ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 146

Coliform Mastitis in the Sow

Clinical Immunological studies around parturition

Ingrid Österlundh

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

Coliform mastitis in the sow.

Clinical immunological studies around parturition.

Ingrid Österlundh

Akademisk avhandling som för vinnande av veterinärmedicine doktorsexamen kommer att offentligen försvaras i Ettans föreläsningssal, Klinikcentrum, Ultuna, fredagen den 24 januari 2003, kl. 09.15. Av fakultetsnämnden utsedd opponent: Professor Wolfgang Leibold, School of Veterinary Medicine, Hannover, Tyskland.

Abstract

In the sow, acute coliform mastitis is the major cause of dysgalactia at parturition, a period when normal milk production is paramount for the survival of newborn piglets. Polymorphonuclear leukocytes (PMN) that phagocytose and kill bacteria is the first line of defence by the immune system against *E. coli* infection in the mammary gland. The overall aim of this thesis was to elucidate this aspect of the sow immune system around parturition in order to gain a better background understanding of the disease.

The course of events regarding the number and function of leukocytes in the blood circulation and locally in the mammary gland was described in healthy sows around parturition. At parturition, PMN reached a peak in both blood and mammary secretion. The phagocytic capacity per PMN showed no change, nor did opsonic activity in serum, while the opsonic activity in mammary secretion increased after parturition. The sum effect of the variations on mammary gland defence mechanisms is not clear. The concentrations of oestradiol-17β and cortisol were determined, and variations were seen in blood and mammary secretion.

Directed migration and phagocytosis of PMN in colostrum and milk were compared. Chemoattractant properties of the two types of secretion were similar, while the phagocytic capacity of PMN was lower in colostrum, which may make sows more susceptible to coliform mastitis during the early postparturient period. The concentrations of oestradiol-17β and cortisol were greater in colostrum.

In a study of experimentally induced mastitis, prepartum opsonic activity in serum and functional capacity of PMN in the blood circulation were compared between sows that remained clinically healthy and sows that developed coliform mastitis after intramammary *E. coli* inoculation at parturition. The pre-inoculation chemotaxis, phagocytosis and expression of CD18 adhesion molecules did not differ between the two groups of sows. In contrast, the pre-inoculation opsonisation of *E. coli* tended to be better in serum from non-affected sows. Thus specific opsonins likely contribute to local protection of the mammary gland. Possibly, prepartum opsonic activity in serum can be used to predict predisposition to develop clinical mastitis in the peripartal sow.

To conclude, no clear-cut evidence for a markedly depressed function of PMN was found in the mammary gland at parturition. No impaired functional capacity of PMN in the circulation was found before parturition in sows that developed mastitis after inoculation. However, the same sows had less opsonic activity for *E. coli* in serum before inoculation than non-affected sows, which might contribute to a susceptibility to clinical coliform mastitis.

Keywords: sow, mammary gland, leukocyte, neutrophil, granulocyte functions, coliform mastitis, *E. coli*, parturition, oestradiol-17β, cortisol, experimental mastitis

Distribution:
Swedish University of Agricultural Sciences
Department of Obstetrics and Gynaecology
SE-750 07 Uppsala, Sweden

Uppsala 2003
ISSN 1401-6257
ISBN 91-576-6363-7

Coliform Mastitis in the Sow

Clinical immunological studies around parturition

Ingrid Österlundh
Department of Obstetrics and Gynaecology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Veterinaria 146

ISSN 1401-6257
ISBN 91-576-6363-7

Tryck: SLU Service/Repro, Uppsala 2003

To my parents

Margareta and Carl-Gustaf

Abstract

Österlundh, I. 2003. *Coliform mastitis in the sow. Clinical immunological studies around parturition.*
Doctor's dissertation
ISSN 1401-6257, ISBN 91-576-6363-7

In the sow, acute coliform mastitis is the major cause of dysgalactia at parturition, a period when normal milk production is paramount for the survival of newborn piglets. Polymorphonuclear leukocytes (PMN) that phagocytose and kill bacteria is the first line of defence by the immune system against *E. coli* infection in the mammary gland. The overall aim of this thesis was to elucidate this aspect of the sow immune system around parturition in order to gain a better background understanding of the disease.

The course of events regarding the number and function of leukocytes in the blood circulation and locally in the mammary gland was described in healthy sows around parturition. At parturition, PMN reached a peak in both blood and mammary secretion. The phagocytic capacity per PMN showed no change, nor did opsonic activity in serum, while the opsonic activity in mammary secretion increased after parturition. The sum effect of the variations on mammary gland defence mechanisms is not clear. The concentrations of oestradiol-17β and cortisol were determined, and variations were seen in blood and mammary secretion.

Directed migration and phagocytosis of PMN in colostrum and milk were compared. Chemoattractant properties of the two types of secretion were similar, while the phagocytic capacity of PMN was lower in colostrum, which may make sows more susceptible to coliform mastitis during the early postparturient period. The concentrations of oestradiol-17β and cortisol were greater in colostrum.

In a study of experimentally induced mastitis, prepartum opsonic activity in serum and functional capacity of PMN in the blood circulation were compared between sows that remained clinically healthy and sows that developed coliform mastitis after intramammary *E. coli* inoculation at parturition. The pre-inoculation chemotaxis, phagocytosis and expression of CD18 adhesion molecules did not differ between the two groups of sows. In contrast, the pre-inoculation opsonisation of *E. coli* tended to be better in serum from non-affected sows. Thus specific opsonins likely contribute to local protection of the mammary gland. Possibly, prepartum opsonic activity in serum can be used to predict predisposition to develop clinical mastitis in the peripartal sow.

To conclude, no clear-cut evidence for a markedly depressed function of PMN was found in the mammary gland at parturition. No impaired functional capacity of PMN in the circulation was found before parturition in sows that developed mastitis after inoculation. However, the same sows had less opsonic activity for *E. coli* in serum before inoculation than non-affected sows, which might contribute to a susceptibility to clinical coliform mastitis.

Keywords: sow, mammary gland, leukocyte, neutrophil, granulocyte functions, coliform mastitis, *E. coli*, parturition, oestradiol-17β, cortisol, experimental mastitis

Author's address: Ingrid Österlundh, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Centre for Reproductive Biology, SLU, PO Box 7039, SE-750 07 Uppsala, Sweden

Contents

Appendix

Papers I – IV

The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Österlundh, I., Holst, H., & Magnusson, U. 1998 Hormonal and immunological changes in blood and mammary secretion in the sow at parturition.
Theriogenology 50, 465-477.

II. Österlundh, I., Holst, H., & Magnusson, U. 2001 Effect of mammary secretions on functions of polymorphonuclear leukocytes in pigs.
American Journal of Veterinary Research 62, 1250-1254.

III. Österlundh, I., Hultén, F., Johannisson, A. & Magnusson, U. 2002 Sows intramammarily inoculated with *Escherichia coli* at parturition: I. Functional capacity of granulocytes in sows affected or non-affected by clinical mastitis.
Veterinary Immunology and Immunopathology 90, 35-44.

IV. Österlundh, I. & Magnusson, U. Can the opsonic activity in serum be used to predict the resistance to clinical mastitis in the peripartal sow?
Manuscript submitted for publication.

Abbreviations

BLAD	bovine leukocyte adhesion deficiency
C3b	opsonic fragment of complement factor 3
CD	cluster of differentiation
CM	coliform mastitis
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetra-acetic acid
FITC	fluorescein isothiocyanate
KRG	Krebs Ringer buffer
ME	metabolisable energy
MJ	megajoule
PBS	phosphate buffered saline
PMN	polymorphonuclear leukocyte
PPDS	postpartum dysgalactia syndrome
WBC	white blood cell (leukocyte)

Introduction

Background

Normal milk production in the newly farrowed sow is of vital importance for the survival of newborn piglets. The major cause of disturbance in milk production during the early peripartal period is coliform mastitis, part of postpartum dysgalactia syndrome (**PPDS**). Coliform mastitis is caused by various strains of *Escherichia coli* (***E. coli***) that infect the mammary gland. The development of clinical mastitis is restricted to the first days after parturition. Why the disease occurs only during this limited period is not known.

