group C (severe mastitis, 17 cows, SCC ⩾400 000 cells/ml in at least one quarter). Infected and uninfected quarters in groups B and C were analysed separately. Viability of PMN leucocytes was significantly (P=0·0012) lower in group A (72·6%) than in healthy quarters of group C (84·0%). Lowering the SCC limit of healthy quarters to <50 000 cells/ml (group A: all quarters within the udder) revealed striking differences between samples of groups B and C: in addition to varying differential cell counts and viabilities, CL activity of group $B_{<50}$ (2929 CL units/million PMN) was markedly lower than that of the other groups (5616 in group $A_{<50}$ and 6445 CL units/million PMN in group $C_{<50}$). These results allow the conclusion that the infection of one udder quarter influences the cell activity of neighbouring quarters. When the SCC threshold for healthy quarters was reduced to 50 000 cells/ml, greater differences in cell activities were detected between healthy udders and healthy quarters of infected udders.

Keywords: Udder defence activity, bovine PMN, chemiluminescence.

The study of udder immune defence mechanisms has become part of research on subclinical mastitis (Allen et al. 1972; Jain, 1976; Burvenich et al. 1995). Milk contains the following immune cells: polymorphonuclear leucocytes (PMN), lymphocytes and macrophages (Paape et al. 1981; Wever & Emanuelson, 1989; Schröder & Hamann, 2005). These immune cells are differently distributed in normal and mastitic milk, and the presence of PMN seems to play a central role in the defence of the mammary gland (Burvenich et al. 1994; Paape et al. 2002). The main task of PMN is to defend against bacteria at the beginning of an acute inflammatory process (Jain, 1976; Paape et al. 1979; Vangroenweghe et al. 2005). Not only the number of PMN increases enormously, but also their level of defence activity (Targowski, 1983; Craven & Williams, 1985; Paape et al. 2003). PMN ingest particles and bacteria and set off a series of immune reactions (Burvenich et al. 2003). One of these reactions is the oxidative burst (Babior, 1984), including high oxygen consumption (Rossi & Zatti, 1964) and the release of reactive oxygen species like superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Weber et al. 1983; Lein & Paape, 1993; Mehrzad et al. 2001c).

PMN activity can be measured by the chemiluminescence (**CL**) reaction (Allen et al. 1972), which is

*For correspondence; e-mail: roswitha.merle@tiho-hannover.de

Table 1. Cow groups, based on highest quarter SCC. A: all quarters with SCC <100 000 cells/ml (11 cows); B: at least one quarter with SCC 100 000–400 000 cells/ml (8 cows); C: at least one quarter with SCC >400 000 cells/ml (17 cows)

Group	A	B			C	
	$n=44$†	$n=32$†			$n=64$†	
Subgroup	$A_{<100}$	$B_{<100}$	$B_{100–400}$	$C_{<100}$	$C_{100–400}$	$C_{>400}$
	$n=44$†	$n=17$†	$n=15$†	$n=12$†	$n=19$†	$n=33$†
Redefined group	$A_{<50}$	$B_{<50}$		$C_{<50}$		
	$n=28$†	$n=11$†		$n=10$†		

† n = quarters

characterized by the oxidation of H_2O_2 under emission of photons, which are then detected in a luminometer (Allen et al. 1972). The higher the CL activity per million PMN, the stronger the defence reaction. CL is used in human and veterinary medicine and in dairy research, mainly to investigate blood PMN (Lohuis et al. 1990; Hoeben et al. 2000a, b). Some researchers also use PMN isolated from milk to measure CL activity (Weber et al. 1983; Dosogne et al. 1999; Mehrzad et al. 2001b).

Previous studies on udder defence mechanisms have dealt with physiological variations (Mehrzad et al. 2001b) in the respiratory burst activity: *i.e.*, lactation stage (Mehrzad et al. 2002; Piccini et al. 2005), metabolism (Hoeben et al. 1997) and drugs (Paape et al. 1990a, b; Hoeben et al. 2000a). Several studies also focused on changes in the defence activity caused by mastitic events (Lohuis et al. 1990; Hoeben et al. 2000a).

