INFLUENCE OF FREQUENT REGROUPING AND SOCIAL STATUS ON BEHAVIORAL, ENDOCRINE AND IMMUNE RESPONSES OF GROUP-HOUSED PREGNANT SOWS

Christiane Schalk

Institute of Animal Science

University of Hohenheim

Behavioral Physiology of Livestock

Prof. Dr. Volker Stefanski

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Christiane Schalk

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Leitung des Kolloquiums:	Prof. Dr. Andrea Knierim
Berichterstatter, 1. Prüfer:	Prof. Dr. Volker Stefanski
Berichterstatter, 2. Prüfer:	Prof. Dr. Eberhard H.K.F. von Borell
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FOR WILMA

Dogs look up to us. Cats look down on us. Pigs treat us as equals.

Winston Churchill

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ACTH	Adrenocorticotropic hormone
ADI	Average dominance index
APC	Antigen-presenting cell
AU	Arbitrary units
CBG	Corticosteroid-binding globulin
CD	Cluster of differentiation
CRH	Corticotropin-releasing hormone
CTL	Cytotoxic T cell
DC	Dendritic cell
DS	David’s Score
DV	Dominance value
GC	Glucocorticoid
HPA	Hypothalamic-pituitary-adrenal
HR	High-ranking
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
KLH	Keyhole limpet hemocyanin
LR	Low-ranking
MHC	Major histocompatibility complex
MR	Middle-ranking
MT	Mixed treatment
N:L ratio	Neutrophil:lymphocyte ratio

NK cell	Natural killer cell
NON-MT	Non-mixed treatment
PBMC	Peripheral blood mononuclear cells
RI	Rank index
SD	Standard deviation
SEM	Standard error of the mean
SNS	Sympathetic nervous system
TCR	T cell receptor
TH cell	T helper cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
wk	Week

CHAPTER 1

INTRODUCTION AND LITERATURE OVERVIEW

1 INTRODUCTION AND LITERATURE OVERVIEW

1.1 GENERAL INTRODUCTION

The social organization of wild boars (*Sus scrofa*) is characterized by dominance-subordinate relationships resulting in clear and stable dominance hierarchies (McGlone, 1986). From an evolutionary perspective, the establishment of these dominance relationships is beneficial for the regulation of access to mates, food or territory (Chase and Seitz, 2011; Douglas et al., 2017; Lindquist and Chase, 2009; Otten et al., 1999; Williamson et al., 2016). Female wild boars usually live in stable family units consisting of four to six sows and their offspring, with groups led by an older sow (Graves, 1984). Among group members there only exists a low frequency of overt aggressions and threats and they usually have no contact with unfamiliar conspecifics to avoid conflict and fights (Meese and Ewbank, 1973). A similar social structure still exists in domestic pigs kept under semi-natural conditions (Graves, 1984; Stolba and Wood-Gush, 1989).

In this respect, increased legislative, consumer and retailer awareness of modern agricultural practices and animal husbandry led to the prohibition of individual housing of sows in gestation stalls (Matthews and Hemsworth, 2012) by prescribing group-housing from four weeks after mating to one week before parturition by law since 2013 (EU directive 2001/88/EC).

In contrast to their wild ancestors, in domestic sows, aggressive encounters occur regularly as commercial housing settings involve and enforce regrouping (“mixing”) of animals (Arey and Edwards, 1998; Kongsted, 2004). Therefore, the natural social structure can often not be realized in the commercial housing systems for sows. Farm animals are generally able to cope with quite diverse environments, but it should not be expected that livestock adapts easily to all situations without effects on welfare or health status (Sachser, 2001). Every regrouping of animals or change of group composition partially is associated with the establishment or adjustment of a dominance hierarchy. This in turn provokes aggressive behavior and, therefore, may adversely affect sow welfare, particularly because of its effects on injuries, claw lesions, pain, and fear (Arey, 1999; Puppe et al., 2008; Verdon et al., 2015). Although the formation of hierarchies by agonistic encounters is a natural behavior in pigs, this process is known to result in social stress by an activation of different stress systems, e.g. the hypothalamic-pituitary-adrenal axis (Coutellier et al., 2007; Couret et al., 2009) (see also 1.5). The subsequent release of neuroendocrine signals like glucocorticoids has the potential to alter several immune functions and immune cell numbers in the blood and therefore stress-induced immunomodulations may be directly associated with animals’ health, reproduction, embryonic development and economic losses (Grün et al., 2013; von Borell et al., 2007; Greenwood et al., 2014). Previous research on pregnant sows primarily focused on the stressfulness of housing environment in general (e.g. space allowance, group-size and individual housing). Whether and how the large discrepancy between natural environment and artificial group-housing affects pregnant sows’ behavior, stress hormones and especially the distribution and functionality of blood leukocyte subpopulations represents a major research gap in the field of stress assessment of housing conditions in pig production.

1.2 GROUP-HOUSING OF PREGNANT SOWS

Since the introduction of new European legislation prohibiting individual housing of pregnant sows in crates, the transition of pregnant sows to group-housing systems poses new challenges for housing management. Particularly with regard to animal welfare, housing of pregnant sows in groups is less restrictive and, compared to crate-housing, better enables the animals to perform natural needs like locomotion, exploration and direct social behavior (Brown and Seddon, 2014). They are also allowed to spatially separate defecating, eating and resting areas according to their biology (Pedersen, 2018). Moreover, group-housed sows show less abnormal bone and muscle development and better cardiovascular fitness (Brown and Seddon, 2014; Karlen et al., 2007). Nevertheless, group-housing also presents some disadvantages. Individual feeding and monitoring of sows becomes more difficult and one central welfare problem seems to be stress and injuries caused by aggression, particularly after group formation and for feed access (Chapinal et al., 2010).

Available commercial group-housing systems vary considerably in several aspects like terms of feeding (in groups or individually, simultaneous or sequentially), floor (straw, slats, concrete) and the total space allowance provided. The main differences generally relate to the number of sows accommodated or the stability of the social group. In stable groups, sows are grouped once after service and group composition stays constant during the entire pregnancy. In contrast, when sows are kept in dynamic groups, the composition of groups changes at regular intervals by continuous introduction and removal (mixing) of pregnant sows of different gestational stages (Jungbluth et al., 2005; Durrell et al., 2002).

Social instability caused by mixing of animals is a stressful condition for animals of many mammalian species (Capitanio and Cole, 2015; Sachser et al., 1998; Stefanski, 2000; Otten et al., 2002) and the following sections will present a short overview of the neuroendocrine regulation of immunity by stress and pregnancy. Afterwards, the current knowledge of the effects of mixing and social stress on behavior and physiology in model species and pigs is summarized.

1.3 The immune system with special focus on the pig

The immune system consists of physiological processes helping to protect the organism against pathogens like viruses or bacteria and to maintain the integrity of the body. To distinguish harmful foreign antigens from endogenous substances, a complex array of protective mechanisms is involved to recognize foreign structural features and to neutralize pathogens (Sacks et al., 1999; Chaplin, 2010). In mammals, the mechanisms permitting recognition of microbial, toxic, or allergenic structures are composed of an innate and adaptive part, both of which include humoral and cellular components.

The innate immune response represents the first line of defense and is performed by cells of both hematopoietic and non-hematopoietic origin. Myeloid cells involved in innate immune processes include macrophages, dendritic cells (DC), mast cells, neutrophils, basophils, eosinophils, and natural killer (NK) cells. Epithelial cells lining the respiratory, gastrointestinal and urogenital tract complete the cellular innate immune defense (Murphy et al., 2009). Humoral components include complement proteins, acute phase proteins and mannose-binding lectin (Sacks et al., 1999). The innate immune system is characterized by rapid inflammatory responses in case of pathogen exposure and plays an essential role in activating the subsequent adaptive immune response. Cells of the innate immune system have the ability to distinguish between pathogens and self- or non-pathogenic structures and typically recognize pathogens by pattern recognition receptors such as Toll-like receptors (TLR) on the surface of the cells. TLR activate tissue-resident macrophages to produce pro-inflammatory cytokines, including tumor-necrosis factor α (TNF-α), interleukin-1β (IL-1β) and IL-6, which coordinate local and systemic inflammatory responses. TNF-α and IL-1β, in turn, activate the local endothelium to induce vasodilation and increase the permeability of the blood vessel, allowing serum proteins and leukocytes to be recruited to the site of infection (Kick et al., 2011; Medzhitov, 2007).

Porcine neutrophil granulocytes represent 40 – 55% of blood leukocytes and are highly specialized, short-living phagocytes that act as a first line of defense against various pathogens including bacteria and fungi. Neutrophils are able to kill pathogens intracellularly by phagocytosis and extracellularly through degranulation and by release of antimicrobial peptides. A second group of granulocytes, - eosinophils - is mainly detectable in the skin and mucosa of lung and gastro-intestinal tract. They only make up 2 – 4% of leukocytes in pigs. Compared to neutrophils, they are of minor importance in their function as phagocytes but play a major role in the extracellular defense of multi-cellular parasites which cannot easily be phagocytized due to their size (Mair et al., 2014).

Pro-monocytes in the bone marrow, monocytes in the blood stream, as well as macrophages and DC in the tissue altogether form a system of phagocytic cells, commonly called mononuclear phagocyte system. Monocytes make 4 – 6% of blood peripheral leukocytes. The expression of specific cell surface molecules is important to mediate functions including antigen recognition, cell activation and phagocytosis. The migration of monocytes into the tissue is associated with functional and morphologic differentiation into macrophages (10 – 15% of total immune cells in tissue) which synthetize different biologically active substances (e.g. lysozyme, acid phosphatase, elastase, collagenase, and complement factors). Those stimulate components of both the innate and the adaptive immune system, support tissue remodeling, and mediate extracellular defense against pathogens and tumor cells. Moreover, macrophages are responsible for specific antigen-uptake, processing and presentation to T cells in the spleen and lymph nodes. DC play a major role in antigen-transport from the site of entry at the skin and mucosa to the lymphoid tissue (Mair et al., 2014).

NK cells represent 5 – 15% of blood mononuclear cells and play a major role in the cellular innate immune system as they are mainly responsible for defense and killing of viruses and intracellular bacteria and parasites. Virus-infected cells that cannot be recognized by cytotoxic T cells are often vulnerable to NK cells which provide a second defense mechanism. NK cells use similar mechanisms for killing of infected cells like perforins and granzymes but do not require a pre-activation by cytokines or antigen-presentation (Gerner et al., 2009).

The adaptive immune response is responsible for mounting long-lasting and antigen-specific defense mechanisms. Key regulators of the adaptive immune system include the effectors of cellular immune responses, T lymphocytes, and the antibody-producing B lymphocytes, forming the humoral part of the adaptive arm. T cells are responsible for recognizing a high number of foreign antigens and are involved in the regulation of humoral immunity, cell-mediated cytotoxicity, and delayed hypersensitivity reactions. They support B cell development, recognize and destroy virus-infected cells, activate phagocytes, and control the intensity and quality of an immune response. For the recognition of foreign structures, T cells express specific cell surface antigen receptors – the T cell receptor (TCR) – which allow the differentiation of T cells into αβ- and γδ- T cells based on the expressed domains of the TCR chains (Murphy et al., 2009). The distinguishing co-receptor molecules further differentiate T cells by their expression of cluster of differentiation (CD) molecules – CD4 or CD8 referred to T helper (TH) cells ($CD4^+$) and cytotoxic T cells (CTL) ($CD8^+$). TCR αβ-T cells recognize small peptide fragments only if bound to a major histocompatibility complex (MHC) molecule. $CD4^+$ T cells recognize antigens presented on MHC class II molecules on antigen-presenting cells (APC) and B cells, while $CD8^+$ T cells recognize antigens presented on MHC class I molecules on APC such as DC and macrophages (Murphy et al., 2009). The majority of γδ-T cells express neither CD4 nor CD8, however, there are some γδ-T cells that express CD8 (Gerner et al., 2009). Upon contact and binding to an antigen, B and T cells differentiate from naive into potent effector cells with various activities such as killing, cytokine production, or antibody production. After clearance of the pathogen, some of these differentiated cells survive and develop a long-lasting immunological memory (Gerner et al., 2015).

The porcine immune system is characterized by some unique characteristics of the T cell system. Beside the $CD4^+CD8^-$ TH cell phenotype known from other species, the extrathymic occurrence of a second population of $CD4^+$ TH cells co-expressing the α-chain of the CD8 molecule was demonstrated in healthy pigs (Saalmüller et al., 2002; Charerntantanakul and Roth, 2006). These $CD8\alpha^+$ TH cells are considered to be antigen-experienced and activated memory TH cells that origin from naive $CD8^-$ TH cells and respond to foreign antigens with proliferation and the expression of CD8α and MHC class II molecules (Charerntantanakul and Roth, 2006; Saalmüller et al., 2002). Beside $CD8\alpha^+$ TH cells, a substantial proportion of blood γδ-T cells was demonstrated in pigs (Saalmüller et al., 2002; Gerner et al., 2009; Charerntantanakul and Roth, 2006). Whereas peripheral γδ-T cells represent 0.5 – 2% of lymphocytes in mice and rats (Haas et al., 1993), they mount up to 21% among peripheral blood lymphocytes in 12 month old pigs (Yang and Parkhouse, 1996). Similar to $CD8\alpha^+$ TH cells, porcine $CD8^+$ γδ-T cells seem to acquire CD8α during activation and maturation processes (Gerner et al., 2009). Although their role in immune defense is not completely understood yet, it becomes increasingly evident that functional properties of these cells include cytotoxic activity, cytokine production, and antigen-presentation (Charerntantanakul and Roth, 2006).

1.4 PREGNANCY AND THE IMMUNE SYSTEM

It is important to note that progressing pregnancy is associated with some substantial immunological alterations which are supposed to protect the fetus from harmful maternal immune activity (Luppi, 2003; Ramsay, 2018). Studies in model species demonstrated an activation of the complement system as well as of certain components of the innate immune system by an enhanced number of circulating granulocytes and monocytes in order to leave the maternal defense intact. In contrast, adaptive immune functions are suppressed which is suggested to achieve a successful pregnancy (Luppi, 2003; Meeusen et al., 2001; Stefanski et al., 2005; Sacks et al., 1999; Kühnert et al., 1998; Kwak-Kim et al., 2014; Pazos et al., 2012). The down-regulation of adaptive immune responses includes decreasing numbers of peripheral CTL during the first trimester of pregnancy whereas TH cell numbers decrease during the last third of pregnancy which is presumed to protect the fetus from destruction by maternal immune response (Watanabe et al., 1997; Nakamura et al., 1997). This pregnancy-unique immune status is crucial for reproductive performance by achieving a successful pregnancy but also for maintenance of maternal health (Robinson and Klein, 2012).

A number of factors have been proposed to explain the effects of pregnancy on T and B cell functions. Increasing production of maternal and placental products (e.g. estrogen or progesterone) are known to have immunomodulatory properties and modulate T cell reactivity and production of cytokines (Grossman, 1985). The shift towards a TH2-mediated immune response and the diminished cytotoxic activity of CTL and NK cells in pregnant organisms seems to be related to an increased progesterone sensitivity of some lymphocyte subsets during pregnancy (Szekeres-Bartho et al., 2001; Szekeres-Bartho et al., 1990).

In sows, previous research during pregnancy primarily focused on changes and alterations of humoral and cellular local immune responses in uterine lymph nodes, the endometrium or the mammary gland (Ziecik et al., 2011; Bischof et al., 1996; Bischof et al., 1995; Salmon et al., 2009; Kaeoket et al., 2001). Two early studies from Georgieva (1984) and Schollenberger et al. (1992) aimed to examine blood T-lymphocytes in sows during pregnancy, but were hindered by the fact that pig-specific monoclonal antibodies for flow cytometric analyses were not available at this time. Moreover, the reported effects were different from the previous mentioned studies in other species. Georgieva (1984) found no changes for blood TH cells during pregnancy while Schollenberger et al. (1992) show an increase of blood TH and B cells. More recent studies revealed that the course of blood lymphocytes and granulocytes throughout gestation might be equal as in other mammals (Pacheco and Salak-Johnson, 2016; Couret et al., 2009; Grün et al., 2013). However, those previous studies did not differentiate between important lymphocyte subsets such as $CD4^+$ T, $CD8^+$ T, B and NK cell numbers which are known to be particularly sensitive to pregnancy-induced modifications and did not include analyses of blood immune cell numbers during the first trimester or the entire gestation. Moreover, as sows differ in physiological characteristics (e.g. gestational length, litter sizes, *in utero* development time-line) (Merlot et al., 2008), findings from humans and rodents in this concern cannot be easily transferred to pigs. Thus, it becomes very clear that these profound physiological adaptations need to be further clarified in sows during pregnancy, especially for the evaluation of stress-induced immune alterations.

1.5 STRESS AND THE IMMUNE SYSTEM

Numerous studies have demonstrated that stressful stimuli have the potential to affect the neuroendocrine-immune network comprised of the immune-, central nervous- and endocrine system in animals and humans (Maes et al., 1997; Sachser, 1987; Stefanski, 2000; von Borell, 1995; Glaser and Kiecolt-Glaser, 2005). Since Hans Selye (1936) first presented the concept of stress in a biological context as follows: "*the exposure of an organism to an acute nonspecific nocuous agent induces a typical syndrome, the symptoms of which are independent of the nature of this agent*", the concept has undergone several modifications. Modern definitions e.g. from Dhabhar and McEwen (1997) described stress as a "*constellation of events, which begins with a stimulus (stressor), which precipitates a reaction in the brain (stress perception), which subsequently results in the activation of certain physiologic systems in the body (stress response)*". Stressful social stimuli generally activate the hypothalamic-pituitary-adrenal (HPA-)- axis and the sympathetic nervous system (SNS), resulting in an increased release of glucocorticoids (GC) and catecholamines (Glaser and Kiecolt-Glaser, 2005). The activation of the HPA-axis results in the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus which stimulates adrenocorticotropic hormone (ACTH) secretion in the anterior pituitary gland. The release of ACTH into the systemic circulation leads to a secretion of GC (e.g. cortisol) from the cortex to the adrenal glands (Webster Marketon and Glaser, 2008; Webster et al., 2002). The activation of the SNS leads to the secretion of the neurotransmitter acetyl-choline at sympathetic nerve endings in the adrenal medulla which induces the release of the catecholamines epinephrine and norepinephrine (Webster Marketon and Glaser, 2008). The release of stress hormones from the adrenal cortex and medulla can lead to modifications of further physiological responses like the immune system and induce changes in leukocyte distribution (Sapolsky et al., 2000; Webster Marketon and Glaser, 2008) (**Figure 1**).