Physiological changes in the sow during parturition are considerable, and a variety of hormones play a regulating role. The mediating hormones are for instance relaxin, oxytocin, and the steroid hormones progesterone, oestrogens and cortisol. A close relationship exists between reproduction regulating hormones and the immune system in several species (review Grossman, 1984). A rapid local immune response is needed to defend the mammary gland against bacteria. It can be hypothesised that part of the aetiology of coliform mastitis is an impaired immune defence in the mammary gland in the peripartum period.

Aspects of coliform mastitis in the sow

Coliform mastitis (**CM**) plays a major role in PPDS (Bertschinger, 1999), the most common acute disease in Swedish sows. Coliform mastitis occurs worldwide, and although it has long been recognised in pig practice in Sweden (Ringarp, 1960; Hermansson *et al.*, 1978) and other countries (Jorsal, 1986; Wegmann, Bertschinger & Jecklin, 1986) with intensive pig production, the aetiology has not been fully revealed.

The milk production in diseased sows might be half that of healthy sows, and the piglets suffer as well as the dam (Ross *et al.*, 1975; Bertschinger, 1999). The first clinical symptoms of "farrowing fever" are increased temperature and listlessness. The sow also prefers sternal recumbency and loses interest in the piglets. Consumption of feed and water is reduced. Although severe cases exist in which sows are stiff, dizzy and even comatose, the mortality rate of affected sows is low and the disease is self-curing. In general the symptoms do not last more than 2 to 3 days. The behaviour of the piglets is an early indication of the disease, dysgalactia and starvation result in restless, squeaky piglets that try to suck frequently but still look thin (Bertschinger, 1999). Observed systemic changes in the sow are the result of endotoxin production (Bertschinger, 1999) and the ensuing release of acute-phase mediators in the mammary gland (Tizard, 2000). Only some affected mammary glands can be detected by clinical examination (Persson, Pedersen Mörner & Kuhl, 1996b) and subclinical CM can be present in apparently healthy sows (Pedersen Mörner, 2001). It has been proposed that puerperal mastitis is under-diagnosed in the field and that the impact on milk

production and performance of offspring may be underestimated (Pedersen Mörner, 2001).

The average incidence for PPDS in Sweden was reported to be 12.8% in 1978, with a variation among individual herds from 0.5% to 50% (Hermansson *et al.*, 1978), while an extensive Danish study including 80,000 farrowings in 1986, yielded an incidence of 9.5% of farrowings (Jorsal, 1986). The incidence in Sweden at present may approximate 5 to 10% of sows, while individual herds exist with a considerably higher incidence (personal communication). In a long-term Swedish study a group of 78 sows was followed through several lactations, more than half of the sows were diagnosed with PPDS, while the incidence was 21% calculated on number of farrowings (Persson *et al.*, 1989). Special investigations are required to give the true incidence of coliform infection in diseased sows, for example bacteriological examination of colostrum (Wegmann, Bertschinger & Jecklin, 1986), or necropsies to determine gross lesions of mastitis (Ross *et al.*, 1981). Even though the term "coliform" covers the bacterial genera *Escherichia, Klebsiella, Enterobacter*, and *Citrobacter,* the organisms most often identified are *E. coli* (Bertschinger, 1999).

The route of infection is likely galactogenous (Pedersen Mörner, Faris & Krovacek, 1998). The origin of strains may be the flora of the sow or environmental, and a vast number of different *E. coli* strains have been identified (Bertschinger, 1999). Only one common virulence factor, serum resistance, was identified in *E. coli* isolates recovered from milk of sows with coliform mastitis, this was found in 42 out of 44 (95%) of investigated strains (Pedersen Mörner, Faris & Krovacek, 1998). Serum resistance is considered an important virulence factor since it allows bacteria to evade the bactericidal activity of serum complement. In the mammary gland, the bacteria are located in the ductular and alveolar lumina, with little surface adherence (Bertschinger, 1999). In a sow with CM, the influx of neutrophils to the mammary gland is massive. The time at which spontaneous bacterial invasion into the mammary gland occurs is not known, as bacteria can be found both before and after parturition. Neither is it known whether mammary glands are more susceptible to infection at a certain time period.

Preventive measures for CM include feeding regimens and good hygiene in the pen. Bedding material should be checked if cases of CM accumulate, since high bacterial contamination of teats is undesirable. Drastic reduction of the feeding ration shortly before parturition is a widespread practice (Bertschinger, 1999). It has been suggested that the reduced feed intake is effective through reduced contamination of the lying area. The belief that overly high feeding ration close to parturition is a risk factor is long-standing in Sweden (Persson *et al.*, 1989) and feeding recommendations have been adjusted accordingly. The current recommendation for a sow of 160 to 200 kg is a daily ration of 33–35 MJ metabolisable energy (ME), 8 g crude protein per MJ ME, during the last 3 weeks of gestation (Simonsson, 1994).

There are few scientific reports on induction of specific immunity in the sow for CM, and the vast variety of bacterial strains identified indicates that achieving immunity might be problematic, as does the observation that the mammary glands do not appear to develop local resistance against subsequent infections (Bertschinger & Bühlmann, 1990; Bertschinger, 1999). On the other hand, cows can be immunised for CM by *E. coli* J5 vaccin (review Dosogne, Vangroenweghe & Burvenich, 2002), while vaccination of sows against urinary tract infections can increase overall lactation performance (Pejsak, Tarasuik & Jochle, 1988). Thus the prospect of systemic immunisation as a way to prevent CM in sows is yet uncertain.

In Sweden, the symptomatic treatment of sows with the diagnosis PPDS commonly includes oxytocin, chemotherapeutics, and sometimes anti-inflammatory drugs. The piglets need supportive care. Sows in Sweden farrow approximately 2,2 times a year and average litter size is 11 to 12 piglets.

The farrowing sow

The lenght of gestation in the sow is 112 to 116 days. Considerable physiological changes occur during farrowing, regulated by a variety of hormones, such as relaxin, oxytocin, and the steroid hormones progesterone, oestrogens and cortisol (Britt, Almond & Flowers, 1999). Blood concentrations of oestrogens increase from about day 80 of gestation to a peak value prior to parturition, whereupon they rapidly decrease to reach basal levels by 72 hr after parturition. Blood concentration of progesterone remains fairly constant throughout pregnancy until a decline begins approximately 2 to 3 weeks before parturition (Molokwu & Wagner, 1973; Robertson & King, 1974; Baldwin & Stabenfeldt, 1975).

In close connection with farrowing, the polytocous sow must quickly produce copious amounts of milk to meet the needs of all piglets (Klopfenstein, Farmer & Martineau, 1999). While hardly any mammary secretion can be obtained before parturition, around parturition large amounts are suddenly present, a sign of approaching parturition. The swift onset is due to increased synthesis of milk components by the epithelial cells and to a serum transudate leaking from the circulation through junctions in the mammary epithelium. The withdrawal of progesterone is considered to be the hormonal signal that initiates the increased synthesis of milk components, and prolactin also plays a key role in lactation in the sow as in other species (Klopfenstein, Farmer & Martineau, 1999). Hormonal data regarding the sow mammary gland and its secretions are scarce.

In the sow, several immunological parameters in the blood changes in the peripartum period, some of which may indicate immune depression. Despite the increase in total number of blood leukocytes and polymorphonuclear leukocytes (**PMN**) at parturition, blood concentration of both lymphocytes and active, phagocytic monocytes decrease and reach their lowest number the day before parturition (Magnusson & Fossum, 1988). The number of Ig-bearing monocytes at parturition is half that present 3 days earlier, and a decline in serum levels of gammaglobulin is seen during the weeks up to parturition, followed by a regain

after parturition (Magnusson & Fossum, 1988, 1990). As very few studies of the immune defence in the sow mammary gland have been performed, it is uncertain how the local immune defence is affected and whether or not changes in the blood parallel those in other body compartments, such as the mammary gland (Tsuma & Magnusson, 1992; Magnusson & Greko, 1998).