The present study details the udder-health relevant cell activity of PMN from infected quarters, from neighbouring quarters and from quarters of healthy udders. In addition to determining CL activity, we evaluated the somatic cell count (**SCC**), viability and differential cell count from milk samples of healthy, moderately affected and severely infected mammary glands. The goal was to investigate the defence activities in healthy udders, infected and non-infected quarters of infected udders, and to compare the results from these three groups. We expected that the groups would differ in viability, differential cell count and CL activity.

Material and Methods

Study design

Three groups of cows were classified cytobacteriologically: a healthy reference group A (11 animals, SCC limit <100 000 cells/ml, no pathogen, $n=44$ quarters); group B (moderate mastitis, 8 cows, SCC ⩾100 000 and <400 000 cells/ml in at least one quarter); and group C (severe mastitis, 17 cows, SCC ⩾400 000 cells/ml in at least one quarter). Most of the infected quarters of groups B and C were bacteriologically positive, but some quarters had unspecific mastitis on the day of investigation. Group C included four animals with only three quarters that secreted milk, as one quarter was missing. Infected and uninfected quarters of groups B and C were analysed separately (Table 1).

The non-infected quarters of groups B and C (SCC <100 000 cells/ml) were redefined as group $B_{<100}$ ($n=17$) and $C_{<100}$ ($n=12$). Groups $B_{100–400}$ ($n=15$) and $C_{100–400}$ ($n=19$) contained the samples with SCC of 100 000–400 000 cells/ml, while group $C_{>400}$ ($n=33$) contained those from quarters with SCC ⩾400 000 cells/ml. Furthermore, those quarters of groups $B_{<100}$ and $C_{<100}$ that had an SCC <50 000 cells/ml were redefined as group $B_{<50}$ ($n=11$) and group $C_{<50}$ ($n=10$). In group $A_{<50}$, all quarters of the udder contained <50 000 cells/ml ($n=28$).

Animals and milk sampling

This study included 36 cows from two farms. Animals were selected on the basis of results of a 3-week (farm B) pilot survey and on continuous surveillance data (farm A). All animals were in lactation for at least 60 d.

After cleaning and disinfection of the teat, 10 ml foremilk was collected in sterile glass tubes for cytobacteriological analysis. Afterwards 500 ml quarter milk was manually withdrawn in glass bottles for cell isolation.

Cell isolation

Somatic cells from 500 ml milk were isolated according to Schröder et al. (2005). In brief, the samples were diluted in 500 ml phosphate-buffered saline (PBS) and centrifuged 1000 ***g*** at 4 °C for 15 min. The supernatant was carefully removed and the remaining cell pellet was resuspended in PBS and centrifuged three times with decreasing speed (400, 300, 200 ***g***). The resulting cell pellet was stored on ice in 0·5 ml PBS and diluted to $4{\cdot}0\times10^6$ cells/ml if necessary (cells counted by: Haematology Analyzer, Nihon Kohden, Bad Homburg, Germany).

Preparation of a smear and microscopic differential cell count

The smear was prepared with the aid of a modified coffee grinder for smooth distribution of the cells (Schröder et al. 2005). After air drying, the smear was stained with Hemacolor (Merck Eurolab GmbH, Darmstadt, Germany).

https://doi.org/10.1017/S002202990600238X Published online by Cambridge University Press

Table 2. Means ($\bar{x}$) and standard deviations (SD) of different cytological data of the quarter groups A, $B_{<100}$, $B_{100–400}$, $C_{<400}$, $C_{100–400}$, and $C_{>400}$

Group		A	$B_{<100}$	$B_{100–400}$	$C_{<100}$	$C_{100–400}$	$C_{>400}$
Log SCC	$\bar{x}$	^{a}4·31	^{a,b}4·39	^{c}5·30	^{b}4·55	^{c}5·31	^{d}5·91
	SD	0·28	0·36	0·18	0·29	0·18	0·27
PMN, %	$\bar{x}$	^{a}27·2	^{b}45·8	^{b,c}58·7	^{b,c}57·4	^{c,d}71·7	^{d}81·9
	SD	21·9	22·2	24·6	20·9	24·5	13·9
Lym†, %	$\bar{x}$	^{a}23·8	^{a,b}19·1	^{b,c}9·6	^{a,b}17·2	^{b,c}8·5	^{c}4·8
	SD	18·6	10·2	8·1	12·0	9·0	5·3
Mac‡, %	$\bar{x}$	^{a}46·3	^{a,b}32·3	^{a,b}31·2	^{b,c}22·5	^{b,c}19·5	^{c}12·9
	SD	23·2	15·7	21·8	10·1	15·8	9·3
Viability, %	$\bar{x}$	^{a}72·6	^{a,b}77·3	^{a,b}82·7	^{b}84·0	^{b}83·2	^{a,b}82·0
	SD	12·1	10·3	12·1	6·1	9·1	14·2
CL§	$\bar{x}$	^{a,b}5389	^{a}3843	^{b,c}9290	^{a,b,c}7068	^{b,c}10 110	^{b,c}10 541
	SD	6358	3285	3952	6090	5845	5566

