Dissertation

Form, function and relevance of social relationships in a dynamic dairy cow group

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Eidesstattliche Erklärung

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt, keine anderen als die angegebenen Hilfsmittel benutzt und alle aus ungedruckten Quellen, gedruckter Literatur oder aus dem Internet im Wortlaut oder im wesentlichen Inhalt übernommenen Formulierungen und Konzepte gemäß den Richtlinien wissenschaftlicher Arbeiten zitiert und mit genauer Quellenangabe kenntlich gemacht habe.

Abstract/ Kurzfassung

For gregarious beings, social relationships matter and can be a source of positive emotions, health and resilience, possibly depending on social complexity and relationship quality. Life history, ecology and social organisation and structure predestine cattle to have developed complex individualised and valuable social relationships. Dairy cow housing and management with dynamic, unstable and discontinuous groups however is presumably unsuitable to promote or just to allow them. Dyadic relationships in dynamic dairy cow herds are not well understood and possible beneficial effects have not been exploited yet, although there is growing interest in considering related phenomena such as 'social-buffering' in farm animal health and welfare. The present dissertation therefore aims at investigating form, function and relevance of social relations among dairy cows in a commercial dynamic group. To this end, we collected data in the early and high lactating group at the research farm of the Institute of Animal Science, Prague, and focused on a representative set of cows comprising all animals that were introduced after calving and in parallel cows matched by breed and lactation already present in the group. The present work (1) characterises dimensions of social relationships among dairy cows in terms social interactions and spatial proximity, (2) investigates effects of the presence of familiar group mates present on lying behaviour and synchrony of cows freshly introduced into the resident group, and (3) analyses heart rate and heart rate variability during resting to complement conclusions from behavioural data. (1) Social relationships were characterised by four dimensions representing (interacting during) feeding, displacement success, resting and allogrooming. Cows were found to differentiate between partners and have preferred associations depending on context. Long-term familiarity and continuity of shared experience were associated with the intensity of relationships, i.e.regarding investment of time and energy. (2) After integration into the group, primiparous fresh cows lied less and behaved less synchronous at the dyadic level than their matched residents, while no such effects were present in multiparous cows. Further, with increasing number of early familiar peers present, fresh primiparas' dyadic synchrony increased, however lying times decreased, while multiparas' group synchrony increased. (3) Similarly, heart rate (HR) and heart rate variability (HRV) patterns indicated an aroused physiological state in freshly introduced primiparous cows as compared to a more adaptable and relaxed physiological state in resident primiparas, and in contrast a more relaxed state in freshly introduced multiparous cows as compared to resident multiparas. The presence of familiar animals did not affect HR and HRV measures indicative of stress in freshly introduced primiparas, but positively influenced resident primiparas.

In conclusion the results support the notion that dairy cows actively maintain dyadic relationships. They suggest that especially early-built relationships can persist in the long-

term in dynamic dairy herds, and that long-term familiarity is a predictor of higher intensity of social relationship, but not exclusively of affiliative behaviours. Short-term familiarity instead did not seem to be relevant to the cows. In the challenging situation of introduction into the milking group after calving the presence of familiar peers had no relaxing effects on primiparas, but on cows that were already experienced through age, or habituated through residence. We therefore conclude that a dynamic social environment allows stable and continuous relationships among dairy cows, but that they are easily covered or suppressed by more urgent issues such as metabolic demands or stress through unknown situations, possibly hindering beneficial effects as well. To estimate their full potential however, further investigations about form and function of relationships and how they are related to husbandry aspects are needed. This may lead to recommendations that aim at promoting harmonic situations and positive effects of social companionship to improve animal welfare, instead of avoiding conflict-laden situations and negative effects.