Immune cells generally circulate continuously from the blood, though various organs, lymphatic vessels and nodes, and back into the blood, which is essential for maintaining an effective immune defense network (Dhabhar, 2002). Therefore, the numbers of leukocytes in the blood provide important information on leukocyte distribution in the organism and the activation state of the immune system. High catecholamine concentrations are known to increase neutrophil and NK cell numbers rapidly and dramatically in the circulation whereas blood T and B cell numbers decrease (Dhabhar, 2014; Mills et al., 1997; Dimitrov et al., 2010; Benschop et al., 1996). Glucocorticoids are described as the major mediators of the changes in leukocyte distribution, acting through normal immune cell surveillance and trafficking mechanisms. Leukocytes exit from the blood into other organs (e.g. bone marrow, skin, mucosal lining, gastro-intestinal and urogenital tracts, lung, liver, and lymph nodes) as part of an adaptive stress response (Dhabhar et al., 1996). Such a redistribution of leukocytes results in a decrease in blood leukocyte numbers.

However, acute and chronic stress are known to affect the immune response in different ways. Studies show that acute stress, lasting for a period of seconds to hours, induces biphasic changes by an initial increase followed by a decrease in blood lymphocyte and monocyte numbers, while blood neutrophil numbers generally increase during stress (Dhabhar, 2002; Dhabhar et al., 1995; Stefanski and Engler, 1998). After termination of the stressor, blood lymphocyte and monocyte numbers rise to pre-stress baseline levels again. In contrast, a stress response that continues for several hours to weeks or months causes extended adaption to maintain homeostasis of an organism (Dhabhar and McEwen, 1997; Kick et al., 2011). As a result, excessive circulating GC levels can induce a general immunosuppression and a shift towards a TH cell type 2-mediated immune response (Elenkov, 2004). These deteriorating effects on the organism during chronic exposure to stress hormones with consequences on the distribution of immune cells were described for many species and included a decrease in absolute numbers of blood TH cells, CTL, NK cells, B cells, and monocytes (Bartolomucci, 2007; Stefanski and Engler, 1998; Stefanski et al., 2005; Engler et al., 2004).

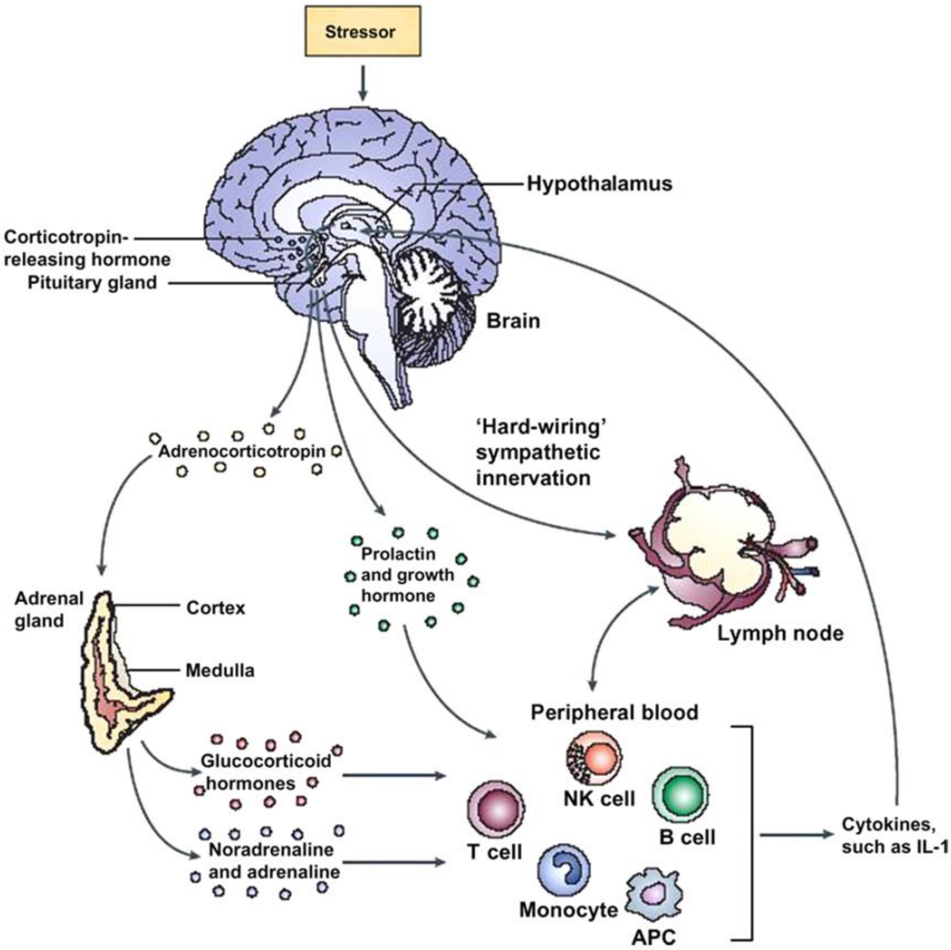

Figure 1. Stress-associated modulation of the hormone response by the central nervous system. Upon experiencing a stressor, the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system are activated resulting in release of glucocorticoids and catecholamines which are able to modulate various aspects of the immune system (Webster Marketon and Glaser, 2008; Glaser and Kiecolt-Glaser, 2005).

Therefore, a physiological stress response mediated by the activation of the HPA-axis and the sympathetic adreno-medullary system can be determined by measuring the level of secreted hormones or peptides in body fluids like blood, urine or saliva (Tuchscherer et al., 2010; Broom et al., 1995). Thus, the analysis of immunological alterations can provide further insight into the stress status of an individual (Salak-Johnson, 2007; Kick et al., 2011).

Various studies assessed the effects of stress on pigs in the different phases of production from gestation to the finishing or fattening phase, suggesting that different stressors of modern livestock husbandry cause modulations of important immune cell numbers and functionality in pigs (**Table 1**). Stressors include temperature variations, photoperiod manipulation, space restriction, novel environments, handling and transportation (Kick et al., 2011; von Borell, 2001).

Table 1. Stress effects on important immune cell numbers and functionality of the immune system in pigs during the different phases of production.

Stressor	Production phase	Effect	References
ACTH challenge (i.v.)	Gilts	↓ Blood lymphocytes ↑ Blood neutrophils ↑ N:L ratio	Salak-Johnson et al. (1996)
Transportation (4 h)	Growing pigs	↓ Blood lymphocytes ↑ Blood neutrophils ↑ N:L ratio	McGlone et al. (1993)
Novel environment	Finishing pigs	↓ Blood lymphocytes ↑ Blood neutrophils ↑ N:L ratio	Krebs and McGlone (2009)
Individual housing in crates	Pregnant sows	↓ Blood lymphocytes ↓ Blood T cells ↑Antibody response	Grün et al. (2013) Grün et al. (2014)
Space allowance (3.3 m^2)	Pregnant sows	↑ Blood lymphocytes ↓ Blood neutrophils ↓ N:L ratio	Salak-Johnson et al. (2012)
Transportation and space allowance	Weaned pigs	↑ N:L ratio (↓ space) ↑ N:L ratio (transport)	Sutherland et al. (2009)
Isolation (9 days, 2h/day)	Piglets	↓ Lymphocyte proliferation	Kanitz et al. (2004)
Isolation (4 h)	Piglets	↓ Plasma TNF-α levels ↓ Percentage $CD4^+$ cells ↑ Percentage $CD8^+$ cells	Tuchscherer et al. (2009)
Cold / Heat	Piglets / Growing pigs	↓Antibody response	Blecha and Kelley (1981) Morrow-Tesch et al. (1994)
Indoor / Outdoor rearing	Growing pigs	↑Antibody response (outdoor rearing)	Rudine et al. (2007)
Cold	Growing pigs	↑ NK cytotoxicity	Hicks et al. (1998)

↑ = increase / higher
↓ = decrease / lower

In general, under stressful conditions, immune function in pigs may be impaired. This is shown by a down-regulation of blood lymphocytes, up-regulation of neutrophils, increased neutrophil:lymphocyte (N:L) ratio and an altered antibody or proliferative response (**Table 1**). However, this overview also illustrates that most studies focused on piglets and growing-pigs while stress assessment of sows so far was underrepresented. Even though lymphocyte subsets such as cytotoxic T cells, naive or antigen-experienced TH cells and γδ-T cells play an essential role for the acute immune defense of the organism, cytokine production or formation of the long-lasting immune memory (Charerntantanakul and Roth, 2006), these cell types are still, for the most part, missing in these investigations. Therefore, to draw conclusions on stress-induced immunomodulation in pigs – or especially in pregnant sows – would be premature as long as a detailed investigation on numbers and distributions of the distinct T cell subsets, B cells and natural killer cells is missing.

1.6 Social mixing, behavior and the immune system

Chronic exposure to glucocorticoids, as mentioned above (see section 1.5), can have adverse effects on the organism, including immunosuppression through alteration of distribution and functionality of immune cells (Dhabhar et al., 1995; Dhabhar, 2009). Stressful animal housing conditions such as mixing of unacquainted animals or the formation of new groups can strongly influence physiology and behavior of laboratory animals (Engler and Stefanski, 2003; Bartolomucci, 2007; Stefanski and Engler, 1998). Colony housing or social confrontations have repeatedly been shown to reduce the numbers of blood TH cells as well as CTL, decrease lymphocyte proliferation and alter antibody production in rodents (Stefanski, 2000; Engler et al., 2004). Social stressors, with respect to behavior, are characterized by the occurrence of agonistic, injurious and stereotypic behavior as well as of behavioral inactivity (von Borell, 1995; Martínez-Miró et al., 2016; DeVries et al., 2003).

Therefore, dysregulations in immune functions as well as excessive aggressive behavior became valuable indicators for the assessment of stressful situations (Kongsted, 2004; de Boer et al., 2016; Martínez-Miró et al., 2016; von Borell, 1995). Studies on swine that evaluate the effects of social mixing on the immune system have primarily focused on piglets or growing pigs so far (de Groot et al., 2001; Bacou et al., 2017; Sutherland et al., 2006). Differences in the immune responses were reported in young barrows after pairwise mixing at approximately 6 weeks of age. After vaccination with pseudorabies virus, antigen-specific lymphocyte proliferation, IgM, IFN-γ and IL-10 responses were lower in mixed barrows compared to unmixed controls, whereas immune responses of mixed gilts did not differ from immune responses of control gilts (de Groot et al., 2001). Deguchi and Akuzawa (1998) reported a suppression of the mitogen-induced lymphocyte proliferation for at least 26 days after grouping littermates with unknown piglets. Damgaard et al. (2009) showed increased neutrophil numbers and an increased N:L ratio in piglets introduced to groups with frequent exchange of group members compared to groups with consistent group compositions. In contrast, Moore et al. (1994) did not find any differences in the N:L ratio between growing pigs either housed in stable or dynamic groups, but N:L ratio was higher under both conditions when compared to control animals which were not exposed to any unknown conspecifics.

Existing data from pregnant sows show that mixing increases aggressions as well as cortisol concentrations (Arey and Edwards, 1998; Ison et al., 2014; Poletto et al., 2014; Verdon et al., 2016; Knox et al., 2014), but no immunological differences in response to mixing were detected so far. Couret et al. (2009) analyzed the immune system in pregnant gilts with respect to an unstable environment, but could not detect an effect of repeated pairwise mixing during late pregnancy on antigen- and mitogen-induced lymphocyte proliferation, antigen-specific IgG titers and peripheral lymphocyte and neutrophil numbers in mixed gilts. Stevens et al. (2015) reported no effects of mixing on the ratio of neutrophils to lymphocytes in pregnant sows.

In this concern, it has to be noted that no study on the effect of mixing in pregnant sows included in-depth analyses of particular stress-sensitive important blood immune cells such as various T cell subsets. For example, antigen-experienced TH cells and CTL were not considered so far although they play an essential role for the immune defense of the organism, as systemically reduced cell numbers might have negative consequences for the immune response towards specific antigens and the resistance to viral infections (Grün et al., 2013).

Thus, it cannot be concluded that pregnant sows might be resistant to a stress-induced immunomodulation due to the limited number of studies and the observation that other potentially stressful housing environments (e.g. space allowance, group-size) indeed affect the immune systems of pregnant sows (Hemsworth, 2013; Salak-Johnson et al., 2012; von Borell et al., 1992). Salak-Johnson et al. (2012) showed that a decreased space allowance increased the numbers of neutrophils and NK cells as well as the N:L ratio in gestating sows, which resembles a picture of stress-induced immunomodulation. Grün et al. (2014; 2013) reported that individual housing of pregnant sows in crates especially affected the number, distribution and functionality of lymphocytes as opposed to group-housed sows. Based on this immunological profile of lower numbers of T cells, CTL and naive TH cells as well as later antigen-specific cytokine production and higher cortisol concentrations in individually housed pregnant sows, it can be suggested that different housing systems represent differently stressful conditions.

To evaluate the effects of social mixing in pregnant sows, detailed investigations on numbers and distributions of distinct T cell subsets, B cells and NK cells are needed to further clarify immunological consequences of social stress during gestation.

1.7 SOCIAL STATUS, BEHAVIOR AND THE IMMUNE SYSTEM

As mentioned above, individuals living in groups develop social hierarchies to structure their society and to determine priority of access to key resources (DeVries et al., 2003). Previous studies showed an influence of social rank on behavior, health, aging and fitness measures, but also on other important aspects of physiology including circadian rhythm, brain development, endocrine status, and immunity (Holekamp and Strauss, 2016; Creel, 2001). Moreover, evidence from group-living species revealed that there is an interplay between social status and the neuroendocrine-immunological response to social stress leading to differences between dominant and submissive animals (Avitsur et al., 2003). Social reorganization was associated with lower body weight, higher cortisol levels and increased risk of infection in nonhuman primates with low social status (Cohen et al., 1997).

Among growing pigs subjected to mixing stress, lymphocyte proliferation and total IgG were greater in dominant pigs than in subordinates (Tuchscherer et al., 1998). De Groot et al. (2001) showed a higher lymphocyte proliferation in mixed dominant pigs compared to mixed subordinates. Additionally, existing data from several studies clearly found that social status also has an impact in sows on reproduction as well as on endocrine and behavioral responses. Low or intermediate social rank was associated with increased cortisol concentrations (Zanella et al., 1998; Tsuma et al., 1996; Mendl et al., 1992; Li et al., 2017), decreased litter size and farrowing rates, higher levels of received aggression and injuries as well as less weight gain, and poorer body condition (O'Connell et al., 2003; Li et al., 2017; Hoy et al., 2009; Kranendonk et al., 2007). However, despite increasing evidence from growing pigs reporting a social status-associated lack in immune reactivity and current findings from other species suggesting that social rank or dominance is one important factor contributing to normal pattern of immune alterations during pregnancy (Stefanski et al., 2005; Chebel et al., 2016), data on immunological consequences related to social rank in pregnant sows are limited. Pacheco and Salak-Johnson (2016) reported greater plasma cytokine levels (IL-12), a lower N:L ratio and a tendency for reduced percentage of blood neutrophils in submissive pregnant sows as compared to dominants, while numbers of total white blood cells, percentages of lymphocytes, monocytes and eosinophils were not affected. Other studies failed to detect any effect of social status on immune cell numbers such as total number of blood granulocytes and lymphocytes as well as on lymphocyte proliferation and antibody response (Couret et al., 2009; Mendl et al., 1992; Zhao et al., 2013).

However, similar to the missing knowledge of social mixing effects on blood immune cells, a detailed investigation on pregnancy-associated alterations in response to social status on numbers and distributions of immunologically highly relevant T cell subsets, B cells and NK cells during the entire pregnancy is not available.

1.8 MEASURING DOMINANCE STATUS

The social status or rank within dominance hierarchies is often associated with many aspects of animals' physiology (Chase and Seitz, 2011) and, therefore, analysis of dominance relationships is increasingly gaining interest in livestock species.

To derive a social hierarchy from observations of the social interactions between a pair of animals, the frequency of wins and defeats are arranged in a winner-loser-matrix (Martin and Bateson, 2007). Based on the outcome and number of acted and received aggressive behaviors of an animal, an individual dominance index can be calculated which aims to derive a simple numerical value that reflecting an individual's social status within a group (Bayly et al., 2006). The identification and choice of an appropriate index to construct dominance hierarchies remains critical as assessment of social hierarchies is influenced by several factors like group characteristics (type of society, stability) as well as observation situation, period and length (Hemelrijk et al., 2005) which has to be taken into account in order to generate reliable datasets.

It has already been noted that measuring different behaviors may result in hierarchy alterations (Boyd and Silk, 1983; Bradshaw et al., 2000). Following Drews (1993) all forms of agonistic behavior clearly refer to dominance and indices vary in their response to characteristics of the input data. Dominance may initially be determined by the outcome of a contest, but then maintained through daily interactions such as displacements from feeding or resting areas, agonistic displays, or submissive behavior. Aggressive interactions do not necessarily represent the highest proportion of social behavior, but socio-positive relationships were not considered for dominance measurement in species other than primates (Silva et al., 2016).

Pigs for example use an avoidance-order to diminish their aggressive outcome in social interactions (Patt et al., 2012; Jensen, 1982). Sows were shown to develop additional behavioral mechanisms to regulate their social relationships, as evidence exists that sows rely on overt agonistic interactions to a lesser extent than younger pigs (Puppe et al., 2008). This emphasizes that dominance measurement should focus on species typical behavior and depending on that respective behavior, different hours of observation are required (Feczko et al., 2015). In this concern, Bayly et al. (2006) postulated that in groups where all animals interact with each on a regular basis, simple indices can be just as appropriate as complex ones, while more sophisticated methods are required for groups in which animals test their dominance outcome less frequently.