† Lym = lymphocytes
‡ Mac = macrophages
§ CL activity/1 million PMN
Values within a row without a common superscript letter indicate significant differences between the groups (Ryan-Einot-Gabriel-Welsch Multiple Range Test, level of significance $P<0·05$)

Oil immersion was used to differentiate 100 cells into PMN, lymphocytes, macrophages and epithelial cells.

Viability

Viability of PMN was analysed by flow cytometry (FACSCalibur, Becton Dickinson, Heidelberg, Germany) using propidium iodide (Sigma Aldrich Chemie GmbH, Steimheim, Germany), which binds to the DNA of membrane-defective cells.

Chemiluminescence

Chemiluminescence was determined in a Luminoscan Ascent luminometer (Labsystems, Helsinki, Finland), which has a sensitivity of <1 fmol ATP per well. A reaction solution was prepared with Dulbecco's PBS containing 666 nmol/ml luminol (5-amino-2,3-dihydro-1,4-phthalazindion; Sigma Aldrich) and 1000 ng/ml PMA (Sigma Aldrich), and adjusted with NaOH to a pH value of 10·0. A white microtitre plate (Nunc GmbH, Wiesbaden, Germany) containing 50 µl cell suspension per well was introduced into the luminometer. The dispenser of the luminometer added 150 µl reaction solution to each well immediately before the detection of light emission was started at 38 °C. Values were recorded at 90-s intervals as relative light units (RLU). After 45 min of measurement, the area under the curve (AUC) was taken as the final result. The study included 12 blind samples without cells and multiple (5–7) samples of each cell suspension, the means of which were determined (SD <5·0%). Results were documented as CL activity/1 million PMN.

Statistical analysis

Statistical tests were conducted with SAS 8e 2002 software (SAS Institute Inc., Cary NC, USA). Different SAS procedures were applied to run Ryan-Einot-Gabriel-Welsch Multiple Range Test, Student's *t* tests and tests based on the Pearson correlation coefficient.

Results

Data for the different groups are shown in Table 2. SCC in healthy quarters of mastitic cows ($C_{<100}$) tended to be higher than in the reference (A), even though the same definition was applied to the three groups on a quarter level (A, $B_{<100}$, $C_{<100}$). The difference in the SCC between the reference (A) and the healthy quarters of cows with severe mastitis ($C_{<100}$) was confirmed statistically ($P<0·05$). This difference between the reference quarters and the results of all other groups became even more distinct in view of the differential cell count. The percentage of PMN was significantly lower in group A than in groups $B_{<100}$ and $C_{<100}$ ($P=0·0058$ and $P<0·0001$, respectively), and the percentage of macrophages differed significantly between the groups A and $C_{<100}$ ($P<0·0001$).

There was a significant difference in viability between the reference (A) and groups $C_{<100}$ and $C_{100–400}$ ($P=0·0012$ and $P<0·0001$, respectively). CL activity was significantly lower in group $B_{<100}$ than in group $B_{100–400}$ ($P=0·0004$). There were no significant differences in CL activities of the groups A, $B_{<100}$ and $C_{<100}$, but SD was high. The correlation coefficient (r) between SCC and PMN was 0·70 ($P<0·0001$); between SCC and macrophages, −0·53 ($P<0·0001$); between SCC and CL activity, 0·42

https://doi.org/10.1017/S002202990600238X Published online by Cambridge University Press

Table 3. Means ($\bar{x}$) and standard deviations (SD) of different cytological data of groups $A_{<50}$, $B_{<50}$, and $C_{<50}$