Wer in Gruppen lebt, für den zählen soziale Beziehungen etwas, können Quelle sein für positive Emotionen, Gesundheit und Widerstandskraft, wobei diese Zusammenhänge wahrscheinlich mit dem Maß an sozialer Komplexität und Beziehungsqualität einhergehen. Rinder sind hinsichtlich ihrer lebensgeschichtlichen Merkmale, Ökologie und sozialen Organisation prädestiniert dafür, während ihrer Evolution komplexe individualisierte und wertvolle soziale Beziehungen entwickelt zu haben. Die Haltungsbedingungen und das Management von Milchkühen hingegen ist wohl oftmals ungeeignet, kontinuierliche Beziehungen zu fördern oder auch nur zu erlauben. Auf Paarebene sind Beziehungen in dynamischen Milchkuhherden nur wenig verstanden und mögliche positive Effekte bleiben ungenutzt, obwohl Phänomene wie ‚social buffering' auch im Bereich von Tierwohl und -gesundheit von zunehmendem Interesse sind. Die vorliegende Dissertation zielt in diesem Zusammenhang darauf, Form, Funktion und Bedeutung von sozialen Beziehungen unter Milchkühen in einer dynamischen Gruppe zu untersuchen.

Die Daten wurden an der Forschungsfarm des Institute of Animal Science, Prag, in der Gruppe der früh- und hochlaktierenden Kühe erhoben. Um ein möglichst umfassendes und repräsentatives Bild zu erhalten, dienten alle Kühe als Fokustiere, die nach der Abkalbung in die Gruppe eingegliedert wurden, sowie parallel dazu schon in der Gruppe anwesende Kühe, nach Rasse und Laktationsnummer passend zugeordnet. In der Analyse wurden (1) Dimensionen sozialer Beziehungen unter Milchkühen anhand von sozialen Interaktionen und räumlicher Nähe charakterisiert, (2) Effekte bereits anwesender bekannter Tiere auf das Liegeverhalten und die Synchronität frisch eingegliederter Kühe untersucht, sowie (3) ergänzend die Herzfrequenz und Herzfrequenzvariabilität in Ruhephasen analysiert.

(1) Vier Dimensionen charakterisierten soziale Beziehungen und repräsentierten dabei (Interaktionen während der) gemeinsame(n) Futteraufnahme, Verdrängungen, Ruhen sowie soziale Fellpflege. Kühe präferierten bestimmte, kontextabhängig unterschiedliche Partner. Bekanntheit über lange Zeiträume und Kontinuität in der gemeinsamen Erfahrung waren mit der Intensität der Beziehung assoziiert, d.h. mit der aufgewendeten Zeit und Energie, aber nicht auf ausschließlich als gemeinhin positiv betrachtete Art und Weise. (2) Primipare frisch integrierte Kühe lagen nach der Eingliederung kürzer und waren auf Paarebene weniger synchron als die schon anwesenden Vergleichskühe, während ein solcher Effekt bei multiparen Tieren nicht beobachtbar war. Bei primiparen Tieren war weiters zu beobachten, dass sie sich umso synchroner auf Paarebene verhielten, je mehr Tiere in der Gruppe anwesend waren, die sie seit frühester Zeit kannten, aber umso kürzer ruhten. Bei multiparen Kühen hingegen war ein positiver Effekt hinsichtlich der Gruppensynchronität zu beobachten. (3) Ein ähnliches Muster bestätigte sich hinsichtlich der Analyse von Herzfrequenz (HR) und Herzfrequenzvariabiltät (HRV). HR und HRV frisch eingegliederter primiparer Tiere wiesen auf einen erregten Status hin, HR und HRV der bereits anwesenden Tiere auf einen anpassungsfähigen und entspannten Status. Umgekehrt schienen frisch integrierte multipare Tiere gegenüber ihren bereits anwesenden Gruppengenossinnen hinsichtlich HR und HRV entspannter. Die Anzahl bekannter Tiere in der Gruppe hatte keinen Effekt auf die HR und HRV von frisch eingegliederten, aber auf die von bereits anwesenden primiparen Kühen positive Effekte.