There is a close relationship between the reproduction-regulating hormones and the immune system in several species (review Grossman, 1984). In the cow, physiological changes in oestradiol and progesterone blood values during the normal oestrus cycle are associated with alterations in PMN function (Roth *et al.*, 1983). In the pig, *in vivo* and *in vitro* studies have shown that oestrogens can modulate some immune functions, such as number and phagocytic capacity of leukocytes (Magnusson & Einarsson, 1990; Magnusson, 1991; Magnusson & Fossum, 1992). Immune parameters in pigs are also influenced by genetic variation, and estimated heritabilities are sufficiently high to allow for genetic selection (Edfors-Lilja *et al.*, 1994; Vaiman, Chardon & Rothschild, 1998).

The incidence of diseases increases in the peripartal period for many animals. In the cow some immune defence mechanisms are depressed for more than a month around calving, and depressed neutrophil functions are associated with pariparturient disorders (Cai *et al.*, 1994; Detilleux *et al.*, 1995; Mallard *et al.*, 1998). It is uncertain whether the hormonal fluctuations in the peripartal period are responsible for the altered immune responsiveness (review Mallard *et al.*, 1998). However, the endocrine network are fundamental in regulation of the normal function of the immune system (review Burvenich *et al.*, 1999). In the sow, susceptibility to experimental *E. coli* infection has been associated with impaired function of blood PMN (Löfstedt *et al.*, 1983), the cause of which is still unclear.

Polymorphonuclear leukocytes are of vital importance in the defence against bacterial infection, and lack or dysfunction of PMN can lead to recurrent and life-threatening infections (Abramson & Wheeler, 1993; Bogomolski-Yahalom & Matzner, 1995). One example is the congenital neutrophil disorder leukocyte adhesion deficiency found in humans (Abramson & Wheeler, 1993), dogs, and cattle. Known as BLAD in the bovine species, this lethal defect inherited as a recessive trait in Holstein cattle causes retarded growth and recurrent infections due to a lack of CD18 expression of leukocytes (Gerardi, 1996).

Neutrophils and the immune system

Some general aspects of the immune system, inflammation, and neutrophils are described below to give a general background of the studies in the present thesis and the *in vitro* methods that were used to assess neutrophil functions and to estimate defence against coliform mastitis.

The survival of an animal depends on the successful defence of the body against microbial invasion (Tizard, 2000). The immune system, which comprises several interlinked defence mechanisms, can be divided into non-specific, innate

immunity and adaptive, specific immunity. Immune responses are produced primarily by leukocytes of several types (Roitt, Brostoff & Male, 2001).

Acquired, adaptive immune responses are highly specific for a particular pathogen, conferring memory that inhibits or reduces disease on subsequent exposure to the infectious agent (*cf.* Tizard, 2000; *cf.* Roitt, Brostoff & Male, 2001). Lymphocytes are wholly responsible for the specific recognition of pathogens and central to all adaptive immune responses. B-lymphocytes produce antibodies, whereas T-lymphocytes have a wide range of activities where some destroy virus-infected or abnormal cells and others interact with B-lymphocytes and antigen-presenting phagocytic cells through cytokines. Most immune responses to infectious organisms are made up of a variety of innate and adaptive immune responses.

The innate, non-specific immune system with complement and phagocytic cells form the first line of defence against microorganisms when physical barriers have been overcome. Phagocytic leukocytes, *i.e.* monocytes, tissue macrophages and polymorphonuclear neutrophils, bind to invading microorganisms, internalise them, and kill them (*cf.* Roitt, Brostoff & Male, 2001). The complement system is a group of about 20 serum proteins whose overall function is the control of inflammation. Inflammation is a process characterised by increased blood supply, increased capillary permeability, and migration of leukocytes, where the innate defence mechanisms are united and focused on a site of infection to prevent the spread of infection in the body (Tizard, 2000). The majority of leukocytes during early inflammation are neutrophils. Later on, lymphocytes start to generate adaptive immune responses.

In the adult pig, neutrophils constitute about 40% and lymphocytes about 50% of the total leukocyte count of 11–22 x 10^9 cells per litre. Neutrophils are the major constituent of the granulocyte population, another denomination of polymorphonuclear leukocytes. Neutrophils, as do all leukocytes, originate from stem cells in the bone marrow, where they differentiate, mature, and are stored as a pool of band and mature neutrophils (*cf.* Abramson & Wheeler, 1993). From the bone marrow the cells egress to the blood stream where they constitute two pools of approximately the same size, the freely circulating cell pool and the marginated pool of neutrophils that slowly roll along the endothelium in small blood vessels. When the rolling cells reach an area of tissue damage or inflammation, they enter the tissue. Neutrophils released from the bone marrow have a lifespan of a few days. During bacterial infections, the number of circulating neutrophils is multiplied by increased release from the bone marrow and by demargination.

The process by which neutrophils eliminate invaders through phagocytosis can be divided into four stages: chemotaxis, adherence, ingestion, and digestion (*cf.* Tizard, 2000; *cf.* Roitt, Brostoff & Male, 2001). Impairment in any stage will lead to an increased susceptibility to bacterial infections. Within minutes after an inflammatory stimulus, neutrophils will be recruited from the circulation. Selectins and integrins, adhesive proteins expressed by blood vessel endothelial cells activated by an inflammatory stimulus, bind to receptors on the neutrophils. The

neutrophils initiate a directed migration into the tissue, called chemotaxis, towards chemotactic molecules released by injured tissue and by bacteria. At the site of infection, the neutrophils must bind to the bacteria. For efficient binding, the bacteria need to be opsonised, coated with molecules that promote phagocytosis, primarily antibodies and the complement protein C3b. The neutrophils then adhere to the bacteria via their specific opsonin receptors, and the bacteria can be engulfed. Finally, the ingested bacteria are destroyed, either by oxidising radicals or by lytic enzymes from intracellular granules. Oxidising radicals are generated in a process called the respiratory burst. When antibody binding triggers the neutrophils, their oxygen consumption is radically increased to produce bactericidal products, such as hydrogen peroxide and hypochlorite ions.

Both neutrophils and macrophages can be "activated", for example the cytokine interferon-γ greatly enhances the respiratory burst, and the capacity for chemotaxis and killing of bacteria. In order to interact with the environment, cells need many different surface receptors. For neutrophils, the most relevant are receptors for opsonins (CD35, CD11b/CD18, CD32) and receptors that mediate cell attachment, integrins (CD11a/CD18, CD11c/CD18) (Tizard, 2000).

Study rationales

Susceptibility to, or a successful defence against, a bacterial infection in the mammary gland is dependent both on the virulence of the bacterial strain involved, and the ability of the immune system to raise a fast and efficient response. The first line of defence by the immune system against *E. coli* bacteria in the mammary gland is by the PMN, and the process of inflammation (Müller-Eberhard, 1989). Regarding the sow, literature on PMN function in the blood circulation in general, and in the mammary gland in particular, is scarce (Evans et al., 1982; Tsuma & Magnusson, 1992; Magnusson & Greko, 1998). Thus, it can be hypothesised that the susceptibility of some sows to *E. coli* infection in the peripartal period can be explained partly by an insufficient number of vital PMN in the mammary gland. The studies in this thesis were performed to clarify whether or not this is the case.

Aims

The overall aim of the present work was to elucidate some aspects of immune defence in the sow around parturition, with special focus on the mammary gland, to gain a better background understanding to the development of clinical coliform mastitis at this time. The specific aims were to

- map the course of events with regard to 2 steroid hormones, and number and function of leukocytes in blood and mammary gland secretion, in healthy sows around parturition,

- compare the function of neutrophils in colostrum and normal mammary gland secretion,

- compare prepartum neutrophil functions in sows that developed clinical coliform mastitis and sows that remained clinically healthy after intramammary *E. coli* inoculation at parturition, and to

- establish suitable *in vitro* methods to evaluate neutrophil function in the pig.

Methodological considerations

General design of the experiments

The thesis comprises 3 studies: a longitudinal study on healthy sows around parturition (Paper I); a study in which the effect of colostrum and milk on neutrophil function was assessed *in vitro* (Paper II); and a study in which clinical coliform mastitis was induced by inoculation. Prepartum neutrophil functions were evaluated in sows that developed clinical coliform mastitis or remained clinically healthy after inoculation at parturtion (Paper III–IV).