Many methods are used to produce a dominance hierarchy from a matrix, but a consistent approach for dominance measurement still does not exist due to the variety of introduced indices to calculate individual's social rank. In addition, no recommendations for sampling interval, observation length and types of behavior have been reached. Although dominance hierarchy is a central feature in many studies of animal behavior, it is still difficult to decide which ranking method might be the best and most realistic one to produce a sufficient amount of data in order to generate the most reliable social hierarchy.

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CHAPTER 2

OVERVIEW AND RESEARCH QUESTIONS OF THE INCLUDED MANUSCRIPTS

2 OVERVIEW AND RESEARCH QUESTIONS OF THE INCLUDED MANUSCRIPTS

The overall aim of the present doctoral project was to evaluate the effects of social mixing and rank position of group-housed sows on behavior as well as on the endocrine and immune system during gestation. A study with pregnant sows was designed to resemble some aspects of commercial housing conditions to investigate the influence of frequent changes of group composition on numbers and functionality of blood leukocyte subpopulations in combination with analyses of agonistic behavior and the endocrine status for comprehensive stress assessment. In order to contribute to filling the knowledge gap in respect to pregnancy-associated immunomodulation in sows, blood immune cell numbers were analyzed during all trimesters of gestation and the impact of social status on these modifications was assessed. To clarify the rank-dependent modulations on the immune system, it was necessary to elucidate methodical details related to dominance measurement. The objectives of the manuscripts can be briefly characterized as follows.

MANUSCRIPT 1: Effects of repeated social mixing on behavior and blood immune cells of group-housed pregnant sows (*Sus scrofa domestica*)

The natural social structure of sows can often not be realized in commercial housing systems. In modern animal husbandry, housing of sows in dynamic groups is a common procedure and involves frequent regrouping or mixing of unfamiliar sows which raises several welfare and health concerns due to social stress. Against this background, effects on the immune system are likely to occur, but were not investigated in pregnant sows in detail so far. Therefore, group composition of sows was frequently changed over a certain period in order to determine possible persistent effects on the number, distribution and functionality of distinct blood leukocytes and lymphocyte subsets, plasma cortisol concentrations and aggressive behavior. The overall aim was to investigate whether repeated social mixing even of familiar pregnant sows has the potential to act as chronic social stressor with consequences on sows' health and welfare.

The manuscript was published in *Livestock Science*.

MANUSCRIPT 2: Pregnancy-associated alterations of peripheral blood immune cell numbers in domestic sows are modified by social rank

The maternal immune system is characterized by unique alterations in numbers and functionality of certain blood immune cells in order to achieve a successful pregnancy and maintain health of the dam. It became clear from humans or model species that some of these immunological changes occur during early gestation but also during the last trimester, but no detailed investigation on immunologically highly relevant blood lymphocyte subsets during the entire pregnancy in domestic sows exist. Moreover, evidence shows a rank-dependent influence on behavior, physiology and reproductive performance of sows. Based on the same group-housed multiparous sows as used in MANUSCRIPT 1, an in-depth analysis of number and distribution of blood leukocyte subpopulations and plasma cortisol concentrations was performed during all trimesters of pregnancy. Besides, this manuscript emphasized rank-dependent pregnancy-associated immunological changes of sows to determine whether the social status is an important factor that affects welfare and health of sows within group-housing environments.

The manuscript was published in *Animals.*

MANUSCRIPT 3: What, when and how? The influence of group stability and observational procedures on comparability of dominance indices in sows

Social status within a dominance hierarchy is often associated with aspects of animals' physiology, health, and reproductive performance. Therefore, in livestock production there is increasing interest in the analysis of dominance relationships and social networks. The influence of social rank on immunomodulations and endocrine changes during pregnancy of group-housed sows was investigated in MANUSCRIPT 2 and showed social rank-associated effects. Considering the essential role of social status or rank on animals' behavior and physiology, the necessity to choose the appropriate measurement for calculation of dominance relationships becomes evident. Since no consistent recommendation for measuring dominance exists so far, a variety of dominance indices to rank individuals was introduced. Besides, according to the experience from MANUSCRIPT 1 and 2, observing animal behavior to quantify dominance relationships requires considerable time and effort. In this manuscript, pregnant sows of the same experiment as in MANUSCRIPT 1 and 2 were used as a model system to compare various dominance indices that based on different methodical aspects concerning types of observed behavior as well as observation duration and situation under varying group stability conditions. The overall aim was to investigate whether several indices of medium complexity are comparable and equally applicable for determination of dominance relationships.

The manuscript was submitted to *Applied Animal Behaviour Science.*

CHAPTER 3

INCLUDED MANUSCRIPTS

3 INCLUDED MANUSCRIPTS

In the following chapter, the manuscripts included in the present doctoral thesis are presented. MANUSCRIPT 1 and 2 were published and MANUSCRIPT 3 was submitted in international peer-reviewed journals.

Effects of repeated social mixing on behavior and blood immune cells of group-housed pregnant sows (*Sus scrofa domestica*)

Christiane Schalk, Birgit Pfaffinger, Sonja Schmucker, Ulrike Weiler, Volker Stefanski

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

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Abstract

The objective of this study was to assess whether frequent re-grouping (“social mixing”) poses a potential welfare problem and affects aggressive behavior, stress hormones and leukocyte subsets of sows during gestation. Pregnant sows (German Landrace, n=40) were housed in groups of 5 animals each and were assigned either to a repeated social mixing treatment with an interchange of 2x2 sows between groups twice a week over a period of eight weeks or remained undisturbed in their original composition. Five blood samples of all sows were collected before, during, and after the mixing period and distribution of blood leukocyte subpopulations, mitogen-induced lymphocyte proliferation and plasma cortisol concentrations were evaluated. During the entire mixing period, higher levels of aggressive behavior ($p \leq 0.05$) were caused by mixing. In comparison to baseline values pre mixing, lymphocyte numbers were lower in mixed sows ($p \leq 0.05$) due to lower antigen-experienced T helper cells, cytotoxic T cells and natural killer cells. Granulocytes and cortisol concentrations were not affected ($p > 0.05$), but mixed sows showed a higher proliferative response of lymphocytes.

These findings show that repeated social mixing not only resulted in an increase of aggressive behavior, but also in altered immune cell numbers of the adaptive immune system and suggest an adverse influence of frequent mixing on the immune system, even of familiar pregnant sows.

Pregnancy-associated alterations of peripheral blood immune cell numbers in domestic sows are modified by social rank

Christiane Schalk, Birgit Pfaffinger, Sonja Schmucker, Ulrike Weiler, Volker Stefanski

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

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Abstract

A shift from adaptive to innate immune functions occurs during pregnancy. Besides, there is evidence for a rank-dependent influence on the immune system. This study investigates gestation-induced and rank-associated immunomodulations in sows during pregnancy. Five blood samples of 35 low (LR), middle (MR), or high-ranking (HR) sows were collected throughout pregnancy to evaluate the distribution of various blood leukocyte subpopulations and plasma cortisol concentrations. During the last trimester of pregnancy, a decrease of numbers of blood natural killer (NK), T cells, cytotoxic T cells (CTL), $CD8^{+}$ $\gamma\delta$ T cells, and B cells were found. Number of blood neutrophils and plasma cortisol concentration increased before parturition. B cells and monocytes were affected by social rank as MR sows showed higher numbers than LR sows. There also was a tendency for plasma cortisol concentrations to be higher in MR sows compared to LR sows.

Pregnancy-associated alterations in the immune system also exist in sows and seem to be rank-dependent, as especially middle-ranking sows display signs of stress-induced immunomodulations.

What, when and how?
The influence of group stability and observational procedures on comparability of dominance indices in sows

Christiane Schalk, Sonja Schmucker, Volker Stefanski, Birgit Pfaffinger

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

The manuscript has been submitted to *Applied Animal Behaviour Science*.

Abstract

Analysis of dominance relationships is increasingly gaining importance in livestock production. However, no consistent approach exists due to a variety of sociometric indices. The aim was to validate the comparability of four rank indices (dominance value, average dominance index (ADI), David´s score and rank index) based on distinct types of behaviour. Behaviour of sows with (MT) or without (NON-MT) a repeated social mixing treatment was continuously analysed in three different situations (during constant social conditions, after frequent changes of group composition or at competitive feeding). Indices were compared by *Spearman's rank correlation*. For MT sows, all indices were highly positively correlated for standard and for mixing situation. NON-MT sows revealed high correlation coefficients as well, but results were not equally consistent, mainly due to increased numbers of unknown relationships. For ADI, best comparability could be seen between standard and feeding situation in both treatments. The study demonstrates that comparability of indices was influenced both by group stability and by observational procedures (situation and duration), emphasising the importance of considering these factors in observation planning. In order to avoid the risk of high values of unknown relationships, it is advantageous to include not only aggressive, but also affiliative interactions or interactions with a further resource (e.g. a feeding situation) in observations for dominance measurements. We presume our findings in sow groups to be generally transferable to dominance measurement of other (group-housed) livestock species with comparable social systems and under similar housing conditions.

Key words

Affiliative behaviour; aggressive behaviour; mixing; pig; social hierarchy; sociometric methods

1. Introduction

Particularly for animal welfare and health research there is increasing interest in the analysis of dominance relationships in livestock species in order to gain more knowledge on the interplay between the dominance status and stress physiology (Ott et al., 2014; Büttner et al., 2015a; Büttner et al., 2015b). Social status or rank within dominance hierarchies is often associated with many aspects of animals' physiology, weight gain, health, and reproductive performance (Chase and Seitz, 2011).

In order to derive a social hierarchy from observing pair-wise interactions of animals, the data of agonistic encounters are usually arranged in a winner-loser matrix (Martin and Bateson, 2007). Choosing which types of social interactions to record and include into a matrix is critical, because different dominance indices vary in their response to characteristics of the input data.

According to Langbein and Puppe (2004), not only aggressive interactions but also submissive reactions should be considered in social hierarchy measurement. Moreover, little attention has been paid to the fact that social groups in many mammalian species are not only consisting of competitive agonistic, but also of affiliative relationships (Whitehead, 1997; Val-Laillet et al., 2009). Therefore, socio-positive interactions followed by submissive behaviour might also be related to the outcome of the dominance hierarchy.

In general, the assessment of social hierarchies depends on several factors like group characteristics (type of society, stability) or observation period (Hemelrijk et al., 2005; Strauss and Holekamp, 2019). For smaller group sizes simple indices can be just as appropriate as complex ones, especially when all animals interact with each other regularly (Bayly et al., 2006). Different, more sophisticated methods are required for groups in which animals test their dominance outcome less frequently (Bayly et al., 2006). Under these conditions, the determination of the proper sampling interval and observation length is crucial to avoid production of sparse data sets and to generate reliable results (Daigle and Siegford, 2014; Lendvai et al., 2015). This is especially true because observing and quantifying animal behaviour requires considerable time and effort (Martin and Bateson, 2007).

With respect to livestock species, social dominance is a multidimensional phenomenon occurring in all farm animals and finds its reflection in a dominance hierarchy. Aggression or other types of agonistic behaviours typically lead to a fight or flight response to determine dominant– subordinate relationships, especially when unacquainted animals first meet each other (Langbein and Puppe, 2004). The introduction of new animals into established groups often leads to an increase in agonistic behaviour as has been described for most farm animals, e.g. cows, pigs, sheep and goats (reviewed in Patt et al., 2012). In pigs, the intensification of animal production over the last decades has often resulted in a discrepancy between natural and artificial environment (Abeyesinghe et al., 2013). Especially for pregnant sows, regrouping or mixing of unfamiliar animals is difficult to avoid, leading to more aggressive behaviour, fights and injuries due to new dominance hierarchy establishment or adjustment (Meese and Ewbank, 1973; Arey and Edwards, 1998; Puppe, 1998; Arey, 1999; Kongsted, 2004; Rhim et al., 2015; Schalk et al., 2018). Social rank is one important factor contributing to sow welfare as evidence revealed that social status affects the neuroendocrine-immunological response as well as the reproductive performance of sows (Zanella et al., 1998; Hoy et al., 2009a; Zhao et al., 2013; Pacheco and Salak-Johnson, 2016; Schalk et al., 2019).

Considering this essential role of dominance assessment, it is surprising that no consistent approach exists in behavioural analysis. The concept of dominance has been developed continuously and several sociometric measures were introduced, but standardized recommendations of analysing social dominance have not been achieved, especially in farm animals (Langbein and Puppe, 2004). Thus, considerable inconsistencies in the used methodology may impair obtained results and interpretations.

It is important to recognise that previous studies only focused on behaviour associated with aggression, while socio-positive interactions or different competitive situations have rarely been included so far (Hemelrijk et al., 2005; Bayly et al., 2006; Puppe et al., 2008; Bang et al., 2010). Moreover, the influence of group stability and of observational situations (e.g. during competitive feeding or mixing) on comparability of different dominance indices is still poorly investigated.

The aim of the current study was to investigate the appropriate measurement of dominance relationships within different observational situations in pregnant sows with varying group stability. Therefore, the sows were housed either under constant social conditions or with frequent changes of group composition. We examined the correlations of four popular dominance indices based on different types of social behaviour in sows. In detail, we analysed what types of social interactions (aggressive only, or aggressive and affiliative) should be included into index calculation, and whether these indices based on different behaviours provide similar results. Additionally, we assessed whether the four indices lead to comparable results regarding the social hierarchy. Furthermore, we compared one prominent dominance index (ADI) based on aggressive interactions between different situations (standard, mixing and competitive feeding) to examine whether the calculated indices among these observational procedures are similar.

2. Material and methods

2.1. Animals and housing

The present study investigated the behaviour of 40 multiparous German Landrace sows (parity number 4.3 ± 1.8) in two independent replicates with 20 females each. Animals were kept in the same building under comparable and controlled environmental conditions according to the ethical and animal care guidelines (HOH 29/13, RP Tübingen, Germany). At the start of the experiment, they were 819.4 ± 319.5 days of age and weighed 229.9 ± 41.4 kg. The sows were kept at the Agricultural Experiment Station of the University of Hohenheim (Location Lindenhöfe, Eningen, Germany) in groups of five animals each, balanced by parity, age and body weight at beginning of the experiment.
The animals were housed in pens which provided an area of 15–17 m^2 with concrete flooring and solid wooden walls for visual protection. Straw and sawdust for bedding and manipulation were provided daily after removal of the soiled bedding material. Sows had *ad libitum* access to water by a nipple drinker and were fed restrictively once a day at 0830 h by trough feeding located at the front area of the pen with a barely-wheat-oat-based diet according to the actual requirements for pregnant sows. Body condition and health status of the sows were monitored regularly.

2.2. Experimental design

Animals were arranged in groups of five animals directly after service and were allocated to one of the two treatments (Not-mixed, NON-MT; Mixed, MT). Animals of both treatments were housed in this group composition over a period of four weeks. The sows of NON-MT groups stayed in constant group composition during the entire experiment, while MT groups were socially mixed (Figure 1). Over a period of eight weeks, between two MT groups an interchange of 2x2 randomly selected sows was performed twice a week (on Monday and Thursdays at 1030 h) starting on Thursday according to defined directives (Schalk et al., 2018). Prior to the mixing events, straw and sawdust were provided for diversion in both treatment groups. After a period of 16 mixing events, MT groups were again housed in their initial group composition. For individual identification, all sows were marked with a commercial coloured spray on back and both sides of the body twice a week (on Mondays and Thursdays at 0930 h). One MT sow and one NON-MT sow had to be excluded in the course of the experiment due to leg injuries and termination of pregnancy, respectively. No further medical treatment was necessary during the experiment.

2.3. Behavioural recording

Behaviour of animals was recorded by video cameras (Viewex-350/WS; Monacor International, Bremen, Germany) which were located on the ceiling above each pen and ensured a full view of the entire pen. The present study is based on social interactions of sows during three different situations:

Situation 1 (STD, observation for 4 h): To investigate a standard social situation, we chose a time point as distant as possible after the mixing events and where no feeding took place, i.e. 48 h after a social mixing or at corresponding time points.

Situation 2 (MIX, observation for 2 h): In order to investigate a situation which affords the opportunity to observe high numbers of aggressive interactions, the groups were observed directly after a social mixing in the MT groups (or at corresponding time-point in the NON-MT groups). As we knew from preliminary observations that interactions between animals decrease after 2 hours, we concentrated on the first 2 h after the mixing events. In addition, this time window provided the opportunity to directly compare 2 h observations to 4 h observations in the NON-MT groups.

Situation 3 (FEED, observation for 30 min): We included the observation of a feeding situation which represents a situation of special context and with high conflict potential.

The frequency of social interactions during the three different situations was analysed at defined time points during (4th and 12th mixing) and after (post mixing) the mixing phase (Figure 1). Observed behaviours included aggressive, affiliative and submissive interactions acted by one sow towards another receiving sow in the pen. Behaviours and their descriptions are listed in the ethogram shown in **Table 1** (modified after Jensen, 1980; O'Connell et al., 2004; Horback and Parsons, 2016).

2.4. Data analysis

The numbers and the outcomes of the recorded interactions per group (in its current composition) were transferred to 5x5 dyadic interaction matrices. Based on clear criterions for winner and loser three different variants were calculated as follows:

Variant 1 (V1): In order to investigate whether the consideration of aggressive behaviour independently of the reaction of the receiver affects the sociometric indices, we included all performed aggressive behaviour. The acting sow received a value of 1 for aggressive behaviour against another sow, independently of whether the receiver showed a reaction or not. The acting sow receives another value of 1 if the challenged sow withdrew, avoided or fled.

Variant 2 (V2): In this variant we set the strictest specifications by only including aggressions which were followed by submissive behaviour. The acting sow showed aggressive behaviour against another sow and received a value of 1 only if the challenged sow withdrew, avoided or fled.