Insgesamt unterstützen unsere Ergebnisse die Annahme, dass Kühe ihre Beziehungen aktiv beibehalten und dass insbesondere früh gebildete Beziehungen auch in dynamischen Herden über die Zeit bestehen können. Die Intensität der Beziehung – nicht aber in rein affiliativer Form – hängt mit der Dauer der Bekanntschaft zusammen, Kurzzeitbekanntschaften scheinen nicht relevant zu sein. In der herausfordernden Situation, nach der Abkalbung in die zu melkende Herde eingegliedert zu werden, hatte die Anwesenheit bekannter Tiere keinen beruhigenden Effekt auf Erstkalbende. Hingegen waren positive Effekte bei Tieren zu beobachten, die entweder schon eingewöhnt oder durch ihr Alter erfahren waren. Wir schließen daher, dass eine dynamische soziale Umwelt stabile und kontinuierliche Beziehungen zwar erlaubt, dass diese aber leicht durch dringlichere Themen wie hohe Stoffwechselbedarfe oder Stress durch unbekannte Situationen verdeckt oder unterdrückt werden – und damit ggf. auch mögliche positive Effekte. Um das volle Potential sozialer Beziehungen einschätzen zu können, braucht es weitere Untersuchungen über ihre Form und Funktion sowie auch die Abhängigkeit in Bezug auf Aspekte des Haltungssystems und Managements. Dies kann dann zu Empfehlungen führen, die weniger darauf abzielen, konfliktgeladene Situationen und negative Effekte zu vermeiden, sondern vielmehr darauf, harmonische Situationen und positive Effekte sozialer Gemeinschaft zu fördern.

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1 Foreword – Social relationships matter

"[S]ocial connections are really good for us, and […] loneliness kills. It turns out that people who are more socially connected to family, to friends, to community, are happier, they're physically healthier, and they live longer than people who are less well connected. And the experience of loneliness turns out to be toxic. […] And we know that you can be lonely in a crowd and you can be lonely in a marriage, so […] it's not just the number of friends you have, and it's not whether or not you're in a committed relationship, but it's the quality of your close relationships that matters. […] And good, close relationships seem to buffer us from some of the slings and arrows of getting old. Our most happily partnered men and women reported, in their 80s, that on the days when they had more physical pain, their mood stayed just as happy. But the people who were in unhappy relationships, on the days when they reported more physical pain, it was magnified by more emotional pain."

In his TED talk, Robert Waldinger speaks of the main conclusions he and his current and past colleagues draw from the Harvard Study of Adult Development: started in 1938, about every two years 724 men in two groups with distinct socio-economical background completed questionnaires, gave interviews and handed out medical information (in 2017, 19 of them still did). He continues: "So this message, that good, close relationships are good for our health and well-being, this is wisdom that's as old as the hills." (Waldinger, 2015).

The significance of social relationships, social embeddedness and social support is comparably long known to the scientific fields traditionally dealing with these phenomena such as sociology, (cultural) anthropology, ethnology or psychology. Still, a systematic scientific approach and interest in the underlying mechanisms began only recently, especially in the area of animal science – and when compared to age of hills... For a few decades now, increasing attention has been paid in a variety of scientific and non-scientific fields to the phenomena of the social share of health, resilience and welfare, including animal science. Alongside, the present dissertation aims to add knowledge to the understanding of the relevance, the function and functioning of dairy cows' social life and experience. The work is embedded in the broader scope of evaluation and improvement of animal husbandry systems and innovations in animal welfare assessment.

2 General introduction

2.1 Animals' social relationships

In the animal kingdom, group-living is widely spread and ranges from simple temporary aggregations to complex multigenerational and individualised societies. The level of sociality relates less to the spatial and temporal distribution of individuals, but to their interactions at various scales. All mammals interact socially, as mothers and their offspring have to communicate and interact intensively (and fertilisation in advance requires some social exchange, too). And while traditionally sociality only begins beyond interactions in the context of reproduction, following a rather uncommon perspective even solitary living mammals can be viewed as social over large distances: Orangutans (*Pongo spec.*), mouse lemurs (*Microcebus spec.*) and loris (*Lorinae*) for example are defined as 'solitary but social' (Sussman, 2003), and solitary carnivores' social behaviour only recently began to gain attention, bearing evidence for an underlying 'hidden' complex social system (Elbroch et al., 2017; Graw et al., 2019).