The materials and methods used are described in detail in the specific papers. Comments of a general nature follow below.

Animals

The sows used in the longitudinal study (Paper I) and in the inoculation study (Paper III–IV) were all primiparous crossbred sows (Swedish Landrace x Swedish Yorkshire). They were purchased from a commercial herd and arrived at the clinic four at a time, 5 to 6 weeks before parturition to ensure that they were healthy and acclimatised to the environment at the time of farrowing. The sows were housed at the clinic in individual pens with straw bedding and fed according to the Swedish feeding ration, *i.e.* 3.0 kg pellets per day. In the inoculation study (Paper III–IV), the ration was increased to 3.5 kg pellets per day approximately 2 weeks before parturition to make the sows more susceptible for mastitis. All sows farrowed naturally. The longitudinal study comprised a total of 8 animals divided into 2 batches, while the inoculation study comprised 16 animals divided into 4 batches.

In the *in vitro* study (Paper II), mammary secretion was collected from Yorkshire sows of different parities, housed at the University Research Centre Funbo-Lövsta outside Uppsala.

To assess the test samples, leukocytes were needed in some of the *in vitro* assays (Paper I, II & IV). In these cases, blood samples were collected from female pigs over 6 months of age, not included elsewhere in the studies. The pooled pig serum used as a standard or control sample in assays for granulocyte functions, was also prepared from blood samples collected from adult non-lactating female pigs.

The Ethical Committee for Animal Experiments, Uppsala, Sweden, approved the experimental designs.

Sample collection and processing

Blood samples were collected in all studies (Paper I–IV), and mammary secretion samples were collected in the first two studies (Paper I–II).

In the longitudinal study (Paper I), blood samples were collected through a silastic catheter inserted in the jugular vein. In the other studies the samples were collected by jugular venipuncture into tubes containing heparin, EDTA, or no

additive. The catheter facilitated the collection of blood samples during an extended period, while venipuncture was chosen to minimise the risk of premature neutrophil activation prior to assessing neutrophil function, as well as to minimise the risk of contamination from the catheter. After collection, the blood samples were further processed for use the same day, or were centrifuged, frozen, and stored as serum or plasma until assayed.

The mammary secretion samples were collected in as sterile a manner as possible and either processed to retrieve milk cell suspensions for use the same day or centrifuged to yield cell-free samples. These were frozen and stored as whole or fat-depleted samples until assayed. All mammary secretion samples were bacteriologically examined via culture on blood agar, and none had bacteriological indications of infection.

Total and differential cell counts

The total number of white blood cells (**WBC**) per litre was counted with an automatic blood cell analyser, Sysmex F 800 (TOA, Kobe, Japan), and differential WBC counts were determined by manual counting of stained blood smears, in the longitudinal study (Paper I). The total number of milk cells per litre in mammary secretions was counted on stained smears, while differential milk cell counts were carried out by fluorescence microscopy.

In the inoculation study (Paper III), both the total number of WBC per litre and the differential WBC counts were determined with an automatic blood cell analyser, Cell-Dyn 3500 (Abbot, Wiesbaden, Germany), adjusted for pig blood.

Three methods for assessing neutrophil function *in vitro*

One of the specific aims of this work was to establish suitable *in vitro* methods for studying neutrophil function in the pig. Several methods have been previously used in other species, each with their own advantages and disadvantages. Below is a brief description and account of the methods selected for the studies in this thesis to assess chemotaxis, phagocytic capacity, opsonic activity, and expression of surface adhesion molecules of PMN.

Phagocytosis and opsonisation assay

A chemiluminometer can be used to determine the oxidative metabolic activity of neutrophils. The respiratory burst generates a photon emission, a light signal that magnified by luminescent substances such as luminol or lucigenin can be recorded (Steele, 1991). The assay requires live phagocytic cells and a target particle, and yields a relative measure either of phagocytic capacity of leukocytes in a sample, or inversely, of the opsonic activity in a sample for a specific target particle.

An ML 3000 luminometer (Dynatech Laboratories, In Vitro, Solna, Sweden), representing a method based on luminol-enhanced chemiluminescence, was used to assess phagocytic capacity of neutrophils (Paper I–III), and opsonic activity in serum (Paper I & IV) and mammary secretion samples (Paper I). The assay had

previously been adapted for pig samples, but in these studies some additional modifications were developed.

To assess phagocytic capacity for neutrophils in the circulation, whole blood samples were used in the first study. In the other studies, a granulocyte suspension was prepared by separating polymorphonuclear cells from the mononuclear cells and platelets by centrifugation through a low-viscosity medium (Ficoll-paque, Pharmacia-Biotech, Uppsala, Sweden). The protocol used for whole blood samples (Paper I) was well established (Magnusson & Einarsson, 1990; Magnusson & Holst, 1998). In the following studies (Paper II–III) we aimed to distinguish the abilities of granulocytes in the samples from other whole blood components. In addition, the quenching effect of erythrocytes in whole blood samples was avoided. The number of cells in the purified granulocyte suspension was standardised, diluted to a concentration of 5×10^6 cells/ml. The same granulocyte suspension was used in the cell migration assay.

To support the vitality of the cells, buffers of a slightly different composition were used: phosphate buffered saline (PBS), Gays solution, and Krebs ringer buffer. The phagocytic capacity of cells in the milk was assessed in a cell suspension prepared from the mammary secretion samples (Paper I).

Two different target particles, zymosan and *E. coli*, were used for comparison in the assay, since it was previously known that the opsonisation may vary for different targets. Zymosan (Paper I–IV) is a commonly used target particle in *in vitro* assays, while *E. coli* (Paper II & IV) represented a pathogenic microbe.

When the opsonic activity in serum and fat-depleted samples from mammary secretion was assessed leukocytes were needed, and blood samples were thus collected from sows not included in the studies. The leukocyte suspension was prepared by centrifugation of the blood samples, collection of the buffy coat containing leukocytes, and dilution of these cells to a concentration of 5×10^6 cells/ml. While assessing the serum opsonic activity in Paper IV, leukocytes from two different sows were used for each test sample to reduce the possibility of a single sow related effect of the leukocytes interacting with the test sample values.

Luminol-enhanced chemiluminescence in a 96-well plate was regarded a suitable method for detailed examination of granulocytes in whole blood and mammary secretion samples, with the advantage of enabling examination of several samples in the same assay (Steele, 1991).

Cell migration assay

The capacity of neutrophils for directed migration, chemotaxis, was measured in a 48-well chemotaxis chamber (Neuro Probe Inc, Cabin John, MD, USA). In this assay, a chemoattractant is added to wells below a semipermeable filter and a granulocyte suspension is added to wells above the filter. The whole chemotaxis chamber is incubated while the cells are allowed to migrate within the filter against a gradient of chemotactic molecules towards the bottom wells. The

distance of the cells' directed migration is then measured by use of a light microscope after staining of the filter. In the second study, I established this method for use in pigs by determining a suitable concentration for the granulocyte suspension, adjusting the incubation time, and developing a staining protocol for the cellulose nitrate filter. The method was used to assess the chemoattractant properties of sow colostrum and milk (Paper II) and to measure the pre-inoculation directed migration of neutrophils collected from sows that after inoculation at parturtion developed clinical disease or remained clinically healthy (Paper III).

The 48-well chemotaxis chamber was regarded a suitable tool for examination of granulocyte migration where several samples together with controls could be included in the same assay (Falk, Goodwin & Leonard, 1980; Richards & McCollough 1984). In addition, small sample volumes were required and the granulocyte suspension could be prepared in the same way for use in both the chemotaxis chamber and in the chemiluminometer (Paper II–III).

Assay to assess expression of adhesion molecules

Flow cytometry is a technology used for measurement of soluble substances or properties of cells in suspensions. In cell analysis, flow cytometry can yield information about relative size, internal complexity and fluorescence intensity. Thousands of cells stained either with fluorescent dyes or fluorophore-labelled antibodies pass the light source, a laser beam, one by one, and values for forward scatter (size), side scatter (internal complexity), and fluorescence intensity are measured. In this way cells can be analysed for several different properties such as morphology, surface antigens and proteins, DNA and RNA content, and phagocytic and killing abilities. Leukocyte traits can thus be differentiated by this method.