Group		$A_{<50}$	$B_{<50}$	$C_{<50}$
Log SCC	$\bar{x}$	[a]4·14	[a]4·15	[b]4·44
	SD	0·17	0·16	0·24
PMN, %	$\bar{x}$	[a]22·3	[b]37·3	[c]56·7
	SD	14·6	14·5	24·4
Lym†, %	$\bar{x}$	[a]24·5	[a]21·3	[a]19·3
	SD	20·0	8·4	13·3
Mac‡, %	$\bar{x}$	[a]49·6	[a,b]36·6	[b]23·1
	SD	23·0	14·3	10·9
Viability, %	$\bar{x}$	[a]69·3	[a,b]77·1	[b]83·6
	SD	11·7	10·1	6·2
CL§	$\bar{x}$	[a]5216	[a]2667	[a]6445
	SD	6961	1721	5880

† Lym = Lymphocytes
‡ Mac = Macrophages
§ CL activity/1 million PMN
Values within a row without a common superscript letter indicate significant differences between the groups (Ryan-Einot-Gabriel-Welsch Multiple Range Test, level of significance $P<0·05$)

($P<0·0001$); and between SCC and viability, 0·32 ($P=0·0002$).

Differences in groups $A_{<50}$, $B_{<50}$ and $C_{<50}$ mainly occurred between groups $A_{<50}$ and $C_{<50}$ (Table 3). It has to be stressed that the SCC was different in those groups despite the selection conditions. The percentage of PMN was significantly higher both in groups $B_{<50}$ ($P=0·0087$) and $C_{<50}$ ($P=0·0026$) than in group $A_{<50}$, but the differences in the percentage of macrophages and viability are statistically significant only between groups $A_{<50}$ and $C_{<50}$. Furthermore, it should be noted that the mean CL activity was lowest in group $B_{<50}$ and highest in group $C_{<50}$.

Discussion

Cell functionality was determined for milk of healthy (group A), moderately (group B) and severely diseased (group C) udders, and the results for healthy and infected quarters were interpreted separately. SCC of the healthy udder quarters of diseased animals ($B_{<100}$ and $C_{<100}$) were below the physiological threshold of 100 000 cells/ml as defined by the German Veterinary Society (DVG, 1994). SCC even fell within the physiological range (<50 000 cells/ml) as cited by Doggweiler & Hess (1983). However, SCC in the healthy quarters of mastitic cows was slightly higher than in the reference (A). Experimental udder infections with *Staphylococcus aureus* or *Escherichia coli* also led to a rise in SCC in the adjacent quarters (Wever & Emanuelson, 1989; Burvenich et al. 1994; Leitner et al. 1995).

There were high percentages of PMN in the differential cell count in the diseased quarters of the groups $B_{100–400}$, $C_{100–400}$, and $C_{>400}$. It reflects the knowledge of immune defence mechanisms (Lohuis et al. 1990; Burvenich et al. 1994; Hoeben et al 2000a; Paape et al. 2003) that in the beginning of an acute inflammation PMN flow massively into the affected area.

But also in the non-inflamed quarters in groups $B_{<100}$ and $C_{<100}$ the percentage of PMN in the differential cell count was significantly higher than in the reference (A) ($P=0·0058$ and $P<0·0001$, respectively) (Jain, 1976; Paape et al. 1979; Burvenich et al. 1995). Viability in groups $B_{<100}$ and $C_{<100}$ almost reached the values of their neighbouring diseased quarters (Mehrzad et al. 2004).

Correspondingly, in groups $B_{<50}$ and $C_{<50}$ both PMN content and viability were higher than in group $A_{<50}$, indicating the presence of defence activities. It could not be explained satisfactorily, why the lowest mean CL activity was found in group $B_{<50}$. Group C might have consisted of animals with acute infections and a very effective immune system. Previous investigations have mainly dealt with acute or peracute infections and report very high cell activities in the infected udder quarters (Burvenich et al. 1994; Leitner et al. 1995). As no other comparisons between healthy animals and healthy and infected udder quarters have yet been published, further research is necessary to clarify the phenomenon observed here.