Several attempts have been made to categorise species into distinct social systems, however sociality may be best described as a multidimensional continuum. Kappeler (2019) proposed a four-dimensional framework for the study of social systems and complexity. The (1) **social organisation** (referring to the size and composition of a social unit), the (2) **social structure** (defined by the content, quality, and patterning of social relationships emerging from repeated interactions between pairs of individuals belonging to the same social unit), and the (3) **mating** and (4) **care system** represent distinct components of every social system. The complexity of animals' social relationships may be related to all those components. So which factors determine the beneficial potential of social connectedness? How much do social relations matter for animals?

2.2 Perspectives from behavioural ecology

Behavioural ecology explains behaviour in the framework of evolutionary theory and considers behaviour as subjected to evolutionary processes and mechanisms in the same way as e.g. anatomical characteristics are. In this sense, group-living and sociality are understood as evolutionary strategies that offer benefits such as more efficient foraging, shared vigilance, easier monopolisation of resources or reduced vulnerability to predators (Krause and Ruxton, 2002). At the same time, however, being more than one is

accompanied by costs such as higher risk of infection and parasite load (Hoogland, 1979; Pulliam and Caraco, 1984). Further, as behaviour is determined by many 'egoistic'

Figure 1: A front of muskoxes. By far no easy prey: acting as a group accumulates eyes, ears and power. But note that those Muskoxes (Ovibos moschatus) are more closely related to goats than to cattle. (Nunivak Island, Alaskan muskoxen in the 1930s, shown here in defensive formation; U.S. Fish and Wildlife Service, © public domain)

motivations, conflicts of interests and enhanced competition are an inert characteristic of sociality, with the potential to escalate into serious and costly conflicts. All types of social organisation and structure form part of evolutionary strategies to buffer those negative aspects of group-living and to maximise benefits. In individualised and (temporary) stable animal groups, relationships between members can become defined, remembered (by the directly concerned individuals, and potentially also by non-involved bystanders) and stable, thus the battle don't have to be fought each time (and not by each individual). Social hierarchy and its communication through signalling dominance, submission, and appeasement is a prominent example of social structure that helps to control aggression (Preuschoft and Van Schaik, 2000). Avoiding conflicts is one part, however adaptation to sociality likely includes wanting to have company and being part of a group. A growing body of evidence shows that animals do interact in affiliative, cooperative and seemingly altruistic ways, and that they actively seek, maintain and repair positive relationships, frequently even independent of genetic relatedness (e.g. domestic goats, *Capra hircus* (Schino, 1998); spotted hyaenas, *Crocuta crocuta* (Wahaj et al., 2001); bottlenose dolphins, *Tursiops truncatus* (Weaver, 2003); domestic dogs, *Canis familiaris* (Cools et al., 2008); wolves, *Canis lupus* (Cordoni and Palagi, 2008); corvid species, *Corvus spec*. (Fraser and Bugnyar, 2010; Sima et al., 2018); horses, *Equus caballus* (Cozzi et al., 2010)). The concept of post-conflict friendly re-union for example describes a behavioural strategy to reduce anxiety and restore relationships when a conflict has escalated into an aggressive interaction. The proposed relation to relationship quality and value could be confirmed in number of species (de Waal and Yoshihara, 1983; Kappeler, 1993; Cheney and Seyfarth, 1997; Call et al., 1999; Palagi et al., 2008; Thierry et al., 2008; Cozzi et al., 2010). In summary, theoretical models and empirical evidence indicate that evolution favoured long-lasting, individualised, complex and valuable relationships, and at the same time the required cognitive and emotional capacity to recognise and remember group members as well as the social history with them, and among

them (Hamilton, 1964; Aureli and de Waal, 2000; Aureli et al., 2002; de Waal and Tyack, 2003; Kappeler, 2008). The next paragraph hence focuses on the physiological, psychological and emotional fundaments of social relationships in animals.