Analysis of the expression of CD18 molecules on the granulocytes was conducted by a FACStarPLUS flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) in the inoculation study (Paper III). The procedure used here was based on immunofluorescence, with monoclonal antibodies specific for CD18, and secondary antibodies labelled with fluorescein isothiocyanate (FITC) fluorescence. A leukocyte suspension diluted to a concentration of 5 x 10^6 cells/ml was derived from the buffy coat of centrifuged blood samples, after which the cell suspension was handled on ice during the antibody labelling procedure. The labelling protocol was adapted for pig leukocytes in connection with the study. A minimum of 30,000 cells from each sample were registred.

Flow cytometry was regarded a suitable method to measure expression of CD18 adhesion molecules on granulocytes in a heterogenous cell suspension in a rapid and sensitive way (Herzenberg, De Rosa & Herzenberg, 2000).

With these three *in vitro* methods, two of which were established specifically for the studies in this thesis, I could pinpoint three different, vital steps in neutrophil recruitment to and phagocytosis ability at a site of infection.

Steroid hormone determinations

The concentration of steroid hormones was determined in all studies. The purposes were twofold, to describe and compare the concentrations in the blood circulation and mammary secretion around farrowing (Paper I), and in colostrum and milk (Paper II), and if possible correlate hormonal concentration to neutrophil activity (Paper I–III).

The plasma concentrations of oestradiol-17β, progesterone and cortisol were determined by commercial kits, solid phase I^{125} radioimmunoassays Coat-A-Count Estradiol, Coat-A-Count Progesterone, and Coat-A-Count Cortisol, respectively (Diagnostic Products Corporation, Los Angeles, CA, USA). For oestradiol-17β and cortisol, the assays has been previously validated for pig plasma and were used in all studies (Paper I–III), while the assay for determination of progesterone concentration, used in the inoculation study (Paper III), had to be validated for pig plasma in connection with this study.

The concentrations of oestradiol-17β and cortisol were determined in mammary secretion (Paper I–II) using the same commercial kits. The assay for oestradiol-17β was validated for sows both for fat-depleted (Paper I) and whole milk samples (Paper II). The double validation was performed to ensure a parallelism between the two types of samples, and thus to confirm the suitability of fat-depleted samples for steroid hormone analysis, irrespective of the high lipophilic properties of the butterfat pool for unconjugated steroid hormones.

Bacterial suspensions

A bacterial suspension of the characterised *E. coli* strain (serotype O 127) was prepared from a frozen stock culture for the *in vitro* assays (Paper II & IV), and for the intramammary inoculation for Paper III. The strain of *E. coli* was originally isolated from sow milk from a field case investigation.

While needed, the bacterial suspension was prepared daily from a fresh overnight culture and diluted to a standardised concentration, based on the specific optical density measured in a spectrophotometer. The concentration of live bacteria in the suspensions used for inoculation in the mammary glands was confirmed retrospectively by the viable count method.

Clinical assessment of disease

In the third study, we used a model developed at the clinic (Magnusson *et al.*, 2001) to induce clinical coliform mastitis (Paper III). The sows were categorised as affected or un-affected by clinical mastitis at the end of the experimental period, based on clinical examination performed at least once daily. The different clinical observations taken together were habitus, appetite, rectal temperature (>39.5°C),

and thorough palpation of each mammary gland. After being confirmed clinically ill, all 4 sows with coliform mastitis received chemotherapeutic treatment (trimetoprimsulfoxide) and 2 sows received complementary anti-inflammatory treatment (ketoprofen).

Statistical analyses

The data were analysed by using the Statistical Analysis Systems software package (SAS Institute Inc, Cary, NC, USA, 1996). When data were not normally distributed, they were transformed to logarithms to achieve normality, though for clarity these data were converted to anti-logarithmic values before graphical presentation. Analysis of variance of the data from the longitudinal study (Paper I) and the inoculation study (Paper III) were analysed by least squares using the general linear model. To allow repeated observations on the same animal, an animal term was fitted to the model. The paired Students *t*-test was used to compare data between groups in the *in vitro* study (Paper II). Correlations between steroid hormone concentrations and granulocyte traits in the inoculation study (Paper III) were analysed using the correlation procedure.

Results and discussion

The general purpose of all studies included in this thesis was to increase knowledge on immune defence mechanisms in the sow mammary gland in the search for preventive measures for clinical coliform mastitis. The overall hypothesis was that a temporary impairment of the immune defence at farrowing and especially of neutrophil functions plays a role in development of disease. An insufficient number of functional neutrophils in the mammary gland may explain partly why some sows are susceptible to coliform mastitis. Thus, the studies in this thesis were focused on neutrophil function in sows around parturition, assessed in blood and mammary secretions, and in pre-inoculation blood samples from sows that after inoculation developed coliform mastitis or remained clinically healthy. In addition, steroid hormone concentrations around parturition were determined. Few scientific studies have been previously published in this field regarding sows.

Changes in neutrophil functions around parturition

In the first study, the purpose was to map possible changes in the leukocyte population in blood and mammary secretion in healthy sows around farrowing (Paper I). The sampling period comprised 6 days, from 3 days before to 3 days after farrowing, thus both colostrum and regular milk were collected. In blood, the number of PMN increased at parturition, which is in agreement with a previous study (Magnusson & Fossum, 1990), phagocytic capacity per PMN did not change, nor did serum opsonic activity. Therefore, since the concentration of PMN peaked at farrowing and phagocytic capacity per PMN was unchanged the total phagocytic capacity of blood PMN was rather increased than impaired.

Also in mammary secretion the highest concentration of PMN was seen at parturition, and no change was seen in phagocytic capacity per PMN. Opsonic activity in mammary secretion was at the lowest at farrowing and then increased. How these variations effect mammary gland defence mechanisms is not clear, if high concentration of PMN in mammary secretion at farrowing counterbalance a somewhat lower opsonic activity the sum effect may be similar during the first days after parturition. To summarise, a difference in opsonic activity was seen between blood and mammary secretion, but no evidence of impaired immune defence based on *in vitro* assessment was seen in any of the body compartments at parturition in the healthy sows.

In the second study, the purpose was to find out if neutrophils were differently affected by mammary secretions: if colostrum collected at a time when some sows develop coliform mastitis was a more adverse environment for neutrophils than milk collected at a time when sows do not develop clinical coliform mastitis (Paper II). Colostrum and milk samples were collected from sows 2 weeks apart, and the effect of mammary secretions on PMN functions was compared using *in vitro* assays for directed migration and phagocytic capacity.

Chemoattractant properties of the 2 types of mammary secretion *in vitro* were similar and even better than that of the pooled serum control. In the sow it may imply that neutrophils in the blood circulation readily migrate to colostrum and milk even in the absence of chemotactic factors induced by bacteria. The most prominent cell type in colostrum in the sow is PMN, which constitutes approximately 60–70% of the total cell count (Evans et al., 1982; Persson, Pedersen Mörner & Kuhl, 1996a). Two weeks after farrowing the concentration of PMN has declined to approximately 40–50% of the total cell count in milk. The reason to the decline is not likely related to chemoattractant properties.

Phagocytic capacity was lower in colostrum than in milk, which may make sows more susceptible to coliform mastitis during the early postparturient period.

In cows in the peripartum period it has been shown that functions of milk PMN are more impaired than those of blood PMN (Mehrzad *et al.*, 2001). Fat and casein in cow milk can reduce phagocytosis and oxidative burst activity (Dulin, Paape & Nickerson, 1988; Cooray, 1996), and these neutrophil functions are also directly affected by diapedesis across mammary epithelium (Smits *et al.*, 1999). In the sow, a similar effect by mammary secretion on PMN capability is likely to be found, however, it would not explain the difference seen in PMN phagocytosis between colostrum and milk in this study.

To summarise the studies on changes in neutrophil function: the total PMN capacity seems not impaired in blood or in mammary gland secretion in healthy sows peripartum (Paper I). Chemoattractant properties of colostrum and milk were similar, but colostrum seems to hamper PMN phagocytosis (Paper II). Hence, the hypothesis that number of *functional* neutrophils may be insufficient in the mammary gland at farrowing was supported to a certain extent. However, the hypothesis that immune defence in the sow temporary is impaired in the peripartum period was not supported by the results in the studies.