Variant 3 (V3): To investigate whether the consideration of affiliative behaviour affects the sociometric indices, we additionally included affiliative behaviour followed by submissive behaviour. The acting sow showed aggressive or affiliative behaviour against another sow and received a value of 1 only if the challenged sow withdrew, avoided or fled.

2.5 Sociometric measures

Based on the obtained matrices, sociometric measures were calculated in order to analyse dominance relationships at the group level. For each interaction matrix variant, the four following dominance indices were determined by using DomiCalc (de Silva et al., 2017).

1. Average dominance index (ADI): Per pair of individuals, wij is calculated as the number of times a sow i won against another sow j (xij), divided by the total number of agonistic interactions between the two sows, thus wij = xij / (xij + xji). The ADI value of one individual is the average of all its dominance indices with all its interaction partners and varies from 0 to 1, with a higher value indicating a higher dominance rank in the group (Zumpe and Michael, 1986; Hemelrijk et al., 2005).

$$ADI = \frac{1}{n} \sum w_{ij}$$

2. Individual dominance value (DV): The DV is calculated for each sow by the number of wins (W) minus defeats (D) in relation to all fights with other sows in the group over the whole observation period, and varies from -1 (no wins) to 1 (no defeats) (Tuchscherer et al., 1998).

$$DV = (W – D) / (W + D)$$

3. *David`s score (DS):* The DS is calculated according to the following formula:

$$DS = w + w_2 – l – l_2$$

The proportion (Pij) of wins by sow i in its interactions with another sow j is the total number of i defeats j (aij) divided by the total number of interactions between sow i and sow j (nij), thus Pij = aij / nij. The proportion of losses of sow i to sow j is calculated as Pij = 1 - Pij. w represents the sum of proportions of wins by the focal sow, w2 represents the sum of weighted proportions of wins of the individuals against whom the focal sow has won. l represents the sum of proportions of losses by the focal sow, l2 represents the sum of weighted proportions of losses of the individuals against whom the focal sow has lost (Gammell et al., 2003; Hemelrijk et al., 2005; Bang et al., 2010).

4. Rank index (RI): The rank index is determined for each sow based on wins and defeats and the number of other sows in the group pen where w is the number of wins, pw is the number of other sows in the group pen against the sow has won, d is the number of defeats, pd is the number of other sows in the group pen against the sow hast lost and n is the total group size. This index varies from -1 to 1 with a higher value indicating a higher dominance rank in the group (Hoy, 2009; Hoy et al., 2009b).

$$RI = (w * p_w) – (d * p_d) / ((w + d) * (n – 1))$$

For each of the determined interaction matrix variants, the four introduced dominance indices were calculated, respectively, so three different values of each dominance index per sow were obtained altogether (**Table 2**). If an individual was not involved in any social interaction (ADI, RI and DV: index was mathematically not defined, DS: index revealed an invalid value of zero) it was excluded from analysis.

2.6. Statistical analyses

For statistical analyses R programming language version 3.1.0 was used (R Development Core Team). Data sets were analysed separately for treatment and for each observation time-point. In order to compare indices based on different types of observed social interactions, each pair of interaction matrix variants was correlated using Spearman`s rank correlation within each calculated index (V1 vs. V2, V1 vs. V3, V2 vs. V3 for ADI, DV, DS and RI, respectively). In addition, a Wilcoxon rank sum test was applied to compare the determined correlation coefficients between treatments (NON-MT vs. MT).

Furthermore, Spearman`s rank correlations were performed to analyse whether all measured indices within each variant are comparable (ADI vs. DV, ADI vs. DS, ADI vs. RI, DV vs. DS, DV vs. RI and DS vs. RI for V1, V2 and V3, respectively). In order to detect possible agreements among indices of different behavioural observation situations and durations Spearman`s rank correlation was performed using the calculated indices by ADI of interaction matrix variant 1 (STD vs. MIX, MIX vs. FEED, FEED vs. STD only for ADI of V1). All data are expressed as correlation coefficients. Comparisons were considered significant at $p < 0.05$ and a tendency at $p < 0.1$.

3. Results

3.1. Influence of observed social interactions: comparison of dominance indices between matrix variants

In order to analyse whether the same indices based on different social behaviours (aggressive only, or aggressive and affiliative) provide similar results, Spearman`s rank correlation coefficients attributed for each dominance index between all analysed interaction matrix variants (V1 vs. V2, V1 vs. V3, V2 vs. V3) were calculated. This was done for the two observational situations STD (= standard social situation as distant as possible after the mixing events) (**Table 3**) and MIX (= directly after a social mixing) (**Table 4**). In the STD situation, each index comparison revealed significant positive and consistent correlations for NON-MT and MT sows ($p < 0.05$; NON-MT: $\rho = 0.49 – 1.00$; MT: $\rho = 0.83 – 1.00$) for each time-point (**Table 3**).

Similar within the MIX situation, highly significant Spearman`s rank correlations were observed for MT sows ($p < 0.05$; $\rho = 0.90 – 1.00$) at each time-point. Highly significant positive correlations ($p < 0.05$; $\rho = 0.74 – 1.00$) were also recorded for NON-MT sows, except for the comparison of DS between V1 and V2 as well as V1 and V3 which revealed only a tendency ($p < 0.1$; V1 vs. V2: $\rho = 0.58$; V1 vs. V3: $\rho = 0.51$) at the corresponding 12th mixing time-point and no agreement ($p > 0.1$; $\rho = 0.36 – 0.53$) between V1 and V3 for each dominance index post mixing (Table 4). A closer analysis of these results included the effect of treatment by pair-wise comparisons of received correlation coefficients between the three observation variants (V1 vs. V2, V1 vs. V3, V2 vs. V3) for all dominance indices between NON-MT and MT groups during mixing treatment (Supplemental Fig. 1A and 1B). Coefficients of MT groups were higher during the mixing phase at STD and MIX after the 4th and 12th mixing. After mixing treatment (post mixing), MT groups showed higher values ($p < 0.001$) at STD and a tendency ($p < 0.1$) at MIX situation.

At MIX situation, NON-MT groups in particular showed higher numbers of missing indices at post mixing, as well as a high proportion of unknown dyadic relationships of 52.5 – 92.5% (data not shown).

3.2. Influence of index choice: comparison of dominance indices within each variant

In order to assess whether all introduced indices lead to a comparable social hierarchy, Spearman`s rank correlation between pairs of dominance indices were conducted within each interaction matrix variant (V1, V2, V3), respectively. Again, results for NON-MT and MT groups at STD (**Table 5**) and MIX (**Table 6**) situation were tested.
During STD situation, the coefficients varied from 0.78 – 0.99 for NON-MT and from 0.84 – 0.99 for MT groups.
Within MIX situation, comparable coefficients were found between pairs of dominance indices, ranging from 0.75 – 1.00 for NON-MT and from 0.78 – 1.00 for MT groups, although behaviour was analysed only for 2 h.
Independent of respective index pairs or interaction matrix variant, all tested relationships were calculated as positively correlated and significant for both treatment groups during the complete experimental phase ($p < 0.001$; except for few comparisons at MIX situation: NON-MT (12^{th} mixing, post mixing), MT (post mixing): $p < 0.01$).

3.3. Influence of observational procedures: comparison of the average dominance index for different observation situations and durations

Results showed that all indices and variants revealed an evident comparability (see 3.1. and 3.2.). This part of the analysis was carried out only for dominance values calculated by ADI following interaction matrix variant 1 (V1) for different observation situations (STD vs. MIX; MIX vs. FEED; FEED vs. STD), in order to examine whether the calculated indices are similar between these observational procedures (**Table 7**). Overall, Spearman`s rank correlation revealed inconsistent correlations and variation between the situations compared. Depending on behavioural situation, treatment and observation time-point, coefficients varied between 0.10 – 0.88. Agreement was best for FEED vs. STD, all receiving consistent significant correlations ($p < 0.05$) for NON-MT (ρ: 0.57 – 0.75) and MT groups (ρ: 0.51 – 0.88) post mixing as well as during the mixing phase.

NON-MT groups showed a significant correlation of ADI following V1 for the corresponding 4th mixing time-point at STD vs. MIX (ρ: 0.82) and at MIX vs. FEED (ρ: 0.84). Calculated indices were not comparable at STD vs. MIX and at MIX vs. FEED at the corresponding time-points 12th mixing and post mixing.

With the beginning of the mixing phase significant correlations ($p < 0.05$) were found for sows of MT groups at STD vs. MIX (ρ: 0.55 – 0.68) at 4th and 12th mixing, but at MIX vs. FEED only at 4th mixing (ρ: 0.71). In contrast, after the mixing phase there was no agreement for both of these observed situations ($p > 0.1$).

4. Discussion

In the present study, high correlations between four frequently used dominance indices were found and all indices resulted in a comparable social hierarchy. To our knowledge, this is the first study demonstrating the influence of both group stability and observational procedures (i.e. observed behavioural pattern, duration or situation) on comparability of indices in sows used as a model system.

4.1. Influence of group stability on index comparability

Two experimental settings were used to analyse whether constant social conditions or frequent changes of group composition affect index comparability differently. In general, all indices were applicable at STD situation, as all results were consistent for MT and NON-MT sow groups and high correlations could be verified during all experimental phases. Analysis of the MIX situation yielded high correlation values as well. At the post-mixing time-point, however, no correlations were found in NON-MT sows for one interaction matrix variant (V1 vs. V3) at all dominance indices. This is further supported by the fact that, compared to MT sows, NON-MT sows showed lower correlation coefficients at all time-points, which indicates an influence of group stability on dominance index outcome.

In pigs, most aggressive interactions occur within the first hours after regrouping and rank stability is normally reached after two to ten days (Arey and Edwards, 1998). This may explain why we observed agonistic interactions among all sows (or at least between most dyads) sufficient to produce significant correlations for the MT groups. In contrast, the presumably stable dominance hierarchy in NON-MT sows led to a reduction of agonistic interactions, involving the risk of a high proportion of unknown dyadic relationships and data scarcity (Büttner et al., 2019) which may have affected index comparability.

4.2. Influence of observed behaviour on index comparability

The interaction matrix variants (V1 vs. V2, V1 vs. V3, V2 vs. V3) were mostly correlated for all tested indices. However, as mentioned above the comparison between aggressive only (variant 1) and aggressive with affiliative followed by submissive behaviour (variant 3) revealed least comparability within dominance indices in NON-MT sows at post-mixing time point.

One explanation might be, that depending on species, entirely aggressive interactions might not necessarily represent the absolutely highest proportion of social behaviour (da Silva et al., 2016). Affiliative behaviours appear to be used for stabilisation of social relationships, especially in well-established groups of animals, which was already shown in primates (Ryan and Hauber, 2016). In animals other than primates, little attention has been paid to socio-positive relationships. Hemelrijk et al. (2005) showed that hierarchy correlations are weaker at a lower intensity of aggression, as it might also be the case at the end of the experimental phase due to the familiarity of the group members (Puppe, 1998). This might also have led to the increased number of sows with no interactions in this study.

Previous studies in pigs and goats revealed that animal groups regulate their aggression level by an avoidance order, and that newly-introduced animals often avoid contact, which diminishes the aggressive outcome in social interactions (Jensen, 1982; Patt et al., 2012). Although data showed that the ratio of observed interactions to individuals required to infer reliable hierarchies is low (at least 10, ideally 20 interactions) (Sánchez-Tójar et al., 2017), a high number of unknown or tied dyads is a common problem (Büttner et al., 2019). As this represents a limiting factor in behavioural datasets to generate comparable results (Klass and Cords, 2011; Neumann et al., 2011; Douglas et al., 2017), it should obviously be avoided.

In case of animal species which develop additional behavioural mechanisms to regulate their social relationships by establishing and maintaining a hierarchy without the extensive use of overt agonistic behaviour (Puppe et al., 2008; Büttner et al., 2015a), we would therefore like to particularly emphasise the importance of observed behaviour type as well as the inclusion of socio-positive interactions in dominance measurement.

Additionally, compared to pigs at a younger age, sow groups show a high number of unknown relationships and a low percentage of performed mutual agonistic interactions (Puppe et al., 2008; Büttner et al., 2015a). Furthermore, sows are in a state of advanced pregnancy at the last observation points. In this gestational phase sows naturally do not frequently interact but separate from other group members while focusing on nest-building behaviour (Kurz and Marchinton, 1972; Wischner et al., 2009).

In the present study, poorer correlations due to missing values led to our recommendation to include affiliative behaviour (variant 3) or to increase observation time when only aggressive interactions (variant 1) are observed.

4.3. Influence of observational duration on index comparability in NON-MT sows

In this study, the direct comparison of a 2 hour observation period (referring to the corresponding MIX situation) and a 4 hour observation period (referring to the corresponding STD situation) was possible in sows with a persisting stable group composition (NON-MT sows), because the social situation was the same for MIX and STD. It is interesting to note that lower or even no correlations between interaction matrix variants occurred only after an observational duration of 2 hours (referring to the corresponding MIX situation), while analysis over 4 hours (referring to the corresponding STD situation) revealed similar dominance indices. The most likely explanation might be that the double length of the observational session provides sufficient numbers of social interactions and, as a consequence, reduces the risk of unknown or tied relationships. A study in rhesus macaques also showed that, depending on the respective behaviour, different hours of observation are required to reveal reliable social networks (Feczko et al., 2015). In addition, pigs show a biphasic activity peak in the morning and afternoon (Hoy, 1998). As behavioural analysis in this study always started at forenoon, we can assume that for the 4 hours observation at least one activity peak was included.

It is therefore recommended that dominance measurement includes an observation period that involves species-typical activity behaviour. Moreover, analysis should consider the fact that different behaviours require different amounts of data, e.g. analysis of aggressive behaviour in groups with an established social hierarchy requires more observation time than in groups with instable social conditions.

4.4. Influence of observational situation on index comparability

Dominance assessment by continuous observation of social behaviour between all animals over a certain period is a very time-consuming procedure. As a consequence, some authors use competition tests to match each individual with each other in a competitive situation, forcing animals to show agonistic interactions (Benton et al., 1980; Ellard and Crowell-Davis, 1989; Langbein and Puppe, 2004). Some studies showed that dominance ranks calculated by paired-feeding competition tests or even by daily feed order are almost similar to those measured by social interactions in a group situation (Ellard and Crowell-Davis, 1989; Brouns and Edwards, 1994; Kranendonk et al., 2007; Horback and Parsons, 2016). However, other studies failed to show a clear relation between dominance and feeding performance (Veiberg et al., 2004; Kidjo et al., 2016), because it might not always be the most dominant or aggressive animal which has the first access to food, but the most motivated (Drews, 1993). The outcome of these tests may differ from results obtained by continuous observation because they deliver only a competitive order and have to be carefully interpreted in the specific context of acquiring an essential resource (McGlone, 1986).

In this study, high correlations were found for the comparison between a standard social and a feeding situation for both treatment groups, indicating that dominance indices calculated by shorter observations (30 min in the present study) while acquiring an essential resource (e.g. food) are comparable to a 4 hours observation period of a standard social situation. Moreover, during feeding nearly every sow was included in social interactions and the comparison of the standard social and a feeding situation revealed no missing values irrespective of treatment. To include an observational situation like feeding may therefore help to sharpen or clarify the dominance position of an animal, especially in groups with an already established hierarchy and low levels of social interaction.

4.5. Further issues

From our findings, we are able to draw conclusions about the comparability of the analysed indices. Whether one index can lead to a more precise image of the real hierarchy than another is a question which can only be answered by comparing them to theoretical models in future studies. We would like to point out that the dominance measurements investigated in this study are of medium complexity by considering relational aspects. This is a clear advantage over simpler indices, because a single event of winning or losing is unlikely to have a great effect on the outcome (Hemelrijk et al., 2005). We therefore presume that factors like group stability and observational procedures have an even greater effect on the comparability of indices based on more simple ranking methods. The influence on indices with higher complexity (e.g. Elo-Rating) has to be further evaluated.

5. Conclusion

The four rank indices of medium complexity which were validated in the present study were comparable and equally applicable for dominance relationship analysis in pregnant sows under most conditions. We showed that sufficient numbers of social interactions and low values of unknown relationships are an important prerequisite to generate reliable data for dominance measurement. In order to avoid the risk of unknown relationships, particularly in groups with an established dominance hierarchy and only a few interactions between individuals, it is advantageous to include not only aggressive but also social interactions in dominance measurements (variant 3). In addition, it is also advisable to observe interactions with a further resource (e.g. a feeding situation). These suggestions are beneficial as more precise information for dominance rank calculations can be achieved without extended observation time.

We presume that these finding are also transferable to other group-housed livestock species, as the situations observed in the present study (constant social conditions, frequent changes of group composition or competitive feeding) were also described for most farm animals, e.g. cattle, horses, sheep and goats. A more standardized approach to analyse social dominance in farm animals will make it easier to compare various studies within or between livestock species (Langbein and Puppe, 2004). This may help to sharpen the knowledge on social behaviour and dominance relationships of farm animals in order to improve the scientific value of future studies on the effects of social dominance on physiological responses, in particular with regard to welfare concerns.

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Table 1
Ethogram of aggressive, affiliative and submissive behaviours observed in gestating sows from video recordings.