2.3   Potential inclusive benefits

For gregarious animals, the group is an essential resource and as such potentially related to biological fitness, health and welfare. This does not only refer to the above mentioned primary and "external" benefits such as reduced vulnerability, but also to the internal features adaptive to group-living. Emotions, encompassing behavioural, autonomic and endocrinological responses, are supposed to be a driving factor in social behaviour at different scales. They work in the very present individual, are evaluated by aggregated experiences over the lifetime, triggered by physiological and hormonal cascades manifested in the genotype over generations, by reason of enhanced Darwinian fitness. To avoid situations which elicit an uncomfortable emotional state, and to seek for and maintain situations producing a positive one – in other words: the principles of reinforcement – are key mechanisms to form and control behaviour. Social behaviour thus is mediated and motivated by emotions in a way that isolation, threats to social bonds or the risk of social exclusion produce negative emotions such as anxiety, whereas proximity, socio-positive behaviour or assurance of social bonds elicit positive ones (Aureli and Smucny, 2000; Hennessy et al., 2009; Machin and Dunbar, 2011; Panksepp, 2011). These changes in the emotional state have consequences. The phenomenon of 'social buffering' for example describes a stress-reducing effect through the simple presence of a conspecific, even if the stressor itself is of non-social quality (Hennessy et al., 2009; Kiyokawa, 2018). As known from human psychology and related fields, the emotional state is not only affected in the short-term, but may trigger impairing or beneficial long-term effects both on psychological and physiological health and resilience, e.g. through shifts in rating and regulatory systems (reviewed e.g. in Karelina and DeVries (2011)). There is a growing body of research providing evidence for similar phenomena in non-human social animals (reviewed e.g. in Hennessy et al. (2009)), and for the specific underlying physiological mechanisms (reviewed e.g. in Walker and McGlone (2013); Crockford et al. (2014)).

However, social life is complex, and being together with others does not necessarily result in the above mentioned effects. As stated earlier, the social structure of an animal group may basically be described as the aggregated content, quality, and patterning of social relationships, which are formed by interactions between individuals (and through their content, quality, and patterning (Hinde, 1976; Kappeler, 2019). In turn, importance and complexity of individual dyadic relationships differ between species depending on various factors including the type of social organisation, ecological aspects, or brain size (Shultz and

Dunbar, 2007; Pollard and Blumstein, 2012) – and with them the potential of social buffering effects (Hennessy et al., 2009). The more complex relationships are, the more they may differ in importance, the more impact they may have. Even within species or within one and the same group a variety of social and non-social needs, restrictions and experiences diversely influences the characteristics and quality of social relationships – and therefore at the same time possible positive social effects. The next paragraphs focus on where cattle fits into this picture.

2.4 Cattle social organisation and structure

As the last alive aurochs (*Bos primigenius)* was recorded in 1627, direct observations of cattle's wild ancestors are no longer possible. Based on observations of extensively kept or semi-wild domestic cattle groups however (Schloeth, 1961; Hall, 1983; Reinhardt et al., 1986; Hall, 1989), they presumably lived in stable groups consisting of several females, mothers and their female and young male offspring until fully matured, more or less temporarily accompanied by one or several mature bulls. Depending on ecological aspects such as feed abundance, spatial restrictions or seasonality of the mating season, adult bulls may be part of the herd permanently, or stay in individual or shared home ranges where they are visited by moving female herds (Bunzel-Drüke et al., 2008). Male offspring leaves the group (or is forced to leave it) during adolescence or as young adult and builds so-called bachelor groups with males from other families. Also beyond this, cattle groups probably experienced fission-fusion dynamics during foraging, resting, or migrating, within group and between groups, comparable to some deer species (Cervidae) or Africa's large herbivores (Owen-Smith et al., 2020; Szemán et al., 2021). Cattle is long-living, late-matured, and the social hierarchy among females is associated rather with age, i.e. experience, than with body weight as an equivalent of physical strength (Šárová et al., 2013).