There were striking differences between healthy quarters of the groups $B_{<100}$ and $C_{<100}$ and neighbouring diseased quarters, not only in SCC and differential cell count but also in functional properties like viability and CL activity. CL activity of group $B_{100–400}$ was significantly higher than that of group $B_{<100}$ ($P=0·0004$). This could be seen as support for the common assumption that the quarters within an udder react independently. But it might also be an indication that milk from diseased udder quarters essentially contains more mediators, which generate influx and activation of immune cells even if the general defence situation is bad (Jain & Lasmanis, 1978; Guidry et al. 1980; Paape et al. 1981; Riollet et al. 2000). The non-infected quarters even had lower CL activities than the quarters of group A, so that it seems that the high immune reactions are at the expense of the immune protection of the non-infected quarters.

The view that the four quarters of the bovine mammary gland act as independent units is apparently based on the anatomical structure of the mammary gland. Nevertheless, there are some publications which clearly show interactions between udder quarters. Interdependent reactions have been determined between four udder quarters for milk yield (Woolford, 1985; Hamann & Reichmuth, 1991), for the growth of gland tissue (Knight & Peaker, 1991), for milk cell count (Hamann & Gyodi, 1994) and for milk constituents (Hamann et al. 1998; Hamann, 2002). Thus, it seems plausible that udder quarters are interdependent in the CL response and in udder health status. Contradictory results of other studies cannot be used for a direct comparison with ours, mainly because in those studies completely different definitions of udder health were used. Although many experiments have been

https://doi.org/10.1017/S002202990600238X Published online by Cambridge University Press

conducted in recent years, the intention of the experiments and therefore their design differed. Most researchers deal with the peracute phase of experimental infections, *e.g.*, with *Esch. coli* or endotoxin (Lohuis et al. 1999; Mehrzad et al. 2001a, 2004), the functional activity during early lactation (Vangroenweghe et al. 2001; Mehrzad et al. 2002; Piccinini et al. 2005), or with the influence of certain drugs on cell functional activity (Paape et al. 1990a, b). Investigations comparable to our study design found similar results regarding, *e.g.*, the strong correlations between SCC and functional parameters (Lilius & Pesonen, 1990).

Correlations between SCC and differential cell count (PMN, macrophages) were more distinct than correlations between SCC and functional parameters viability and CL activity. However, SCC does not seem to describe completely the defence activities within the udder. To estimate the cellular defence during infectious events in the udder, the determination of functional parameters is recommended.

The results of our study regarding both the differential cell count and the viability of the cells indicate that udder quarters do not seem to act independently but are influenced by infections of neighbouring quarters. These observations cannot be explained by the results of other studies. Our findings thus contradict the results of Wever & Emanuelson (1989), who found no evidence of the interdependence of udder quarters during their investigations of differential cell counts of milk cell suspensions.

Our present results suggest that milk from healthy udders and from infected and uninfected quarters differs in their cell functionality. A similar effect has been reported for milk components in the case of a mastitic event (Hamann, 2002). Those results indicate that the infection of one udder quarter possibly influences the cell activity of neighbouring quarters. When the SCC threshold for the definition of a healthy quarter was reduced to 50 000 cells/ml, the effects observed here became even more distinct.

This study was sponsored by Boehringer Ingelheim Vetmedica GmbH.

References

Allen RC, Stjernholm RL & Steele RH 1972 Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. *Biochemical and Biophysical Research Communications* **47** 679–684

Babior BM 1984 Oxidants from phagocytes: agents of defense and destruction. *Blood* **64** 959–966

Burvenich C, Guidry AJ & Paape MJ 1995 Natural defense mechanisms of the lactating and dry mammary gland. *Proceedings 3rd IDF International Mastitis Seminar, Tel Aviv, Israel* pp. 3–13

Burvenich C, Paape MJ, Hill AW, Guidry AJ, Miller RH, Heyneman R, Kremer WDJ & Brand A 1994 Role of the neutrophil leukocyte in the local and systemic reactions during experimentally induced *E. coli* mastitis in cows immediately after calving. *Veterinary Quarterly* **16** 45–49

Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A & Duchateau L 2003 Severity of *E. coli* mastitis is mainly determined by cow factors. *Veterinary Research* **34** 521–564

Craven C & Williams MR 1985 Defences of the bovine mammary gland against infection and prospects for their enhancement. *Veterinary Immunology and Immunopathology* **10** 71–127