Behaviour	**Description**
Aggressive	
Biting	Biting with teeth at another sow`s head and body. Mouth of the acting sow is open. The attempt is also evaluated.
Head-to-body/head knocking	A rapid heavy thrust or push upwards or sideways with head or snout against another sow`s body or head.
Parallel pressing	Two sows standing side by side and push their shoulders and bodies against each other. With or without biting.
Inverse parallel pressing	Two sows standing face front to front and push their shoulders, bodies and heads against each other. With or without biting and head-to-head knocking.
Following / Chasing	Moving at a walking or running pace more than 3 steps in pursuit of another sow and reducing the distance between both animals to less than 1 m. The receiver sow withdraws or flees.
Displacing	Forcing another sow to leave and avoid its current location, lying place, trough or drinker through only by appearance without any physical contact. The receiver sow avoids.
Displacing during feeding	Forcing another sow to leave the trough during feeding, with or without biting, knocking or pressing. The receiver sow withdraws.
Affiliative	
Nose-to-nose contact	Approaching another sow with nose or snout on nose, snout, head or ears at a close distance (< 10 cm). With or without physical contact.
Nose-to-body contact	Approaching another sow with nose or snout on any part of body with at a close distance (< 10 cm). With or without physical contact.
Ano-genital nosing	Approaching another sow with nose or snout on the genital region with at a close distance (< 10 cm). With or without physical contact.
Submissive	
Avoiding	Result of “displacing”. Leaving and avoiding (> 2 steps) the current location, lying place, trough or drinker caused only by another sow`s appearance, not by physical contact.
Withdrawing	Possible result of any aggressive or affiliative behaviour. Moving away (> 2 steps) from another sow at a walking pace.
Fleeing	Possible result of any aggressive or affiliative behaviour. Moving away (> 3 steps) from another sow at a running pace.

Table 2
Overview of sociometric measures based on different behavioural patterns (aggressive or aggressive and/or affiliative followed by submissive behaviour) between individual A and individual B resulting in three variants of interaction matrices (V1, V2, V3). For each of the determined interaction matrix variants, four dominance indices (average dominance index (ADI), dominance value (DV), David´s score (DS) and rank index (RI)) were calculated, respectively, leading to three different values of each dominance index.

Behaviour		Values for A		
		Variant 1	Variant 2	Variant 3
A → B	B → A			
Aggressive	no reaction/ non-submissive	1	---	---
Aggressive	submissive	1 + 1	1	1
Affiliative	no reaction/ non-submissive	---	---	---
Affiliative	submissive	---	---	1
		↓	↓	↓
Matrix		Interaction matrix (V1)	Interaction matrix (V2)	Interaction matrix (V3)
		↓	↓	↓
Index		ADI DV DS RI	ADI DV DS RI	ADI DV DS RI

Table 3

STD situation: Values of Spearman`s rank correlation coefficients rho (ρ) between pairs of the three observation variants (V1 vs. V2, V1 vs. V3, V2 vs. V3) in pregnant not-mixed (NON-MT) and mixed (MT) sows for each dominance index. Behaviour was analysed for 4 h at 48 h after the 4th and 12th mixing, and for 4 h at the corresponding time-point after the mixing phase (post mixing), respectively.

	4th mixing				**12th mixing**				**Post mixing**			
	ADI	DV	DS	RI	ADI	DV	DS	RI	ADI	DV	DS	RI
NON-MT												
n	*19*	*19*	*19*	*19*	*19*	*19*	*19*	*19*	*18*	*18*	*18*	*18*
V1 vs. V2	0.92	0.92	0.98	0.99	0.87	0.89	0.85	0.91	0.86	0.87	0.81	0.77
V1 vs. V3	0.84	0.83	0.88	0.89	0.89	0.91	0.86	0.92	0.75	0.78	0.49	0.65
V2 vs. V3	0.91	0.89	0.92	0.90	0.94	0.92	0.97	0.97	0.91	0.91	0.68	0.86
MT												
n	*19*	*19*	*19*	*19*	*20*	*20*	*20*	*20*	*19*	*19*	*19*	*19*
V1 vs. V2	0.98	0.98	0.97	0.97	0.90	0.98	0.93	0.97	0.97	0.97	0.95	0.97
V1 vs. V3	0.94	0.91	0.97	0.96	0.90	0.98	0.93	0.97	0.91	0.92	0.83	0.91
V2 vs. V3	0.96	0.92	0.99	0.97	1.00	0.99	1.00	0.99	0.94	0.94	0.84	0.95

All comparisons significant ($p < 0.05$).

Table 4

MIX situation: Values of Spearman`s rank correlation coefficients rho (ρ) between pairs of the three observation variants (V1 vs. V2, V1 vs. V3, V2 vs. V3) in pregnant not-mixed (NON-MT) and mixed (MT) sows for each dominance index. Behaviour was analysed for 2 h directly after the 4th and 12th mixing, and for 2 h at the corresponding time-point after the mixing phase (post mixing), respectively.

	4th mixing				**12th mixing**				**Post mixing**			
	ADI	DV	DS	RI	ADI	DV	DS	RI	ADI	DV	DS	RI
NON-MT												
n	*18*	*18*	*18*	*18*	*12*	*12*	*12*	*12*	*5*^{n}	*5*^{n}	*5*^{n}	*5*^{n}
V1 vs. V2	0.87	0.92	0.97	0.99	0.79	0.83	0.58^{t}	0.88	1.00	1.00	0.92	0.92
V1 vs. V3	0.93	0.94	0.94	0.93	0.77	0.79	0.51^{t}	0.82	0.53^{ns}	0.53^{ns}	0.36^{ns}	0.50^{ns}
V2 vs. V3	0.74	0.81	0.91	0.91	0.90	0.90	0.93	0.95	0.91	0.91	0.92	0.92
MT												
n	*20*	*20*	*20*	*20*	*18*	*18*	*18*	*18*	*6*	*6*	*6*	*6*
V1 vs. V2	0.97	0.95	0.96	0.98	0.97	0.97	0.98	0.96	0.90	0.94	0.91	0.95
V1 vs. V3	0.94	0.92	0.99	0.98	0.93	0.93	0.96	0.92	0.90	0.94	0.91	0.95
V2 vs. V3	0.95	0.96	0.95	0.97	0.96	0.96	0.96	0.97	1.00	1.00	1.00	1.00

Comparisons are significant ($p < 0.05$), except for a tendency^{t} ($p < 0.1$) and for not significant^{ns} ($p > 0.1$).
^{n} = 6 animals were included in analysis between V1 vs. V3, as one sow received no index for V2.

Table 5
STD situation: Spearman`s rank correlation coefficients between pairs of all dominance indices for each of the three observation variants (V1, V2, V3) for not-mixed (NON-MT) and mixed (MT) sows, respectively. Data includes behavioural observations for 4 h at 48 h after 4th and 12th mixing, and for 4 h at the corresponding time-point after the mixing phase (post mixing), respectively.

	4th mixing			12th mixing			Post mixing		
NON-MT	V1	V2	V3	V1	V2	V3	V1	V2	V3
n	*19*	*19*	*19*	*20*	*19*	*19*	*19*	*18*	*18*
ADI vs. DV	0.94	0.97	0.98	0.97	0.99	0.99	0.97	0.99	0.99
ADI vs. DS	0.91	0.81	0.81	0.92	0.92	0.94	0.86	0.89	0.86
ADI vs. RI	0.86	0.81	0.81	0.93	0.92	0.94	0.89	0.90	0.91
DV vs. DS	0.84	0.78	0.78	0.91	0.90	0.93	0.86	0.89	0.80
DV vs. RI	0.88	0.82	0.80	0.91	0.92	0.95	0.89	0.90	0.90
DS vs. RI	0.96	0.97	0.99	0.97	0.96	0.96	0.97	0.96	0.90
MT									
n	*19*	*19*	*19*	*20*	*20*	*20*	*19*	*19*	*19*
ADI vs. DV	0.97	0.97	0.94	0.92	0.95	0.96	0.97	0.98	0.97
ADI vs. DS	0.90	0.89	0.95	0.96	0.93	0.93	0.95	0.92	0.94
ADI vs. RI	0.88	0.88	0.95	0.95	0.94	0.93	0.95	0.95	0.91
DV vs. DS	0.86	0.86	0.93	0.88	0.88	0.89	0.94	0.91	0.89
DV vs. RI	0.84	0.86	0.94	0.95	0.92	0.94	0.97	0.95	0.91
DS vs. RI	0.99	0.97	0.99	0.94	0.97	0.96	0.96	0.93	0.93

Table presents relationships which all were calculated as highly significant (***$p < 0.001$).

Table 6
MIX situation: Spearman`s rank correlation coefficients between pairs of all dominance indices for each of the three observation variants (V1, V2, V3) f not-mixed (NON-MT) and mixed (MT) sows, respectively. Data includes behavioural observations during mixing treatment for 2 h directly after 4^{th} and 1 mixing, and for 2 h at the corresponding time-point after the mixing phase (post mixing), respectively.

	4^{th} mixing			12^{th} mixing			Post mixing		
NON-MT	V1	V2	V3	V1	V2	V3	V1	V2	V3
n	*18*	*18*	*18*	*12*	*12*	*15*	*6*	*5*	*6*
ADI vs. DV	0.95	1.00	0.96	0.93	1.00	1.00	1.00	1.00	1.00
ADI vs. DS	0.95	0.85	0.94	0.86	0.94	0.94	0.90^{x}	0.97^{x}	0.94^{x}
ADI vs. RI	0.93	0.86	0.94	0.92	0.94	0.97	0.90^{x}	0.97^{x}	0.98
DV vs. DS	0.89	0.85	0.91	0.75^{x}	0.94	0.94	0.90^{x}	0.97^{x}	0.94^{x}
DV vs. RI	0.89	0.86	0.92	0.96	0.94	0.97	0.90^{x}	0.97^{x}	0.98
DS vs. RI	0.97	0.98	0.97	0.82^{x}	0.99	0.96	1.00	1.00	0.95^{x}
MT									
n	*20*	*20*	*20*	*19*	*18*	*19*	*8*	*6*	*6*
ADI vs. DV	0.95	0.98	0.97	0.99	0.99	0.98	0.99	1.00	1.00
ADI vs. DS	0.93	0.91	0.95	0.85	0.86	0.83	0.96	0.94^{x}	0.94^{x}
ADI vs. RI	0.94	0.92	0.96	0.89	0.92	0.85	0.96	0.95^{x}	0.95^{x}
DV vs. DS	0.89	0.91	0.93	0.82	0.82	0.78	0.96	0.94^{x}	0.94^{x}
DV vs. RI	0.92	0.94	0.96	0.88	0.90	0.82	0.96	0.95^{x}	0.95^{x}
DS vs. RI	0.99	0.98	0.98	0.97	0.96	0.98	0.99	0.99	0.99

Table presents relationships which all were calculated as highly significant (***$p < 0.001$), except for ^{x} (** $p < 0.01$).

Table 7
Comparison of ADI (average dominance index) for interaction variant 1 (V1) between different observation situations: 2 h directly after mixing (MIX), 48 h after mixing (STD) and during feeding, 72 h after mixing (FEED) for not-mixed (NON-MT) and mixed (MT) sows. Data includes behavioural observations during mixing phase (after 4th and 12th mixing), and at the corresponding time-point after the mixing phase (post mixing), respectively.

	4th mixing		12th mixing		Post mixing	
	n	rho	*n*	rho	*n*	rho
NON-MT						
STD vs. MIX	*17*	0.82***	*12*	0.25^{ns}	*6*	0.10^{ns}
MIX vs. FEED	*18*	0.84***	*11*	0.21^{ns}	*6*	0.11^{ns}
FEED vs. STD	*19*	0.75***	*19*	0.57*	*19*	0.72***
MT						
STD vs. MIX	*19*	0.68**	*19*	0.55*	*8*	0.39^{ns}
MIX vs. FEED	*20*	0.71***	*19*	0.44^{t}	*12*	0.55^{ns}
FEED vs. STD	*19*	0.78***	*20*	0.88***	*19*	0.51*

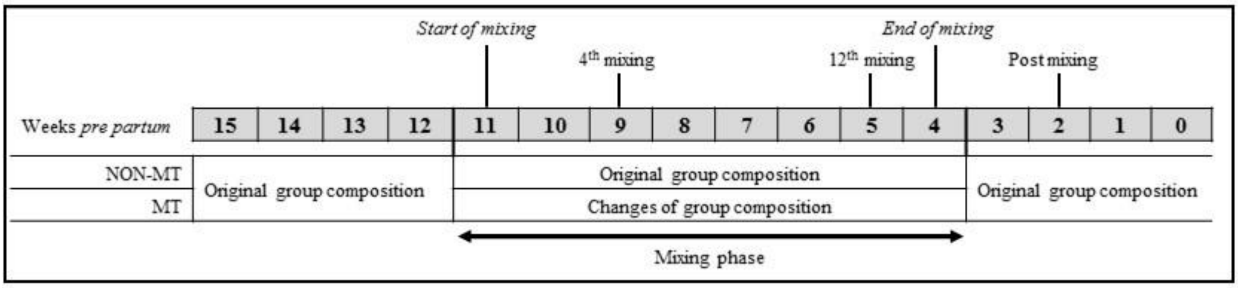
Table presents relationships which were calculated as significant at $p < 0.05$ and $p < 0.1$ as a tendency.

Figure captions

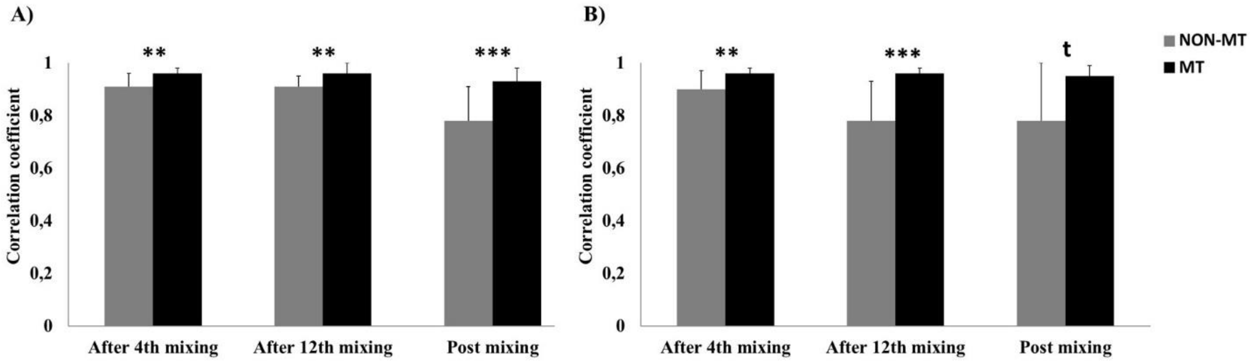
Figure 1. Scheme of the experimental design for pregnant sows with different group stability, housed either under constant social conditions (not mixed, NON-MT) or with frequent changes of group composition (mixed, MT) during pregnancy.

Supplemental Figure 1. Mean (±SD) of correlation coefficients for not mixed (NON-MT, grey bars) and mixed (MT, black bars) sows. Data was calculated at comparison of every interaction matrix variant within each index, (A) 48h after mixing (STD situation) and (B) directly after mixing (MIX situation), during mixing treatment (after 4th and 12th mixing) and at a corresponding time-point after mixing treatment (post mixing), respectively. Asteriks indicate significant differences between coefficients for NON-MT and MT groups: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure 1



Supplemental Figure 1



CHAPTER 4

GENERAL DISCUSSION

4 GENERAL DISCUSSION

4.1 GENERAL INTRODUCTION

The commercial pig production in industrialized developed countries has changed from small family-run farms to production units over the last decades. Along with these changes, arising interest and awareness of animal welfare issues among consumers supported by policy pose substantial challenges for today's pig production. In the European Union, which is responsible for approximately 25% of the world's pig production (EUROSTAT dated May 2019), new legislation during the last years established new housing standards for animal welfare reasons which are not easily fulfilled under the present economic conditions and environmental impacts.

In terms of pregnant sows, the animal welfare legislation prohibited the use of gestating crates since 2013. As sows are known to be social animals that prefer living in groups with well-established social structures and dominance hierarchies, this practice was intended to have a positive effect on animal welfare and to prevent or solve problems inherent to industrial farming so far. Group-housing allows sows to move and to be more active, gives more opportunity for social contact and to spatially separate defecating, eating and resting according to their biology. However, the costs of group-living refer to aggressive encounters in order to establish a dominance hierarchy possibly generating social stress with negative impacts on sows' immunity and health. Moreover, as this required housing standards led to increased costs in commercial swine production, an alarming decline of sow population in Germany of 17.4% from 2.3 to 1.8 million (EUROSTAT dated May 2019) was recorded during the last decade and a further downward trend is expected.

The overall impact of group-housing on welfare is less clear, especially in case of housing sows in dynamic group systems, as the increase in aggressions and the resulting consequences have to be weighed against the increased mobility and expression of natural behaviors. Therefore, there is a growing interest whether this type of housing pregnant sows in groups serves the intended goal of animal welfare and how it may influence physiology and productive performance of sows. Especially with regard to future requirements in sow management and actual disease challenges, a detailed approach measuring behavioral and physiological responses is required to assess the consequences of a dynamic group-housing environment on pregnant sows. This is not only of substantial importance for livestock research in general, but also for pig producers in order to improve health, longevity and productivity of sows and increase animal welfare and profitability for future livestock production.

4.2 MAIN FINDINGS AND RELEVANCE FOR GROUP-HOUSED PREGNANT SOWS

In pig husbandry, frequent changes of group composition by mixing or grouping of unfamiliar animals is a common practice during the entire production cycle. In general, the timing of mixing is determined predominantly by the piggery management system. In most commercial housing settings, all sows are mixed at least once during the production cycle mainly after weaning and/or service. Furthermore, when sows are kept in a dynamic group-housing system, the composition changes regularly by continuous introduction and removal of pregnant sows of different gestational stages. In contrast to their wild ancestors and non-mixed or stable housed domestic sows, fighting and aggression between domesticated sows is relatively common in commercial housing settings.

Social instability created by frequent changes of group composition and rank difference in a social group may represent stressful conditions for mammalian females of many species (Capitanio and Cole, 2015; Sachser et al., 1998; Stefanski, 2000). The present thesis investigated whether social mixing acts as social stressor influencing behavior as well as immunity of group-housed pregnant sows and whether pregnancy-associated alterations of immune cells can be modified by social status (MANUSCRIPT 1 AND 2).