While therefore theoretically predestined to exploit the full potential of social connections, empirical evidence for the functions and value of social relationships among cattle and especially among dairy cows is sparse, and systematic and hypothesis-driven studies on relationship quality in cattle are actually more or less lacking. Impressions such as from the images below (**Figure 2**) illustrate situations that may favour cooperation, and anecdotally, cases of 'friendship' among genetically unrelated cattle have been reported (e.g. Sambraus (1976), but see also Wasilewski (2003)) and are frequently mentioned in standard textbooks on cattle social behaviour (e.g. Bouissou et al., 2001). However, it is not quite clear whether at all, and if to what extent (dairy) cows experience advantages such as food-sharing or support in agonistic interactions through friendly relationships, or disadvantages by loss of such a relationship.

Figure 2: Simmenthal cows confronted with a black bear in Canada. A situation calling for cooperation (© http://www.youtube.com).

2.5   The (social) world of a dairy cow

While defence against predators and competition for mates should not play a role for the domestic cattle in modern production systems, the animals need to cope with competition for feed, restricted space allowance, and unstable artificial groups with high disturbance rate. Functions and functioning of social relationships in such an environment are little understood.

2.5.1   Dairy cow husbandry

For dairy cows, loose group housing is nowadays widely implemented and allows free movement, choice of social partners and expression of social behaviour at a much higher level than tie stalls did (or do). However, the animals are still enclosed and restricted by stall equipment and elements and by management practices. Group structure and social relationships are frequently disturbed. Mother-offspring relationships are non-existent as calves are usually separated immediately or within the first hours after birth and reared artificially. Groups are mostly unstable due to regrouping according to reproductive state or productivity levels, and the age structure is shifted towards rather similar-aged animals, as young stock is kept separately and dairy cows have a comparably short life expectancy (in Austria, the average age reached in 2020 was 6.3 years (Egger-Danner et al., 2020) while the lifespan of cattle amount to up to 20 years). Animals are usually dehorned and consequently adapt their communication towards higher levels of physical interactions (Knierim et al., 2015; Lutz et al., 2019). Additionally, they have to cope with high nutritional and metabolic demands under enhanced competition on locally and temporally predetermined resources, and a diurnal rhythm strongly influenced by artificial pace makers such as milking or fresh feed delivery. This situation may be aggravated through competition for feeding places, as in Austria an animal feeding-place ratio of up to 2.5:1 is permitted when combined with *ad libitum* feeding.

Figure 3: Cows and calves in extensive and intensive systems. (© licencefree from pixabay)

2.5.2 Stress, health and welfare

Studies addressing health and welfare issues in dairy cattle often focus on production-related problems such as metabolic disorders, reduced fertility or lameness. Except obviously harmful interactions or permanent agitation, social behaviour as such traditionally was of less interest. A number of farm management routines however have effects on behaviour that in turn affect health and productivity. Regrouping, large group-size, and high stocking density for example lead to an increase of agonistic interactions combined with a decrease in feeding and lying time, which in turn may affect productivity (Raussi et al., 2005; DeVries and von Keyserlingk, 2006; Huzzey et al., 2006; Gupta et al., 2008; von Keyserlingk et al., 2008a; Hill et al., 2009). Further, negative effects on fertility (Dobson et al., 2001) and a short-term decrease in productivity have been shown (Arave and Albright, 1976; Brakel and Leis, 1976; Fernández et al., 2007 for goats; von Keyserlingk et al., 2008a).