Neutrophil function and predisposition to develop clinical disease

In the inoculation study, the purpose was to compare prepartum immune defence related to neutrophils between sows that after inoculation at parturition developed coliform mastitis and sow that remained clinically healthy (Paper III–IV). Blood samples were collected some days before parturition, and an *E. coli* suspension was inoculated in the mammary glands just before parturition. The sows were categorised in 2 groups after completed experimental period, one category of sows that developed clinical coliform mastitis within 48 h after farrowing, and one category of sows that remained clinically healthy. Pre-inoculation PMN traits were assessed by 3 methods.

Prepartum chemotaxis, phagocytosis and expression of CD18 adhesion molecules of PMN in the blood circulation did not differ between the 2 groups of sows (Paper III). Thus, no difference could be found in PMN traits before farrowing between sows that were to develop disease and those who did not.

Impaired blood PMN functions have been associated with susceptibility to mastitis in an experimental study of coliform mastitis in sows (Löfstedt *et al.*, 1983). Iodination, which is dependent on the oxidative burst, and random migration under agarose was decreased in PMN from sows that had developed mastitis after inoculation/farrowing. No difference was detected before farrowing in blood PMN functions neither in sows from a "susceptible herd" nor in sows from a "resistant herd", which is in agreement with our results.

The reason for the impaired PMN function in the study by Löfstedt *et al.*, (1983) was not clear. However, in the susceptible herd had even non-inoculated sows decreased iodination after farrowing, which was not the case in the resistant herd. Plasma cortisol concentrations 3 and 7 days after farrowing were higher in diseased sows than in non-inoculated sows from the susceptible herd, and higher than in sows from the resistant herd.

It is interesting to compare methods and results in the vast literature on bovine coliform mastitis, although species differences have to be considered. Is it documented in cow that both innate and acquired immune defence mechanisms are at their lowest from 3 weeks precalving to 3 weeks postcalving (Cai *et al.*, 1994; Detilleux *et al.*, 1995; Meglia *et al.*, 2001). Differences in blood neutrophil function between cows with or without peripartal *E. coli* mastitis have been observed (Kehrli, Nonnecke & Roth, 1989), and the key importance of functional host-defence mechanisms for the course of coliform infection in the udder have been reviewed (Kremer, Noordhuizen-Stassen & Lohuis, 1990).

In the cow, pre-inoculation PMN oxidative burst activity has been found to correlate with high susceptibility to experimental *E. coli* mastitis (Lohuis *et al.*, 1990). Preinfection directed migration, but not preinfection chemiluminescense, has been found to be predictive for severity of *E. coli* mastitis (Kremer *et al.*, 1993). Characteristics of PMN responded differently to mastitis depending on the severity of the disease, and a complexity in alteration of PMN functions during mastitis could be demonstrated in cow (Dosogne *et al.*, 1997).

In Paper IV, prepartum opsonic activity was assessed in pre-inoculation serum samples for zymosan and *E. coli.* Opsonic activity for zymosan was similar between sows that developed clinical coliform mastitis and sows that remained clinically healthy after inoculation. Interestingly, the serum opsonic activity for *E. coli* tended to be lower in sows that developed mastitis. It is likely that specific antibodies for *E. coli* in the blood circulation contributed to local protection of the mammary gland. Hence, a high opsonic activity in serum prepartum may predict that the sow is less predisposed to develop clinical coliform mastitis at farrowing.

If the variation in antibody concentration was induced by previous *E. coli* infections rather than constitutional, this may imply that immunisation is a possible way to prevent disease in herds of sows with a high incidence of coliform mastitis, as has been shown for cows (review Dosogne H, Vangroenweghe F, Burvenich C. 2002). However, the difference between the groups of sows in our study had low significance and the individual sow had a significant effect on

variation in opsonic activity. Thus, the finding needs to be confirmed by further studies before any definite conclusion can be drawn.

Variation in opsonic and anti-adhesive activity in colostrum has been seen between sows that had acquired natural immunity to K88-positive *E. coli* and sows that had not (Sellwood, 1982, 1984).

To summarise the studies on neutrophil function and predisposition to develop clinical disease: no difference in pre-inoculation PMN traits was seen between sows that developed clinical coliform mastitis and sows that remained clinically healthy after inoculation. However, diseased sows had a tendency to less serum opsonic activity for *E. coli* before inoculation, which might contribute to susceptibility to clinical coliform mastitis in the peripartum period.

Influence of hormones on neutrophil function

Changes in concentrations of 2 reproduction-regulating hormones were followed around farrowing (Paper I). Hormonal concentrations were determined in all studies in an attempt to correlate hormonal concentration to neutrophil function (Paper I–III).

An oestradiol-17β concentration of approximately 2000 pmol/L in plasma before farrowing decreased below 200 pmol/L immediately after parturition. Plasma cortisol concentration showed a diurnal rhythm with higher concentrations in the morning. A cortisol peak occurred concomitant with expulsion of the foetuses, whereafter the diurnal rhythm was resumed. The concentrations of both hormones were lower in mammary secretion than in plasma. Changes seen in mammary secretion were parallel to those seen in the blood circulation (Paper I). Concentrations of oestradiol-17β and cortisol were greater in colostrum than in milk (Paper II). In the inoculation study no difference in prepartum plasma concentrations of oestradiol-17β, cortisol, or progesterone was seen between the 2 groups of sows, whereas an effect of the individual animal was seen for all 3 hormones (Paper III).

No correlations were established between hormone concentration and PMN functions in any of the studies, however, the studies comprised few animals. To be able to establish clear correlations between these parameters it is likely that a larger number of animals are necessary.

To conclude, the hypothesis of depressed immune defence in the mammary gland related to neutrophils is not supported by the results in this thesis. Based on the different functions examined, it is suggested that it is unlikely that depressed neutrophil function is the cause of increased susceptibility to coliform mastitis in crossbred sows at parturition.

Conclusions

The hypothesis of a depressed immune defence in the mammary gland regarding neutrophil functions is not supported by the results in this thesis.

- In healthy sows, the results do not show an impaired total phagocytic capacity of neutrophils in either blood or mammary secretion, at or shortly after parturition.

- Colostrum seems to be a more adverse environment for neutrophils than normal milk resulting in an impaired phagocytic capacity of neutrophils in colostrum. The colostrum is collected at a time when coliform is likely to develop and its hampering of neutrophil phagocytosis may contribute to a higher susceptibility to coliform mastitis during the early post partum period.

- Changes in plasma concentration of oestradiol-17β and cortisol in the peripartum period are paralleled in mammary gland secretion in sows.

- In sows that developed clinical coliform mastitis after intramammary inoculation of *E. coli* at parturition
  - prepartum capacity of neutrophils, as measured by chemotaxis, phagocytosis, and expression of CD18 adhesion molecules, in the peripheral circulation, was not lower compared to sows that did not develop clinical mastitis.
  - prepartum opsonic activity of serum for *E. coli* tended to be lower, compared to sows that did not develop clinical coliform mastitis. This indicates that specific antibodies in the blood circulation contributed to local protection of the mammary gland and that high opsonic activity in serum before parturition may be predictive of a lower risk to develop clinical coliform mastitis in peripartal sows.

- The *in vitro* methods used in this study were shown to be suitable to evaluate neutrophil function in the pig.

References

Abramson, J.S. & Wheeler, J.G. (eds.) 1993. *The neutrophil.* Oxford University Press. Oxford. 306 pp.

Baldwin, D.M. & Stabenfeldt, G.H. 1975. Endocrine changes in the pig during late pregnancy, parturition and lactation. *Biology of Reproduction 12*, 508-515.

Bertschinger, H.U. 1999. Coliform mastitis. In Straw, B.E., D'Allaire, S., Mengeling, W.L. & Taylor, D.J. (eds) *Diseases of Swine*. Ames: Iowa State University Press. Ames, IA. 457-464.

Bertschinger, H.U. & Bühlmann, A. 1990. Absence of protective immunity in mammary glands after experimentally induced coliform mastitis. *Proceedings 11th International Pig Veterinary Society Congress,* 175.