4.2.1 IMPACT ON BEHAVIOR, STRESS HORMONES AND IMMUNE SYSTEM

Social mixing resulted in increased aggressive interactions immediately thereafter in mixed sows (MANUSCRIPT 1). Notably, 48 h later mixed sows still showed higher frequencies of aggressions than non-mixed sows. Compared to data of Barnett et al. (1996) who observed an average of 40 aggressions in groups of 4 sows only in the first 15 min after mixing, the level of aggression of mixed sows in the present thesis was rather low, but is in accordance with Poletto et al. (2014) and Couret et al. (2009b) who found similar frequencies after mixing pregnant sows. Levels of fighting in sows are described to be highly variable and even depend on individual characteristics of the observed animal (Arey and Edwards, 1998; Krauss and Hoy, 2011; Verdon et al., 2016), which may explain those differences. In addition, agonistic behavior might be a heritable trait (Løvendahl et al., 2005) or can also be affected by feeding system, sow size, previous exposure to group members, group size or pen design and, therefore, may also have triggered the number of aggressions (McGlone, 1986; Puppe, 1998; Weng et al., 1998).

The present results suggest that it may take up to several days after mixing before a new dominance hierarchy is fully established (Arey and Edwards, 1998), leading to the assumption that sows have to re-establish or adjust a social hierarchy by agonistic interactions with each change of group composition (Meese and Ewbank, 1973; Ringgenberg et al., 2012). Moreover, due to the mixing protocol sows had had contact with each other after a few mixings and it is likely to assume that they remembered each other individually (Spoolder et al., 1996; Spoolder et al., 2009; Nawroth et al., 2019). These findings clearly show that dominance relationships need to be reasserted even after mixing of familiar sows.

A further indicator of the negative consequences of social mixing is the accumulation of skin lesions from fighting and received bullying (Turner et al., 2006). Interestingly, although frequencies of aggressions and threats didn't reach excessive levels, an increase of skin lesions was proven in mixed sows of the present thesis (ANNEX 1). Previous studies on group-housed sows also demonstrated injuries and skin lesions as a consequence of social mixing (Couret et al., 2009b; Li and Gonyou, 2013; Ison et al., 2014; Knox et al., 2014; Poletto et al., 2014; Bos et al., 2016) and described them as a direct measure of aggression, which correlate with the incidence of aggressive events (Barnett et al., 1992). This indicates that injurious aggressive behavior appears not only in the first hours after mixing. It is known that pigs show an activity peak not only in the morning but also in the afternoon (Hoy, 1998; Marchant-Forde, 2009), though it seems possible that further fighting also occurred after behavioral observations.

It should be made clear that the results of MANUSCRIPT 1 cannot differentiate for contexts or motivations of aggressive behavior between the individual sows, but it seems likely that group-housing may offer the potential for subdominants to improve their social status while dominants have to defend their own social status irrespective of familiarity. In this context, it is interesting to note that there was no relation between social mixing and social status for numbers of aggressive interactions, as statistical analyses revealed no interaction between mixing treatment and rank position. Generally, dominant animals initiate aggressive interactions more often than subordinates (Creel, 2001; DeVries et al., 2003) and it was also shown for sows that dominant individuals are more aggressive while submissive sows receive more aggressions (O'Connell et al., 2003; Elmore et al., 2011; Pacheco and Salak-Johnson, 2016; Salak-Johnson, 2017).

However, as the number of mixings increased, levels of aggression were lower with every regrouping (MANUSCRIPT 1). This type of decrease was also recorded in other studies on pigs and calves (Veissier et al., 2001; Coutellier et al., 2007). A decline in agonistic behavior could be explained by the fact that especially sows seem to develop additional behavioral mechanisms and adopt new coping strategies to form social hierarchies faster with less agonistic behavior due to prior social experience in order to limit energy costs (Hessing et al., 1994; Veissier et al., 2001; Puppe et al., 2008; Büttner et al., 2015; Rhim et al., 2015).

Aggressive behaviors seem to be an adaptive strategy used to establish a dominance hierarchy which subsequently should promote stability within a group, thereby leading to a social rank order with high, middle and low ranking sows (Hoy et al., 2009b; Krauss and Hoy, 2011; de Boer et al., 2016; Verdon et al., 2016; Rault, 2017). However, problems may occur when the social structure of a group is disrupted or is frequently changed and animals are not able to avoid or escape aggression during the adjustment of the hierarchy (Puppe, 1998; Rault, 2017). Especially if the required adaptability of the animal is high and nearly reaches its limits, their health status or welfare may be impaired (Ohl and van der Staay, 2012). Repeated conflict in unstable social groups was not only shown to increase the risk of injuries, divert energy from reproductive activities, disrupt physiological and circadian rhythms and cause gonadal atrophy and adrenal hypertrophy, but also to compromise the immune system in rodents (Fleshner et al., 1989; Stefanski, 2001; de Boer et al., 2016). Besides, an individual's position in the dominance hierarchy accompanied by the attempt to increase or decrease the rank position can also cause social stress and influence performance and the immune system. The results described in MANUSCRIPT 1 and 2 were found to support these assumptions especially on the immune system.

But before discussing these results, it is worth mentioning that this thesis provided for the first time a detailed picture on blood immune cell numbers during the entire pregnancy in swine (MANUSCRIPT 2). Profound reductions in the number of most blood lymphocyte subsets characterize the immunological profile of pregnant sows mostly at the end of pregnancy. This decrease was due to a combination of a consistent reduction in the number of T cells and T cell subpopulations as well as of B and NK cells which is comparable to previous findings in humans and rodents (Luppi, 2003; Stefanski et al., 2005; Ramsay, 2018), with the particular exception of blood monocytes which changed in a different direction. Some of these pregnancy-induced immune alterations in sows partially occurred even at the beginning of pregnancy (total TH cells and antigen-experienced CD8α^{+} TH cells in sows decreased already from the first trimester onwards), a period that was not part of immunologically oriented studies previously. Interestingly, there was no significant week of pregnancy × housing interaction, indicating that both mixed and non-mixed sows had a comparable similar direction of change during pregnancy. However, frequent changes of group composition caused lower numbers of cytotoxic T cells, antigen-experienced CD8α$^+$ TH cells and NK cells in the blood of pregnant sows (MANUSCRIPT 1) and, moreover, the pregnancy-associated alterations described in MANUSCRIPT 2 were also influenced by social rank especially for blood B cells and monocytes of middle-ranking sows.

Conflict in response to social dominance between animals is generally known to be stressful for animals of many mammalian species with the potential to increase the release of GC (e.g. cortisol) (Sachser et al., 1998; Stefanski and Engler, 1999). GC are glucoregulatory hormones that are synthesized in response to a range of stimuli including stress and are regularly measured in the assessment of animal welfare. In swine, cortisol is a widely used biomarker to detect the stressfulness of housing conditions, as it represents the main glucocorticoid in pigs (Martínez-Miró et al., 2016).

Considering the behavioral consequences and findings on injuries described in the present thesis, it may be surprising that plasma cortisol levels were not elevated in response to the mixing treatment (MANUSCRIPT 1), especially because mixing of sows was already shown to increase cortisol levels in saliva and plasma (Barnett et al., 1996; de Groot et al., 2001; Couret et al., 2009b; Ison et al., 2014; Knox et al., 2014; Verdon et al., 2016). Olsson et al. (1999) and Coutellier et al. (2007) analyzed repeated social regrouping in growing pigs and found increased cortisol levels in saliva after several regroupings. Couret et al. (2009b) detected higher salivary cortisol concentrations between mixed and control pregnant gilts 1 h after the beginning of groupings as well as 19 h later and Grün et al. (2013) previously described lower plasma cortisol concentrations in dynamically group-housed pregnant sows compared to individually housed pregnant sows. However, most data from literature describe acute effects directly after mixing (ranging from 1 to 4 hours after treatment), often after only a single social challenge.

Especially alterations caused by regrouping are known to be influenced by the social status of an animal, as evidence shows a rank-dependent influence on behavior and physiology (Tuchscherer et al., 1998; Kranendonk et al., 2007). Therefore, social status often plays a more decisive role in an animal’s response to a stressor than the stressor itself (Salak-Johnson, 2007). The dominance status is certainly known to affect stress physiology, but so far existing data in sows are not uniform. While some studies show that cortisol concentrations are higher in submissive sows (Tsuma et al., 1996; Li et al., 2017), others found increased levels in sows of intermediate ranking position (Mendl et al., 1992; Zanella et al., 1998) or no effect of social status (Kranendonk et al., 2007). Results of MANUSCRIPT 2 also showed that cortisol levels were just slightly increased in middle-ranking sows compared to low-ranking sows while no differences were found in comparison to high-ranking sows. Thus, whether frequent changes of group composition represent a less stressful condition for pregnant sows or if other factors have influenced cortisol concentrations will be discussed in the following.

The magnitude of a stress reaction shows high individual variation depending on stress duration and intensity, previous experience and behavior of pen mates as well as age and genetics of the individual animal (Otten et al., 2015; Martínez-Miró et al., 2016). Isolation of female pigs for 60 min induced a plasma cortisol concentration of 94 ng/ml, in response to fixation for 5 min a peak plasma cortisol concentration of 108 ng/ml was found, mating led to a peak of greater than 60 ng/ml, and introduction of a sow to a boar resulted in 100 ng/ml (Turner et al., 2002). Collectively, these studies show that there exists a range of normal or appropriate cortisol responses to stressors and although most stress stimuli are known to increase cortisol, in some cases cortisol levels even seems to remain constant. In addition, there exists a large variability in HPA axis activity between individuals (Ralph and Tilbrook, 2016). This was also seen in sows of the present thesis, ranging from 5 – 52 ng/ml. Individual variations can also arise from environmental (temperature, blood sampling method) and intrinsic (genotype, age, circadian rhythm, pregnancy) factors (Mormède et al., 2007). Different authors suggested that repeated exposure to the same stressor results in a decline of cortisol responsiveness, suggesting habituation to that stressful situation (Pignatelli et al., 2000; Sutherland et al., 2006; Coutellier et al., 2007; Couret et al., 2009b; Otten et al., 2015). This was explained by a decrease of the sensitivity of the adrenal axis or by an alteration (Couret et al., 2009b) of the negative-feedback inhibition of the HPA axis due to elevated glucocorticoid concentrations in response to a chronic stressor (Jaferi et al., 2003). Furthermore, as a stress-induced alteration of the regulation of the HPA axis was described to take about eight weeks to develop (Capitanio and Cole, 2015) and the experimental and blood sampling phase of the present work was finished exactly after eight weeks, it remains possible that the mixing phase was too short to detect differences in cortisol.

Particularly a typical pregnancy-induced increase in cortisol at the end of pregnancy (Takahashi et al., 1998; Stefanski et al., 2005; Zhang et al., 2017), which was seen in sows of this thesis (MANUSCRIPT 2), might be responsible for superimposing stress effects of group-housing and therefore alterations in cortisol might not have been detectable. Moreover, inhibitory effects of pregnancy on the sensitivity to stressors have been identified in the endocrine system (Neumann et al., 1998).

An alternative explanation why cortisol levels were not elevated is that differences in corticosteroid-binding globulin (CBG) concertation between sows of both treatments and regardless of social rank position exist which was not analyzed in the present thesis. Due to its lipophilic nature, approximately 90 % of circulating cortisol is bound to proteins, principally albumin and CBG, a specialized glycoprotein that binds cortisol with high affinity and regulates its bioavailability. GC are considered biologically inactive when bound to proteins, as only the free, unbound fraction can cross biologic membranes including the blood-brain barrier and cell membranes to migrate from the blood to the intracellular environment. Greater or lower concentrations of CBG will decrease or increase free cortisol resulting e.g. in lower or no alterations in plasma cortisol levels (Stefanski, 2000; Lay, 2011). During a confrontation situation in rodents, marked reductions in CBG concentrations and unaffected total cortisol concentrations were noted in loser rats, which strongly suggests elevated levels of free cortisol (Stefanski, 2000). These data also show that determination of total cortisol may be insufficient to detect a stress-induced increase in free cortisol concentrations and should be taken into consideration in future studies.

Besides of an HPA axis activation, stress is also known to cause a release of catecholamines through the sympathetic nervous system (Stefanski, 2000; Webster Marketon and Glaser, 2008; Dhabhar, 2009). Kaiser and Sachser (2005) already suggested that the stress response due to unstable social environment might involve the sympathetic adrenomedullary system (Sachser et al., 2011) and not the HPA system, as pregnant guinea pigs living in unstable social conditions also did not show any alterations in serum concentrations of cortisol (Kaiser et al., 2003). Whether or not sympathetic adrenomedullary activity led to increased catecholamine levels in pregnant sows in an unstable social environment has to be examined.

Therefore, the lack of clear differences in measured plasma stress hormone concentrations between treatment groups or rank-positions in this thesis (MANUSCRIPT 1 AND 2) does not necessarily mean that group-housing indeed is no stressful-situation. Aggressive behavior (MANUSCRIPT 1) resulting in superficial skin injuries might be a source of pain or discomfort and are likely to cause a stress response and therefore might be linked to adverse effects on welfare for pregnant sows (von Borell, 1995; Hodgkiss et al., 1998; Kongsted, 2004; Verdon et al., 2015). Besides, the findings of the present work on blood immune cell numbers resemble in many aspects a picture of stress-induced immunomodulation found in other species which has been previously reported in context with social stress (Stefanski and Engler, 1998; Engler et al., 2004). Group-housing of pregnant sows associated with repeated social mixing or rank position may be classified at least as rather mild-stressor (Turner et al., 2002) and only the immune system might be sensitive enough to show long-lasting effects.

4.2.2 IMPACT ON HEALTH, PRODUCTIVITY AND REPRODUCTION

The detailed investigation of immunological, endocrinological, and behavioral effects of housing pregnant sows in groups with or without frequent changes of group composition contributes to gain better knowledge on physiological consequences of these husbandry conditions to provide more or new opportunities to improve animal health, productivity, and reproduction.

Especially an efficient immune responsiveness is essential to prevent infectious diseases and to protect against harmful pathogens and parasitic infections. Social stress was described to be linked to disease or health implications through alterations in host susceptibility and virulence of pathogens as well as in increased amounts of pathogens (Proudfoot and Habing, 2015). Previous research addressing the effects and mechanisms by which social stressors impact immune function in pigs found alterations in lymphocyte numbers (Ruis et al., 2001; Couret et al., 2009b; Tuchscherer et al., 2009; Grün et al., 2013), NK cell cytotoxicity (McGlone et al., 1993; Sutherland et al., 2006), lymphocyte proliferation (Hessing et al., 1994), and response to vaccination (de Groot et al., 2001; Grün et al., 2014). Additionally, repeated regrouping of pregnant sows also caused prenatal stress in their offspring influencing stress regulation and the immune system which resulted in negative effects on growth, physiological adaptability, health and behavior in later life (Couret et al., 2009c; Couret et al., 2009a; Otten et al., 2010; Brunton, 2013; Otten et al., 2015).

The immunological profile in blood of mixed sows of the present thesis was characterized by lower numbers of CTL, antigen-experienced TH cells and NK cells (MANUSCRIPT 1). Interestingly, this work could demonstrate that for most immune cells a certain period of instable housing conditions is required to induce a change, but once manifested, these immunological alterations persist even after the end of the mixing period. As cytotoxic T cells are important for killing cells infected with intracellular pathogens and TH cells are critical in initiating the B cell response resulting in antibody production as well as for immunological memory functioning, altered migration patterns or even the loss of these cells might particularly limit the protection against bacteria and parasites or viral, intrecellular pathogens (Charerntantanakul and Roth, 2006; Chase and Lunney, 2012; Gerner et al., 2015). NK cells are an important component of the innate defence mechanisms and participate in activating the adaptive immune response by production of cytokines (Gerner et al., 2009; Chase and Lunney, 2012; Mair et al., 2014). Thus, the results demonstrate that frequent changes of group composition affect both cell numbers of the innate and the adaptive part of the immune system in the blood and these findings seem to signify at least some effects of chronic stress which are not quickly reversible even after stressor cessation.

Data of the present work also showed that a sow's social status is related to blood immune cell numbers during pregnancy (MANUSCRIPT 2). Immunological consequences related to social rank in pregnant sows are scarcely examined to date (Mendl et al., 1992; Couret et al., 2009b; Zhao et al., 2013; Pacheco and Salak-Johnson, 2016). Most rank-dependent particularities were seen in middle-ranking sows with higher numbers of B cells compared to low-ranking sows and higher numbers of monocytes compared to low- and high-ranking sows (MANUSCRIPT 2). As the impact of rank position on immune cell distribution and functioning has not yet been studied in pregnant sows in such detail, it is apparent that more research is needed to clarify why middle-ranking sows deviate from normal pregnancy-associated immunomodulations and how these effects on immune cell distribution may influence health and disease susceptibility.

Additionally, social stress has been repeatedly shown to influence immune functionality by suppressing lymphocyte proliferation and affecting the ability to mount an appropriate antibody response to vaccination in rodents and pigs (Deguchi and Akuzawa, 1998; Stefanski and Engler, 1999; de Groot et al., 2001; Damgaard et al., 2009). With respect to the results of the present thesis, where lymphocyte proliferation increased in mixed sows (MANUSCRIPT 1) and no treatment effect was observed for specific antibody plasma concentrations following immunization with the neoantigen keyhole limpet hemocyanin (KLH) (ANNEX 2), these findings do not fit in this concept of a stress-induced suppression. At this point of research, it seems possible that instable group-housing conditions have the potential to affect blood immune cell distribution of the innate and the adaptive immune system, but with no negative implications on functionality. However, few previous studies on stressful conditions or regrouping in pigs and rodents also found increased lymphocyte proliferation (Shu et al., 1993; Sutherland et al., 2006; Couret et al., 2009a), but possible mechanisms should be further investigated. Moreover, future studies should additionally focus on phagocytic activity and cytokine secretion profiles to gain more insight into immune functionality of group-housed pregnant sows with frequent changes of group composition.