Deutsche Veterinärmedizinische Gesellschaft (DVG) 1994 Guidelines to combat bovine mastitis on farm level 3rd Edn. Giessen, Germany: German Veterinary Association

Doggweiler R & Hess E 1983 Somatic cell count in the milk of undamaged udders. *Milchwissenschaft* **38** 5–8

Dosogne H, Burvenich C, Freeman AE, Kehrli Jr ME, Detilleux JC, Sulon J, Beckers J-F & Hoeben D 1999 Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. *Veterinary Immunology and Immunopathology* **67** 47–54

Guidry AJ, Paape MJ, Pearson RE & Williams WF 1980 Effect of local immunization of the mammary gland on phagocytosis and intracellular kill of *Staphylococcus aureus* by polymorphonuclear neutrophils. *American Journal of Veterinary Research* **41** 1427–1431

Hamann J 2002 Relationships between somatic cell count and milk composition. Proceedings Animal Health Conference IDF World Dairy Summit 2001, Auckland, New Zealand. *IDF Bulletin* **372** 56–59

Hamann J & Gyodi P 1994 Effects on milk yield, somatic cell count and milk conductivity of short-term non-milking of lactating quarters of cows. *Journal of Dairy Research* **61** 317–322

Hamann J, Gyodi P, Krömker V & Stahlhut-Klipp H 1998 Physiological variation of milk components in bovine udder quarters with special regard to milking frequency. *Proceedings 10th International Conference on Production Diseases in Farm Animals, Utrecht, The Netherlands* p. 313

Hamann J & Reichmuth J 1990 Exogenous influences on the somatic cell count of milk in consideration of the udder's state of health. *Milchwissenschaft* **45** 286–290

Hamann J, Schröder A & Merle R 2005 Differential cell count and interdependence of udder quarters. *Proceedings 4th IDF International Mastitis Conference, Maastricht, The Nederlands* pp. 190–195

Hoeben D, Heyneman R & Burvenich C 1997 Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. *Veterinary Immunology and Immunopathology* **58** 165–170

Hoeben D, Monfardini E, Burvenich C & Hamann J 2000a Treatment of acute *Escherichia coli* mastitis in cows with enrofloxacin: effect on clinical signs and chemiluminescence of circulating neutrophils. *Journal of Dairy Research* **67** 485–502

Hoeben D, Monfardini E, Opsomer G, Burvenich C, Dosogne H, de Kruif A & Beckers J-F 2000b Chemiluminescence of bovine polymorphonuclear leucocytes during the periparturient period and relation with metabolic markers and bovine pregnancy-associated glycoprotein. *Journal of Dairy Research* **67** 249–259

Jain NC 1976 Neutrophil leukocytes and inflammation of the bovine mammary gland. *Theriogenology* **6** 153–173

Jain NC & Lasmanis J 1977 Phagocytosis of serum-resistant and serum-sensitive coliform bacteria (Klebsiella) by bovine neutrophils from blood and mastitic milk. *American Journal of Veterinary Research* **39** 425–427

Knight CM & Peaker M 1991 Mammary gland physiology. *Flemish Veterinary Journal* **62** Suppl. 1 33–42

Leino L & Paape MJ 1993 Comparison of the chemiluminescence responses of bovine neutrophils to differently opsonized zymosan particles. *American Journal of Veterinary Research* **54** 1055–1059

Leitner G, Shoshani E, Berman A, Trinin Z, Feingold D, Winkler M, Shpigel NY & Saran A 1995 Immune response of the mammary gland in dairy cows after experimental infection with *E. coli* or *Staph. aureus*. *Proceedings 3rd IDF International Mastitis Seminar, Tel Aviv, Israel* pp. 39–44

Lilius E-M & Pesonen U 1990 Use of inflammatory cell activities in bovine milk to diagnose mastitis. *American Journal of Veterinay Research* **51** 1527–1533

Lohuis JACM, Schukken YH, Henricks PAJ, Heyneman R, Burvenich C, Verheijden JHM, van Miert ASJPAM & Brand A 1990 Preinfection functions of blood polymorphonuclear leukocytes and the outcome of experimental *Escherichia coli* mastitis in the cow. *Journal of Dairy Science* **73** 342–350