Britt, J.H., Almond, G.W. & Flowers, W.L. 1999. Diseases of the reproductive system. In Straw, B.E., D'Allaire, S., Mengeling, W.L. & Taylor, D.J. (eds) *Diseases of Swine.* Ames: Iowa State University Press. Ames, IA. 883-911.

Bogomolski-Yahalom, V. & Matzner, Y. 1995. Disorders of neutrophil function. *Blood Reviews 9*, 183-190.

Burvenich et al., 1999 Burvenich, C., Paape, M.J., Hoeben, D., Dosogne, H., Massart-Leën, A.M. & Blum, J. 1999. Modulation of the inflammatory reaction and neutrophil defense of the bovine lactating mammary gland by growth hormone. *Domestic Animal Endocrinology 17*, 149-159.

Cai, T.-Q., Weston, P.G., Lund, L.A., Brodie, B., McKenna, D.J. & Wagner, W.C. 1994. Association between neutrophil functions and periparturient disorders in cows. *American Journal of Veterinary Research 55*, 934-943.

Cooray, R. 1996. Casein effects on the myeloperoxidase-mediated oxygen-dependent bactericidal activity of bovine neutrophils. *Veterinary Immunology and Immunopathology 51*, 55–65.

Detilleux, J.C., Kehrli, M.E., Stable, J.R., Freeman, A.E. & Kelley, D.H. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Veterinary Immunology and Immunopathology 44*, 251-267.

Dosogne, H., Vangroenweghe, F. & Burvenich, C. 2002. Potential mechanism of action of J5 vaccine in protection against bovine coliform mastitis. *Veterinary Research 33*, 1-12.

Dosogne, H., Burvenich, C., van Werven, T., Roets, E., Noordhuizen, E.N. & Goddeeris, B. 1997. Increased surface expression of CD11b receptors on polymorphonuclear leukocytes is not sufficient to sustain phagocytosis during Esherichia coli mastitis in early postpartum dairy cows. *Veterinary Immunology and Immunopathology 60*, 47-59.

Dulin, A.M., Paape, M.J. & Nickerson, S.C. 1988. Comparison of phagocytosis and chemiluminescence by blood and mammary gland neutrophils from multiparous and nulliparous cows. *American Journal of Veterinary Research 49,* 172–177.

Edfors-Lilja, I,, Wattrang, E., Magnusson, U, & Fossum, C. 1994. Genetic variation in parameters reflecting immune competence of swine *Veterinary Immunology and Immunopathology 40,* 1-16.

Evans, P.A., Newby, T.J., Stokes, C.R. & Bourne, F.J. 1982. A study of cells in the mammary secretions of sows *Veterinary Immunology and Immunopathology 3*, 515-527.

Falk, W., Goodwin, R.H. & Leonard, E.J. 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *Journal of Immunological Methods 33*, 239-247.

Gerardi, A.S. 1996. Bovine leucocyte adhesion deficiency: a review of a modern disease and its implications. *Research in Veterinary Science* 61, 183-186.

Grossman, C.J., 1984. Regulation of the immune system by sex steroids. *Endocrine Reviews 5*, 435-455.

Hermansson, I., Einarsson, S., Larsson, K. & Bäckström, L. 1978. On the agalactia post partum in the sow. *Nordisk Veterinärmedicin 30*, 465-473.

Herzenberg, L.A., De Rosa, S.C. & Herzenberg, L.A. 2000. Monoclonal antibodies and the FACS: complementary tools for immunobiology and medicine. *Immunology Today 21,* 383-390.

Jorsal, S.E. 1986. Epidemiology of the MMA-syndrome, a field survey in Danish sow herds. *Proceedings 9th International Pig Veterinary Society Congress,* Barcelona, Spain. 93.

Kehrli, M.E., Nonnecke, B.J. & Roth, J.A. 1989. Alternations in bovine neutrophil function during the pariparturient period. *American Journal of Veterinary Research 50,* 207-214.

Klopfenstein, C., Farmer, C. & Martineau, G.P. 1999. Diseases of the mammary glands and lactation problems. In Straw, B.E., D'Allaire, S., Mengeling, W.L. & Taylor, D.J. (eds) *Diseases of Swine.* Ames: Iowa State University Press. Ames, IA. 833-860.

Kremer, W.D.J., Noordhuizen-Stassen, E.N. & Lohuis, J.A.C.M. 1990. Host defence and bovine coliform mastitis. *The Veterinary Quarterly 12,* 103-113.

Kremer, W.D.J., Noordhuizen-Stassen, E.N., Grommers, F.J., Daemen, A.J.J.M., Henricks, P.A.J., Brand, A. & Burvenich, C. 1993. Preinfection chemotactic response of blood polymorphonuclear leukocytes to predict severity of Esherichia coli mastitis. *Journal of Dairy Science 76,* 1568-1574.

Lohuis, J.A.C.M., Schukken, Y.H., Henricks, P.A.J., Heyneman, R., Burvenich, C., Verheijden, J.H.M., van Miert, A.S.J.P.A.M. & 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.

Löfstedt, J., Roth, J.A., Ross, R.F. & Wagner, W.C. 1983. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Esherichia coli* mastitis in sows. *American Journal of Veterinary Research 44,* 1224-1228.

Magnusson, U. 1991. In vitro effects of prepartum concentrations of oestradiol-17β on cell-mediated immunity and phagocytosis by porcine leukocytes. *Veterinary Immunology and Immunopathology 28,* 117-126.

Magnusson, U. & Einarsson, S. 1990. Effects of exogenous oestradiol on the number and functional capacity of circulating mononuclear and polymorphonuclear leukocytes in the sow. *Veterinary Immunology and Immunopathology 25,* 235-247.

Magnusson, U. & Fossum, C. 1988. Variations in number and functional capacity of blood mononuclear cells during the peripartal period in the gilt. *Journal of Veterinary Medicine B Infectious Diseases, Veterinary Public Health 35,* 570–578

Magnusson, U. & Fossum, C. 1990. Numerical variations among blood mononuclear cells during the peripartal period in the gilt. *Journal of Veterinary Medicine B Infectious Diseases, Veterinary Public Health 37,* 459–467.

Magnusson, U. & Fossum, C. 1992. Effect of estradiol-17β treatment of gilts on blood mononuclear cell functions in vitro. *American Journal of Veterinary Research 53,* 1427-1430.

Magnusson, U. & Greko, C. 1998. Assessment of phagocytic capacity and opsonic activity in blood and mammary secretions during lactation in sows. *Journal of Veterinary Medicine B 45,* 353-361.

Magnusson, U. & Holst, H. 1998. Assaying granulocyte phagocytosis by chemiluminescence: Effect of storage time and temperature of blood samples. *Journal of Veterinary Medicine B 45,* 217-222.

Magnusson, U., Pedersen Mörner, A., Persson, A., Karlstam, E., Sternberg, S. & Kindahl, H. 2001. Sows intramammarily inoculated with *Escherichia coli*: Influence of time of infection, hormone concentrations and leukocyte numbers on development of disease. *Journal of Veterinary Medicine B 48,* 501-512.

Mallard, B.A., Dekkers, J.C., Ireland, M.J., Leslie, K.E., Sharif, S., Lacey Vankampen, C., Wagter, L. & Wilkie, B.N. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *Journal of Dairy Science 81,* 585-595.

Meglia, G., Johannisson, A., Petersson, L. & Waller, KP. 2001. Changes in some blood micronutrients, leukocytes and neutrophil expression of adhesion molecules in periparturient dairy cows. *Acta Veterinaria Scandinavica 42,* 139-150.

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

Molokwu, E.C.I. & Wagner, W.C. 1973. Endocrine physiology of the puerperal sow. *Journal of Animal Science 36,* 1158-1163.;

Müller-Eberhard, H.J. 1989. Innate immunity. *Current Opinion in Immunology 2,* 3-4.

Pedersen Mörner, A.E. 2001. *A clinical and bacteriological study of the puerperal Escherichia coli mastitis in the sow.* Licentiate thesis, ISBN 91-576-6013-1, SLU, Uppsala, 31 pp.