Beside their ability to affect the immune system, aggression and fighting following regrouping of sows can cause other problems representing different important welfare concerns and considerable economic issues. The increased activity of group-housed sows was indeed shown to have a positive effect on muscle and bone development (Marchant and Broom, 1996; Schenck et al., 2008), but also resulted in an increased risk for skin lesions, vulva biting, claw problems and lameness (Chapinal et al., 2010; Pluym et al., 2011; Baumann et al., 2012; Bos et al., 2016). Mixed sows of the present work had more skin lesions than not mixed sows (ANNEX 1). Normally these lesions result in no serious health problems. However, combined with the experience of social defeat, this might represent a stressful experience. To clarify this, implications of skin injuries on sows' health or immune system, have to be further investigated by testing whether mixed sows with more severe wounds differ immunologically from mixed sows with no wounds or bites. It was shown in rodents, that wounding had no impact on most immunological changes, as no differences were observed between bitten and not-bitten loser rats after confrontation with a dominant rat (Stefanski and Engler, 1999). Thus, injuries seem not to be the primary cause for immunological changes following psychosocially stressful conditions, indicating that these immunological effects could certainly be an evolutionary adaptation, as fights for social hierarchy position often involve a high risk of injury.

During the experiments for the present thesis only one sow suffered from leg injuries and had to be excluded. Thus, claw problems and lameness could not be confirmed under theses group-housing conditions suggesting that these issues are possibly associated with a bigger pen size in combination with a partly slatted and not complete concrete floor design (Baumann et al., 2012).

For a balanced relationship between productivity and economic performance, a healthy sow herd with best reproductive performance is necessary. Most scientific studies conclude that reproductive performance of group-housed sows is sometimes worse, better, or similar when compared to crate-housed sows (reviewed in McGlone, 2013). It is suggested that stressful situations are able to activate a variety of mechanisms which might suppress reproductive efficiency and may compromise maternal abilities such as hypothalamic, pituitary and ovarian function (von Borell et al., 2007). Another explanation might be, that aggressive confrontations may negatively affect sow reproduction and metabolism by deviating energy resources from these important biological processes (Einarsson et al., 2008). In any case, the complex mechanisms in which stress might influence reproduction are still not well understood. In group-housed sows, maternal stress was shown to impair reproductive performance by reducing the number of piglets born alive, suggesting an increased foetal mortality and decreased farrowing rate (Kongsted, 2004; Couret et al., 2009b; Knox et al., 2014; Choe et al., 2018).

Available results on the relation between rank position and reproductive performance are contradictory, as evidence exists that number of born piglets alive was decreased both in submissive and dominant sows (Hoy et al., 2009a; Zhao et al., 2013). In the present work, no further relationships between social status and the reproductive performance were found in sows (ANNEX 3). As litter size and piglet mortality are highly variable between individuals, it is likely that the number of sows investigated here was not sufficient to obtain significant differences. However, due to the large differences on reproductive performance of group-housed sows reported in previous literature, it seems likely that not only the housing conditions themselves are the main factor on risk and success of fertility and reproduction. In addition, it seems obvious that the type of stressor and its timing in relation to the stage of the reproductive cycle as well as other housing factors (e.g. group size, floor space, nutrition) must also be considered before drawing conclusions about the impact of instable housing conditions on reproduction of sows (Greenwood et al., 2014; Salak-Johnson, 2017).

4.3 DOMINANCE MEASUREMENT AND ITS RELEVANCE FOR LIVESTOCK RESEARCH

Social status or rank within dominance hierarchies is often associated with many aspects of animals' physiology, fitness, weight gain, health, genetic expression and reproductive performance (Chase and Seitz, 2011). The results of the present work also showed that social status furthermore has the potential to affect plasma cortisol levels and influences pregnancy-related alterations in the maternal immune system, especially in middle-ranking sows (MANUSCRIPT 2) – a rank position in which animals were described to try to maintain or improve their rank position (Mendl et al., 1992; Zanella et al., 1998). These results are of substantial relevance for livestock production, indicating that social status is an important factor that may adversely affect welfare and health and should be considered in future studies characterizing effects of social status on the immune system in livestock species within group-housing environments.

Considering the essential role of dominance assessment in this respect and the fact that dominance hierarchies have been one of the best-studied forms of social organization (Chase, 1974; Hawley, 1999; Holekamp and Strauss, 2016), still no consistent approach or recommendation exists to date. Besides, observing and quantifying animal behaviour by continuous observation in terms of dominance assessment requires considerable effort and is a very time-consuming procedure (Martin and Bateson, 2007). For the present work a total of

390 hours of video observations were evaluated, 190 hours thereof only for determination of sows’ individual rank position.

Therefore, a data set of pregnant sows was used as model system to demonstrate whether and how group stability and observational procedures influence dominance assessment by calculation of dominance indices. The overall aim was to derive recommendations for observation planning and index calculation in respect to which behavioural patterns, durations or situations should be observed to generate a reliable dominance hierarchy.

In general, the present thesis's findings (MANUSCRIPT 3) are transferable to dominance measurement of other (group-housed) livestock species with comparable social systems and under similar housing conditions and it could be recommended that dominance measurement should consider an observation period that involves species typical activity behaviour and should focus on the fact that different behaviours require different amounts of data. For groups with frequent changes of group composition, it is essential to await the first phase of rank position fighting in order to generate reliable dominance indices. Stable dominance relationships over an extended period affect index comparability due to a reduction of agonistic interactions over time leading to data scarcity, indicating the importance of considering the respective group characteristics of the observed species for dominance measurement. In general, a high number of unknown or tied dyads is a common problem and limiting factor in behavioural datasets to generate comparable results (Douglas et al., 2017; Klass and Cords, 2011; Neumann et al., 2011) and should certainly be avoided. Additionally, it is also advisable to observe a situation with a further resource (e.g. a feeding situation).

4.4 ASPECTS FOR PRACTICAL SOW HUSBANDRY AND FUTURE RESEARCH

This experimentally controlled study was designed to include some relevant aspects of commercial housing conditions in which sows are frequently re-introduced into their original group after farrowing. It is clear that this study design mimics but not exactly resembles the social environment of sows' actual circumstances on commercial farms. Normally, dynamic group-housing is not used for small groups and requires no changes of group composition several times a week. Besides, sows of the present work had more available space (~ 3m^2/sow) than prescribed by law (~ 2,5m^2/sow). The mixing paradigm of the present thesis has been chosen to assess whether frequent changes of group composition have the potential to act as social stressor in pregnant sows in general, with possible negative effects on health and wellbeing of the animals. Thus, these research results reveal the importance of managing aggression in group-housing systems and can be applied for future recommendations to optimize housing environment and management strategies to implement them practically in a commercial farming environment. Until now, most strategies to reduce aggressions and stress in sows focused on the optimal group size (Gonyou, 2002; Anil et al., 2006; Spoolder et al., 2009; Hemsworth, 2013), floor space requirements (Weng et al., 1998; Spoolder et al., 2009; Salak-Johnson et al., 2012; Hemsworth, 2013; Salak-Johnson et al., 2015; Greenwood et al., 2016; Hemsworth et al., 2016), influences of sow weight and parity (Arey and Edwards, 1998; Kranendonk et al., 2007, 2007; Hoy et al., 2009b; Li et al., 2012; Norring et al., 2019; Roelofs et al., 2019), housing with the presence of a boar (Séguin et al., 2006; Borberg and Hoy, 2009), effect of sedation (Barnett et al., 1993), enrichment (Jensen et al., 2000; Elmore et al., 2011; Greenwood et al., 2019b), different housing and feeding strategies (Douglas et al., 1998; Hodgkiss et al., 1998; Sendig et al., 2004; Anil et al., 2006; Munsterhjelm et al., 2008; Grün et al., 2013; Grün et al., 2014), genetic selection (Løvendahl et al., 2005), and nutrition or feed additives (Poletto et al., 2014; Greenwood et al., 2019a).

Scientific research is still unclear about which group size for gestating sows should be recommended. Group size must be sufficiently large (more than 12 individuals) to have an impact on aggression levels (Andersen et al., 2004). It is suggested that a larger group size may be beneficial to subordinate sows, as it allows animals to avoid and flee from aggressive pigs (Gonyou, 2002).

Space allocation at mixing can be managed to reduce aggression and stress in sows. Studies that focused on aggression at mixing throughout gestation showed that aggression is correlated with floor space, as increased floor space resulted in decreased aggressions and injuries (Weng et al., 1998; Spoolder et al., 2009; Hemsworth, 2013). However, to define an optimal space allowance is difficult since this is affected by floor type and feeding system design. Therefore, it would be worth examining how providing adequate space, or barriers for escape and avoidance, which are known strategies to reduce agonistic interactions and injuries (Marchant-Forde and Marchant-Forde, 2005; Spoolder et al., 2009; Hemsworth, 2013; Spoolder and Vermeer, 2015; Peden et al., 2018), could be optimized for housing sows in dynamic groups and may also be implemented practically in a commercial farming environment.

Social status is known to be positively correlated with sow age, parity and weight (Arey and Edwards, 1998). Sows of higher parity are more dominant and are ranked higher in the hierarchy, suggesting that primiparous sows should not be grouped with older and bigger animals as increased attacks against younger animals were identified (Hoy et al., 2009b). In addition, live weight, relative weight, body condition and back fat thickness were associated with winning percentage, giving heavier animals an advantage. Low winning percentage was related to lower live weight gain, probably due to poorer success in competition for feed (Norring et al., 2019). Thus, sows with low body condition score and submissive ones might need special attention on farms, especially to prevent insufficient feed-intake (Salak-Johnson, 2017). A new strategy of sow husbandry practice could be to segregate between parity/weight and to be flexible to remove ‘aggressive’ or ‘vulnerable’ individuals (Greenwood et al., 2014).

Scientific research on how nutrition can reduce aggression is sparse but a promising strategy. There is some evidence suggesting that additional dietary supplementation of magnesium (O’Driscoll et al., 2013a; O'Driscoll et al., 2013b) or tryptophan (Poletto et al., 2014) may reduce aggressive behavior during mixing of pigs. Tryptophan is the precursor for the synthesis of serotonin (5-HT) and inhibits aggression (Nelson and Chiavegatto, 2001). However, further research is required to establish an effective optimum supplementation as well as dosing level and the associated cost for farmers. Besides, there exist feed-related strategies to reduce aggression in group-housed sows like the provision of foraging materials (e.g., straw, silage), feeding a high fiber diet, or increasing the volume of feed consumed (Greenwood et al., 2019a). Overall, more research on different feeding methods within group housing systems is needed in order to isolate optimum methods for feeding at mixing.

There are many concepts currently being tested attempting to reduce the aggression associated with mixing. The use of enrichment materials is another possibility to minimize aggression in sows. However, research on the effect of enrichment on sow aggression has been less conclusive with studies finding that the provision of straw bedding reduced sow aggression at mixing (Jensen et al., 2000), while others demonstrated that providing straw had no or a heightening effect on aggression (Studnitz et al., 2007; Stewart et al., 2008; Greenwood et al., 2019b).

Taken together, though progress has been made, there are still few explicit recommendations in the scientific literature for the environment into which sows should be mixed, in order to reduce aggression and social stress. Moreover, except for some studies (Salak-Johnson et al., 2012; Grün et al., 2013; Grün et al., 2014) which showed that a decreased space allowance or housing conditions affect the immunological profile of pregnant sows, none of these above mentioned studies combined the investigation of management strategies with the assessment of their immunological consequences.

As aggressive encounters are a natural behavior in sows during hierarchy establishment, it might not be a reasonable future approach to recommend a complete prevention of aggressions and fighting in group-housed sows. Instead, it is more about a combination of environmental concepts to reduce aggressive behavior and to support sows to cope with suboptimal housing conditions in order to ensure that negative effects on the immune system and productivity of sows are minimized. In this respect, a detailed analysis of immunological effects for future studies on mixing or group-housing conditions in sows is highly recommended, as this might help to sharpen and define optimal concepts for sow husbandry. Until then, different management techniques such as providing as much space as possible, separating younger sows from older sows, generating sufficient feed-intake, no mixing during early pregnancy, and reducing the number of limited resources to be fought over can be implemented to improve animal health and welfare (Greenwood et al., 2014; Pedersen, 2018).

Future studies should focus on sow husbandry strategies or health monitoring indicators (Junge et al., 2012) which can be practically implemented on commercial farms to provide conclusive information on the optimal regrouping management but also consider what is economically acceptable and practical for the commercial pork producer. Although pig farmers reportedly have a high interest and regard for animal welfare, changing current practice and implementing strategies to reduce aggression relies strongly on their perception of the situation (Wilson et al., 2014). To date, farmers are faced with a number of welfare problems such as tail biting, lameness, and pain caused by routine husbandry procedures such as ear tagging and tail docking (Peden et al., 2018). Therefore, to communicate and raise awareness that social rank and agonistic interactions influence the immune response of sows and possibly even of their piglets, forms the basis for possibilities to improve animal health and welfare and is also the responsibility of livestock research.

4.5 Conclusion

The present thesis revealed that frequent changes of group compositions induce stress-related immunological changes in pregnant sows. Regrouping of even familiar sows lead to increased aggression and injuries resulting in long-term consequences for the adaptive immune system with the potential to adversely affect welfare and health. Since results showed contradictory or no effects on *in vitro* lymphocyte reactivity and antibody response, further studies should focus on phagocytic activity and cytokine secretion profiles to clarify the effects of a dynamic housing environment on immune cell functionality of pregnant sows.

Additionally, in order to contribute to filling the knowledge gap, this was the first study with a detailed analysis of blood immune cell subsets in sows showing that pregnancy-associated immunomodulations exist in each trimester of pregnancy. Those alterations in the immune system were affected by social status particularly in middle-ranking sows, indicating that social rank can influence the immune system and endocrine status in group-housed sows during pregnancy. Therefore, the necessity to choose the appropriate measurement for calculation of dominance relationships became evident and the present thesis recommended for the first time specific behavioral patterns, durations or situations of behavioral observations which should be considered in observation planning for dominance index calculation in order to generate reliable results.

The overall picture emerging from the current doctoral thesis indicates that a detailed analysis of lymphocyte subsets should ideally cover the entire gestation period for future studies on stress or housing environment in sows under group-housing conditions. To ensure that negative effects on the immune system and productivity of sows are minimized, the combination of different management techniques should be implemented to reduce aggressive behavior in group-housed pregnant sows. Future studies should focus on concepts for sow husbandry that are practical and economic for commercial farms to improve sows' health and welfare.

4.6 REFERENCES

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CHAPTER 5

SUMMARY

5 SUMMARY

In modern animal husbandry, dynamic group-housing of pregnant sows is a common practice and involves frequent regrouping or mixing of unfamiliar sows, which raises several welfare and health concerns. Every regrouping of animals or every change of group composition is associated with the establishment or the adjustment of a new dominance hierarchy, which provokes aggressive behavior, fights and injuries. Although the formation of hierarchies by agonistic encounters is a natural behavior in pigs, this process is known to result in social stress by an activation of different stress systems. The subsequent release of neuroendocrine signals like glucocorticoids (e.g. cortisol) has the potential to alter several immune functions and immune cell numbers in the blood which may be directly associated with animals' health, reproduction, embryonic development and economic losses. Previous research on pregnant sows primarily focused on the stressfulness of the housing environment in general. The effects of frequent regrouping or mixing on pregnant sows' behavior, stress hormones and especially the distribution and functionality of blood leukocyte subpopulations represent a major research gap in the field of stress assessment of dynamic group-housing conditions in pig production.

The aim of the present doctoral thesis was to evaluate whether frequent regrouping acts as a chronic social stressor influencing behavior as well as the endocrine and immune system of group-housed pregnant sows. Special emphasis was put on the question whether frequent changes of the group composition affect blood leukocyte subpopulations to determine possible persistent stress effects of social mixing. A study with 40 pregnant sows was designed to investigate the influence of frequent changes of group composition on numbers and functionality of blood leukocyte subpopulations in combination with analyses of agonistic behavior and the endocrine status for comprehensive stress assessment. Pregnant multiparous sows were housed in groups of five animals. Sows were either assigned to a repeated social mixing treatment with a mutual exchange of two randomly selected sows of two specific groups (2x2) twice a week over a period of eight weeks, or remained undisturbed in their original group. Blood samples of all sows were collected during pregnancy at five time points before, during, and after the mixing period to evaluate the number of blood leukocyte subpopulations, mitogen-induced lymphocyte proliferation and plasma cortisol concentrations. In order to contribute to filling the knowledge gap in respect to pregnancy-associated immunomodulation in sows, blood immune cell numbers were analyzed during all trimesters of gestation and the impact of social status comparing low-, middle-, and high-ranking sows on these modifications was assessed. Behavioral data of pregnant sows of this experiment were used to compare

various recommended dominance indices to rank individuals based on different methodical aspects (types of observed behavior, observation duration or situation, varying group stability) to investigate whether these indices are comparable and equally applicable for determination of dominance relationships.

Results of the current study provided for the first time a detailed picture on blood immune cell numbers during the entire pregnancy in swine and demonstrated that pregnancy-associated alterations in the immune system generally exist in sows. The numbers of T cells, natural killer cells, B cells, cytotoxic T cells, and $CD8^{+}$ $\gamma\delta$- T cells decreased during the last trimester of pregnancy, while neutrophils and plasma cortisol concentrations increased during pregnancy. Those pregnancy-associated alterations in the immune system were affected especially in middle-ranking sows, which had higher numbers of B cells and monocytes than sows with lower ranking positions. Plasma cortisol concentrations also tended to be higher in middle-ranking sows compared to low-ranking sows indicating that social rank can influence the immune system and endocrine status in sows during pregnancy. These findings showed the necessity to choose the appropriate measurement for calculation of dominance relationships and the present thesis recommended for the first time specific behavioral patterns, durations or situations of behavioral observations. These should be considered in observation planning for dominance index calculation in order to generate reliable results.