Mehrzad J, Dosogne H, Meyer E & Burvenich C 2001a Local and systemic effects of endotoxin mastitis on the chemiluminescence of milk and blood neutrophils in dairy cows. *Journal of Veterinary Research* **32** 131–144

Mehrzad J, Dosogne H, Meyer E, Heyneman R & Burvenich C 2001b Respiratory burst activity of blood and milk neutrophils in dairy cows during different stages of lactation. *Journal of Dairy Research* **68** 399–415

Mehrzad J, Dosogne H, Vangroenweghe F & Burvenich C 2001c A comparative study of bovine blood and milk neutrophil functions with luminol-dependent chemiluminescence. *Luminescence* **16** 343–356

Mehrzad J, Duchateau L & Burvenich C 2004 Viability of milk neutrophils and severity of bovine coliform mastitis. *Journal of Dairy Science* **87** 4150–4162

Mehrzad J, Duchateau L, Pyörälä S & Burvenich C 2002 Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition and early lactation. *Journal of Dairy Science* **85** 3268–3276

Paape MJ, Bannerman DD, Zhao X & Lee JW 2003 The bovine neutrophil: Structure and function in blood and milk. *Veterinary Research* **34** 597–627

Paape MJ, Mehrzad J, Zhao X, Detilleux J & Burvenich C 2002 Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. *Journal of the Mammary Gland Biology and Neoplasia* **7** 109–121

Paape MJ, Miller RH & Ziv G 1990a Effects of florfenicol, chloramphenicol and thiamphenicol on phagocytosis, chemiluminescence and morphology of bovine polymorphonuclear neutrophil leukocytes. *Journal of Dairy Science* **73** 1734–1744

Paape MJ, Nickerson SC & Ziv G 1990b In vivo effects of chloramphenicol, tetracycline, and gentamicin on bovine neutrophil function and morphologic features. *American Journal of Veterinary Research* **51** 1055–1061

Paape MJ, Wergin WP, Guidry AJ & Pearson RE 1979 Leukocytes – second line of defense against invading mastitis pathogens. *Journal of Dairy Science* **62** 135–153

Paape MJ, Wergin WP, Guidry AJ & Schultze WD 1981 Phagocytic defense of the ruminant mammary gland. *Advances in Experimental Medicine and Biology* **137** 555–578

Piccinini R, Binda E, Belotti M, Casirani G & Zecconi A 2005 Comparison of blood and milk non-specific immune parameters in heifers after calving in relation to udder health. *Veterinary Research* **36** 747–757

Riollet C, Rainard P & Poutrel B 2000 Cells and cytokines in inflammatory secretions of bovine mammary gland. *Advances in Experimental Medicine and Biology* **480** 247–258

Rossi F & Zatti M 1964 Changes in the metabolic pattern of polymorphonuclear leucocytes during phagocytosis. *British Journal of Experimental Pathology* **45** 549–559

Schröder A & Hamann J 2005 Influence of technical factors on the differential cell count in milk. *Journal of Dairy Research* **72** 153–158

Targowski SP 1983 Role of immune factors in protection of mammary gland. *Journal of Dairy Science* **66** 1781–1789

Vangroenweghe F, Dosogne H, Mehrzad J & Burvenich C 2001 Effect of milk sampling techniques on milk composition, bacterial contamination, viability and function of resident cells in milk. *Journal of Veterinary Research* **32** 565–579

Vangroenweghe F, Lamote I & Burvenich C 2005 Physiology of the periparturient period and its relation to severity of clinical mastitis. *Domestic Animal Endocrinology* **29** 283–293

Weber L, Peterhans E & Wyler R 1983 The chemiluminescent response of bovine polymorphonuclear leucocytes isolated from milk and blood. *Veterinary Immunology and Immunopathology* **4** 397–412

Wever P & Emanuelson U 1989 Effects of systematic influences and intramammary infection on differential and total somatic cell counts in quarter milk samples from dairy cows. *Acta Veterinaria Scandinavia* **30** 465–474

Woolford MW 1985 The relationship between mastitis and milk yield. *Kieler milchwirtschaftliche Forschungsberichte* **37** 224–233

https://doi.org/10.1017/S002202990600238X Published online by Cambridge University Press