Pedersen Mörner, A., Faris, A. & Krovacek, K. 1998. Virulence determinants of *Escherichia coli* isolated from the milk of sows with coliform mastitis. *Journal of Veterinary Medicine B 45,* 287-295.

Pejsak, Z., Tarasuik, K. & Jochle, W. 1988. Immunoprophylaxis against MMA and/or CM in sows with a vaccine against urinary tract infections (Urovac). *Proceedings 10th International Pig Veterinary Society Congress,* 307.

Persson, A., Pedersen Mörner, A. & Kuhl, W. 1996a. A long-term study on the health status and performance of sows on different feed allowances during late pregnancy. II. The total cell count and its percentage of polymorphonuclear leucocytes in pathogen-free colostrum and milk collected from clinically health sows. *Acta Veterinaria Scandinavica 37,* 279–291.

Persson, A., Pedersen Mörner, A. & Kuhl, W. 1996b. A long term study on the health status and performance of sows on different feed allowances during late pregnancy. III. Esherichia coli and other bacteria, total cell content, polymorphonuclear leukocytes and pH in colostrum and milk during the first 3 weeks of lactation. *Acta Veterinaria Scandinavica 37,* 293-313.

Persson, A., Pedersen, A.E., Göransson, L. & Kuhl, W. 1989. A long term study on the health status and performance of sows on different feed allowances during late pregnancy. I. Clinical observations, with special reference to agalactia post partum. *Acta Veterinaria Scandinavica 30,* 9-17.

Richards, K.L. & McCollough, J. 1983. A modified microchamber method for chemotaxis and chemokinesis. *Immunological Communications 13,* 49–62.

Ringarp, N., 1960. *Clinical and experimental investigations into a post-parturient syndrome with agalactia in sows.* Acta Agriculture Scandinavica Supplementum 7. 166 pp.

Robertson, H.A. & King, G.J. 1974. Plasma concentrations of progesterone, oestrone, oestradiol-17β and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. *Journal of Reproduction and Fertility 40,* 133-141.

Roitt, I., Brostoff, J. & Male, D. 2001. *Immunology.* 6th ed. Mosby, London. 480 pp.

Ross, R.F., Zimmerman, B.J., Wagner, W.C. & Cox, D.F. 1975. A field study on coliform mastitis in sows. *Journal of American Veterinary Medicine Association 167,* 231-235.

Ross, R.F., Orning, A.P., Woods, R.D., Zimmerman, B.J., Cox, D.F. & Harris, D.L. 1881. Bacteriological study of sow agalactia. *American Journal of Veterinary Research 42,* 949-955.

Roth, J.A., Kaeberle, M.L., Appell, L.H. & Nachreiner, R.F. 1983. Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. *American Journal of Veterinary Research 44,* 247-253.

Sellwood, R. 1982. *Escherichia coli*-associated porcine neonatal diarrhea: Antibacterial activities of colostrum from genetically susceptible and resistant sows. *Infection and Immunity 35,* 396-401.

Sellwood, R. 1984. Inhibition of adhesion of Escherichia coli K88 antigen by mammary secretions of susceptible and resistant sows. *Veterinary Microbiology 9,* 477-486.

Simonsson, A. 1994. Näringsrekommendationer och fodermedelstabeller till svin. Report. ISBN 1101-377X ; 75. SLU, Uppsala. 71 pp. In Swedish.

Smits, E., Burvenich, C. & Guidry, A.J. 1999. Diapedesis across mammary epithelium reduces phagocytic and oxidative burst of bovine neutrophils. *Veterinary Immunology and Immunopathology 68,* 169–176.

Steele, R. W. 1991. Clinical applications of chemiluminescence of granulocytes. *Reviews of Infectious Diseases 13*, 918-25.

Tizard, I.R. 2000. *Veterinary immunology: an introduction.* 6th ed. WB Saunders, Philadelphia. 482 pp.

Tsuma, V.T. & Magnusson, U. 1992. Opsonic activity in mammary secretion and serum of gilts during the lactation period. *Journal of Veterinary Medicine B 39,* 285-289.

Vaiman, M., Chardon, P. & Rothschild, M.F. 1998. Porcine major histocompatibility complex. *Scientific and Technical Review. Office international des epizooties 17*, 95-107.

Wegmann, P., Bertschinger, H.U. & Jecklin, H. 1986. A field study on the prevalence of coliform mastitis (MMA) in Switzerland and the antimicrobial susceptibility of the coliform bacteria isolated from the milk. *Proceedings 9th International Pig Veterinary Society Congress,* Barcelona, Spain. 92.

Personal communication

Vet. Arne Persson, PhD, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, SLU, Uppsala, Sweden

Acknowledgements

The studies were carried out at the Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Centre for Reproductive Biology (CRU), SLU (Swedish University of Agricultural Sciences), Uppsala, Sweden.
Financial support was provided by Formas (The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning) [formerly the Swedish Council for Forestry and Agricultural Research (SJFR)], Swedish Meats and Stiftelsen Lantbruksforskning, Stockholm.

I would like to sincerely thank *all* who in any way have contributed to this work over the years, and especially:

Stig Einarsson, Head of the Department of Obstetric and Gynaecology, for giving me the opportunity to study at the department, for placing the facilities at my disposal, and for interest.

Ulf Magnusson, my scientific supervisor, for introducing me to the fields of immunology and clinical pig research, and for clear and constructive criticism of papers-to-be.

Henrik Holst, my co-advisor, for kind support from beginning to end and for, to his own surprise, turning into a hormonal expert.

Fredrik Hultén, my co-advisor, joining halfway just in time for the second clinical study, for good co-operation in the barn and for common sense.

Malin Löving, for sharing work with the sows and piglets in the second clinical study and for being the pig healer.

Anders Johannisson, co-author, for introducing me and the samples to flow cytometry in a professional way.

Mari-Anne Carlsson and Åsa Karlsson at the Department of Clinical Chemistry for hormone analyses and positive attitude. Inger Lilliehöök for patiently answering every question on pig-haematology.

Nils Lundeheim and Patrik Öhagen for prompt and clarifying answers to my statistic questions, through deep knowledge giving insights to the unenlightened.

Staff working at the pig stable at Funbo-Lövsta Research Station for help with blood sampling. Isabelle Wigren for very pleasant co-operation with very young piglets.

Staff at the KC-library for helpfully answering questions and ordering books.

Employees at OG; Ulrika Matsson, Mari Wallbring (ladies first), Kjell-Ove Eklund and Ove Gustavsson for taking such good care of the animals, and Carola Jansson for always providing assistance in a cheerful and animal-friendly manner, to all for nice chats in the coffeeroom, Birgitta Berner for skilful handling of administrative matters etcetera!, Åsa Jansson, Annika Rikberg, Karin Selin-Wretling and Lotta Sundström for orderliness in all lab-related matters and a helpful attitude, Heléne Gille for nice chats and for once upon a time keeping the sows asleep, Kjell-Åke Ahlin and Kjell-Ove Eklund for keeping the printer and computer alive, and Bo Fred and Tom Jangby for generous help with all sorts of things. Arne Persson for practical pig knowledge, teachers and researchers who have provided me with new knowledge, and all other people, and students, adding to an interesting environment and making work and breaks pleasant.

A and Lily, and Eva, my roommates, for friendship, understanding and sense of humour, thus making daily life brighter.

All fellow PhD-students at OG and other departments who through the years created a nice working atmosphere, and guest researchers who helped me out whenever I needed and showed me other horizons. I keep the memories of friendship alive!

Staff at SIPAR who let me join their smoothly working team, twice! and share the events and fun in international course work, I learned a lot of things.

My profound gratitude to people giving tangible advice and encouragement during the month when I completed my thesis.

Family in Nyköping, Göteborg, Stockholm and Uppsala, for always being there, for standing invitations, support and love.

Acta Universitatis Agriculturae Sueciae
presents doctoral theses from the Faculty of Agriculture, Landscape Architecture and Horticulture, the Faculty of Forestry and the Faculty of Veterinary Medicine at the Swedish University of Agricultural Sciences. Each faculty has its own subseries, Agraria, Silvestria and Veterinaria, respectively, with separate numbering.

ISSN 1401-6257
ISBN 91-576-6363-7