Repeated social mixing by frequent changes of group composition not only resulted in an increase of aggressive behavior during the entire mixing period, but also in altered immune cell numbers. The immunological profile in blood of mixed sows was characterized by lower numbers of antigen-experienced T helper cells, cytotoxic T cells and natural killer cells. This work demonstrated that frequent changes of group composition affect both cell numbers of the innate and the adaptive part of the immune system, which may weaken immunological memory functioning and reduce the resistance against certain infections in pregnant sows. For most of these immune cells a certain period of instable housing conditions was required to induce a change, but once manifested, these immunological alterations persisted even after the end of the mixing period. Since results showed contradictory or no effects on antibody response and *in vitro* lymphocyte reactivity, further studies should investigate other functional parameters such as phagocytic activity and cytokine secretion profiles to clarify the effects of an instable housing environment on immune cell functionality of pregnant sows.

Although the findings of the present work on blood immune cell numbers resemble in many aspects a picture of stress-induced immunomodulation previously reported in context with social stress, no clear differences in measured plasma stress hormone concentrations between treatment groups or rank-positions were found. Whether frequent changes of group composition or social status represent a less stressful condition for pregnant sows or if other factors have influenced cortisol concentrations needs to be further evaluated.

The overall picture emerging from the current doctoral thesis indicates that frequent changes of group composition and social status have the potential to induce stress-related immunological changes in pregnant sows which might adversely affect sows' health and performance. Future sow husbandry should implement management techniques to reduce aggressive behavior in group-housed pregnant sows to ensure that negative effects on the immune system and productivity of sows are minimized to improve sows' welfare.

CHAPTER 6

ZUSAMMENFASSUNG

6 ZUSAMMENFASSUNG

In der heutigen modernen Tierhaltung ist die dynamische Gruppenhaltung von trächtigen Sauen, welche häufig Neugruppierungen oder das Mischen einander unbekannter Sauen bedingt und damit Bedenken hinsichtlich der Gesundheit und dem Wohlergehen der Tiere aufwirft, gängige Praxis. Jede Neugruppierung der Tiere bzw. jede Veränderung der Gruppenzusammensetzung ist mit der Etablierung oder Anpassung einer neuen Sozialhierarchie assoziiert, was Aggressionen, Rangordnungskämpfe und Verletzungen zur Folge haben kann. Obwohl die Bildung einer Sozialstruktur zum arttypischen Verhalten von Schweinen gehört, wird angenommen, dass dieser Prozess zu einer sozialen Stressbelastung und damit zu einer Aktivierung verschiedener Stresssysteme führen kann. Die darauffolgende Ausschüttung neuroendokriner Signale wie z.B. von Glucocorticoiden (Cortisol) birgt das Potential, die Funktionalität und die Anzahl verschiedener Immunzellen im Blut zu verändern, was in direktem Zusammenhang mit der Tiergesundheit, Reproduktion, Embryonalentwicklung und damit verbundenen wirtschaftlichen Schäden gebracht werden kann. Forschung an trächtigen Sauen konzentrierte sich bisher vor allem auf die Stressbelastung der Haltungsumwelt im Allgemeinen. Die Folgen stetiger Änderungen der Gruppenzusammensetzung von Sauen auf Verhalten, Stresshormone und vor allem auf die Anzahl, Verteilung und Funktionalität von Leukozyten-Subpopulationen im Blut trächtiger Sauen stellt dagegen eine Forschungslücke im Bereich der Belastungsbeurteilung der dynamischen Gruppenhaltung in der Schweineproduktion dar.

Das Ziel dieser Doktorarbeit war es zu untersuchen, ob sich häufige Neugruppierungen als sozialer Stressor für trächtige Sauen in Gruppenhaltung auswirken können und dabei das Verhalten sowie das Hormon- und Immunsystem beeinflusst werden. Der besondere Schwerpunkt lag hierbei auf der Fragestellung, ob stetige Veränderungen der Gruppenzusammensetzung Leukozyten-Subpopulationen im Blut beeinträchtigen, um mögliche dauerhaft persistierende Stresseffekte durch soziale Instabilität zu bestimmen. Dazu wurde in einer experimentellen Studie mit 40 trächtigen Sauen der Einfluss stetiger Veränderungen der bestehenden Gruppenstruktur auf Anzahl, Verteilung und Funktionalität von Leukozyten-Subpopulationen im Blut sowie das Auftreten agonistischer Verhaltensweisen und der Hormonstatus hinsichtlich ihrer Stresswirkung untersucht. Trächtige Sauen wurden in Kleingruppen zu je fünf Tieren gehalten. Soziale Instabilität wurde durch häufige Wechsel der Gruppenzusammensetzung induziert, indem über einen Zeitraum von acht Wochen zwischen zwei Gruppen zweimal wöchentlich jeweils zwei Gruppenmitglieder randomisiert ausgetauscht

wurden, während die anderen Gruppen über den gesamten korrespondierenden Zeitraum in gleichbleibender Gruppenzusammensetzung unter identischen Bedingungen gehalten wurden. Allen Sauen wurden zu fünf definierten Zeitpunkten vor, während und nach der Phase sozialer Instabilität Blut entnommen, um die Anzahl von Leukozyten-Subpopulationen im Blut, die mitogen-induzierte Lymphozytenproliferation sowie die Cortisolkonzentration im Plasma zu bestimmen. Um zusätzlich zur Schließung der Forschungslücke im Bereich der trächtigkeits-induzierten Veränderung im Immunsystem von Sauen beizutragen, wurde die Anzahl der Immunzellen im Blut über alle Trimester der Trächtigkeit bestimmt und der Einfluss der sozialen Rangposition zwischen niedrig-, mittel- und hochrangigen Sauen auf diese Veränderungen ermittelt. Zudem wurden die Verhaltensdaten der trächtigen Sauen dazu verwendet, verschiedene empfohlene Dominanzindices basierend auf unterschiedlichen methodischen Vorgehensweisen (Art der beobachteten Verhaltensweisen, Dauer und Situation der Verhaltensbeobachtungen, unterschiedliche Gruppenstabilität) auf Vergleichbarkeit und Anwendbarkeit zur Dominanzbestimmung zu überprüfen.

Die Ergebnisse dieser Studie liefern erstmals eine detaillierte Darstellung von Immunzellen während der gesamten Trächtigkeit von Sauen im Blut und zeigen, dass trächtigkeits-induzierte Veränderungen im Immunsystem auch bei Schweinen existieren. Die Anzahl von T Zellen, natürlichen Killerzellen, B Zellen, zytotoxischen T Zellen und CD8α^{+} $\gamma\delta$- T Zellen nahmen während des letzten Trimesters der Trächtigkeit ab, wohingegen Neutrophile und die Cortisolkonzentration im Plasma zum Ende der Trächtigkeit anstiegen. Diese trächtigkeits-induzierten Veränderungen waren vor allem in mittelrangigen Sauen beeinflusst, welche eine höhere Anzahl an B Zellen und Monozyten im Vergleich zu niedrigrangigen Sauen aufwiesen. Sauen mittlerer Rangposition verfügten zudem tendenziell über eine höhere Cortisolkonzentration im Plasma als Sauen niedrigeren Ranges, was darauf hindeutet, dass der soziale Rang das Immun- und endokrine System von Sauen während der Trächtigkeit beeinflussen kann. Diese Ergebnisse zeigen außerdem die Notwendigkeit und den Bedarf für eine geeignete Methode zur Bestimmung von Dominanz bzw. Rangpositionen. Diese Studie liefert zum ersten Mal spezifische Empfehlungen welche Verhaltensweisen und Situationen über welchen Zeitraum beobachtet und bei der Planung von Verhaltensbeobachtungen zur Dominanzbestimmung berücksichtigt werden sollten um zuverlässige Ergebnisse zu generieren.

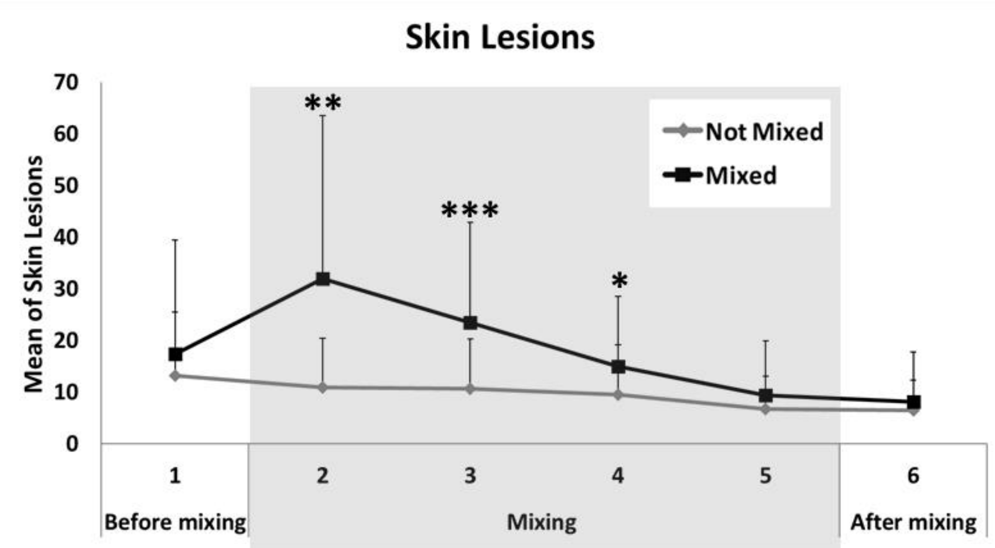
Häufige Wechsel der Gruppenzusammensetzung führten nicht nur zu einem Anstieg aggressiver Verhaltensweisen während der gesamten Phase sozialer Instabilität, sondern auch zu einer Veränderung der Immunzellzahlen im Blut. Das immunologische Profil war durch eine niedrigere Anzahl an antigen-erfahrenen T Helferzellen, zytotoxischen T Zellen und natürlichen Killerzellen charakterisiert. Damit konnte diese Doktorarbeit zeigen, dass sowohl der angeborene als auch der erworbene Teil des Immunsystems beeinträchtigt war, was bei trächtigen Sauen eine schwächere Gedächtnisleistung des Immunsystems oder eine reduzierte Widerstandkraft gegenüber bestimmten Infektionen zur Folge haben könnte. Für die meisten dieser genannten Immunzellen war ein gewisser Zeitraum instabiler Haltungsbedingungen notwendig um diese immunologischen Veränderungen hervorzurufen. Hatten sich diese jedoch einmal manifestiert, blieben sie über das Ende der instabilen Haltungsphase hinaus bestehen. Da die Ergebnisse allerdings auch gegensätzliche oder keine Effekte auf die Reaktivität der Lymphozyten *in vitro* und die Antikörperantwort zeigten, sollten zukünftige Studien auch Phagozytose oder Zytokin-Sekretion berücksichtigen, um die Folgen einer sozial instabilen Haltungsumwelt auf die Funktionalität des Immunsystems trächtiger Sauen näher zu beleuchten.

Obwohl die Ergebnisse dieser Doktorarbeit hinsichtlich des Immunsystems in vielen Punkten das Bild einer, bereits im Kontext von sozialem Stress gezeigten stress-induzierten immunologischen Veränderung wiedergeben, wurden keine klaren Unterschiede in der Cortisolkonzentration zwischen Sauen mit gleichbleibender und Sauen mit wechselnder Gruppenzusammensetzung oder verschiedenen Rangpositionen nachgewiesen. Ob dies nun bedeutet, dass häufige Wechsel der Gruppenstruktur eine eher milde Belastungssituation für trächtige Sauen darstellen, oder ob weitere Faktoren die Cortisolkonzentration im Blut beeinflusst haben, bleibt noch zu klären.

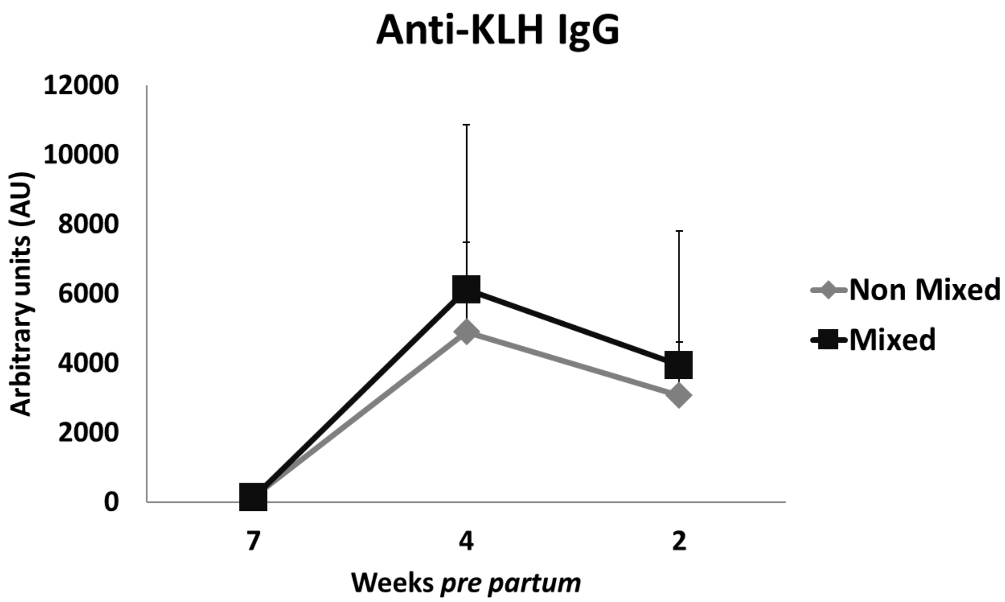
Zusammenfassend lässt sich sagen, dass im Rahmen dieser Doktorarbeit gezeigt werden konnte, dass häufige Neugruppierungen und stetige Wechsel der Gruppenzusammensetzung sowie der soziale Statuts der Tiere über das Potenzial verfügen, stress-assoziierte Veränderungen im Immunsystem hervorzurufen, die sowohl die Gesundheit als auch die Leistung der Sauen beeinträchtigen können. Zukünftig sollten in der Sauenhaltung daher Management- und Haltungsaspekte umgesetzt werden, die aggressives Verhalten zwischen trächtigen Sauen in Gruppenhaltung reduzieren, um die negativen Effekte auf Immunsystem und Produktivität der Tiere zu minimieren und damit das Wohlergehen der Sauen zu verbessern.

ANNEX

Annex 1. Mean skin lesion numbers of mixed and not-mixed sows before, during and after the mixing period. Asterisks indicate significant differences between treatments at the respective time point: *p ≤ 0.05; **p < 0.01; ***p < 0.001.



Annex 2. Arbitrary units (AU) of anti-keyhole limpet hemocyanin (KLH) IgG in blood plasma of non-mixed or mixed sows during mixing period (week 7 and 4 pre partum) and after the mixing period (week 2 pre partum). Anti-KLH IgG titers are given as arbitrary units and values are given as mean (±SD). AU did not differ between sows of the two treatments (p > 0.05).



Annex 3. Effect of social status on reproductive performances of pregnant sows.

Item	Rank			*P* value	
	High ranking	Middle ranking	Low ranking	Rank	Litter size
Weight gain (kg)	73.1 ± 8.4[a]	75.1 ± 3.6[a]	52.9 ± 5.0[b]	0.004	
Gestation length (days)	116.5 ± 0.7[ab]	117.5 ± 0.3[a]	116.1 ± 0.4[b]	0.04	> 0.1
Litter size	12.8 ± 0.9	13.2 ± 0.9	14.5 ± 0.6	0.39	
Stillborn	1.5 ± 0.7	0.9 ± 0.2	0.9 ± 0.2	0.29	0.07
Mummified piglets	1.0 ± 0.6	0.1 ± 0.1	0.08 ± 0.9	0.06	> 0.1
Weaned piglets	10.3 ± 1.0	11.0 ± 0.7	11.6 ± 0.5	0.89	< 0.001
Piglet losses	0.7 ± 0.2	1.4 ± 0.3	1.8 ± 0.5	0.55	0.003
Mean piglet weight (d1)	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	0.3	< 0.001
Mean piglet weight (d21)	6.8 ± 0.3	6.7 ± 0.4	5.9 ± 0.3	0.15	0.0002

Litter size was included in the statistical model as a covariate for gestation length, number of stillborn piglets, mummified piglets, weaned piglets, piglet losses and mean piglet weight. Values are mean ± SEM

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Curriculum Vitae

Personal Data

Name:	Christiane Schalk
Date of Birth:	13.05.1985
Place of Birth:	Ansbach, Germany

Education

11/2012 – 03/2018	Research for PhD, Institute of Animal Science, University of Hohenheim, Stuttgart, Germany Financial support by the H. Wilhelm Schaumann Foundation, Germany and the Faculty of Agricultural Science of the University of Hohenheim, Germany
10/2009 – 06/2012	Study of Agricultural Sciences, University of Hohenheim, Stuttgart, Germany Qualification gained: Master of Science
10/2005 – 06/2009	Study of Agricultural Sciences, Nürtingen-Geislingen University (HfWU), Germany Qualification gained: Bachelor of Science
09/1995 – 06/2004	Gymnasium Dinkelsbühl, Germany Qualification gained: Abitur

Professional Career

Since 04/2018	Employee for Product Management Swine at the Trouw Nutrition Deutschland GmbH, Burgheim, Germany
11/2012 – 03/2018	Scientific staff and PhD student at the Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

Course-Related Activities

2017	Certificate “Research-based learning and Project management” for University Didactics Baden-Württemberg
2014	Course Certificate: Laboratory Animal Science, Category B at the University of Hohenheim (contents in accordance with recommendations of the Federation of European Laboratory Animal Science Associations (FELASA))
2012	Course Certificate: Graduate course “Methods of Livestock Ethology”, GfT, University of Hohenheim, Germany

Practical Experience

09/2007 – 02/2008	Internship at the „Haus der bayerischen Landwirtschaft Herrsching“, Germany
07 – 09/2006	Internship on a commercial pig farm, Söglingen, Germany

Donauwörth, 23.07.2019

Place, Date Signature

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Influence of frequent regrouping and social status on behavioral, endocrine and immune responses of group-housed pregnant sows

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Schalk, C., Pfaffinger, B., Schmucker, S., Stefanski, V. (2016). Group-housing of pregnant sows – Effects of repeated mixing and social status on behaviour and physiological responses. Vortragstagung der DGfZ und GfT, Hannover, Germany

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