The possible consequences of living in an unstable and possibly rather anonymous social environment on emotional and physiological wellbeing, health and resistance, and on the ability to benefit from the group through social buffering of stressful events, or positive emotional experiences, have rarely been considered yet (Jóhannesson and Sørensen, 2000; DeVries et al., 2003a; Rault, 2012). While current recommendations aim at avoiding conflict-laden situations and negative effects, promoting harmonic and positive ones could be the future direction.

2.5.3 (Social) support and resilience

Some investigations in cattle have addressed possible positive short-term effects of social company. In test situations, e.g. during separation (Færevik et al., 2006; McLennan, 2013) or when confronted with a new object or sudden noise (Takeda et al., 2003), calves and heifers were calmer in familiar or preferred company than when tested alone or together with unfamiliar or non-preferred animals. In learning experiments, the presence of (familiar) peers facilitated learning about a new environment (Bailey et al., 2000; Costa et al., 2016; Acevedo-Triana et al., 2017). Regrouping experiments comparing single versus pair- or groupwise integration into a herd provide evidence that the presence of familiar peers

positively influences behaviour and helps to alleviate stress (Bøe and Færevik, 2003; for calves: Færevik et al., 2007; O'Connell et al., 2008; Gygax et al., 2009b). The fact that familiarity might be a critical factor concerning the effectiveness of social buffering has been shown for other species as well (e.g. Kiyokawa et al. (2014) for rats, Hennessy et al. (2008) for guinea pigs, or Kiyokawa and Hennessy (2018) for a review) and is in line with the idea, that relationship quality and social supportive effects are interrelated.

Positive long(er)-term effects have been shown in the context of calf development, showing for example that stable groups positively affect weight gain and reduce disease prevalence (Pedersen et al., 2009), and that early pair-housing of calves prior to grouping allows most social support afterwards (Bolt et al., 2017). In dairy cow research however, studies focusing on effects in the long(er) term are sparse (but see (McLennan, 2013)).

2.6 Approaches to the study of social relationships

As we cannot interview animals about their friends and foes, we need tools to approximate the quality and relevance of their relationships. Beneath controlled test situations, behavioural observations in the "natural" environment are ethologists' main tool.

2.6.1 Behaviour

As mentioned earlier, relationships can be described as content, quality, and patterning of interactions (Hinde, 1976). Encounters with and without bodily contact as well as spatial and temporal associations are adduced to gain information about the specific relationship between two individuals (Whitehead, 2008; Martin and Bateson, 2021). Seemingly same behaviours do not necessarily mean the same depending on the specific context and opponents, and therefore additionally have to be contextualised in relation to individual states as well as the physical and social environment – season, feed abundance, predatory pressure, reproductive state, the presence of young offspring and many other factors may all influence form and function of behaviour. One example in cattle is allogrooming behaviour, that can be interpreted in the context of hygienic aspects (Hart, 2000; Kohari et al., 2009), of bonding and affiliation (Reinhardt and Reinhardt, 1981; Boissy et al., 2007), communication of dominance structure (Reinhardt et al., 1986; Sato et al., 1993), or as coping strategy related to social conflicts (Waiblinger et al., 2002; Val-Laillet et al., 2009) or individual stress (Fraser and Broom, 1990; Tost, 2000). Many detailed observations at individual basis conduced to the current scientific knowledge. A usual rough classification distinguishes between affiliative and agonistic interactions, but this is by far not clear-cut. Even when in competitive situations a winner gains access to some resource and a looser does not, from a single event only careful conclusions can be drawn.

The detailed observation of social relationships is complex, expensive and time-consuming and not even always possible. Individual and group-level observations yield valuable information and approximations about the functioning of the group and the status of its members especially in comparative designs and therefore allow some conclusions about the underlying pattern of dyadic relationship. And finally, to complete the information about relationships gained from directly observable behaviours, a look inside may be useful:

2.6.2 Physiology

To complement the information about relationships gained from directly observable behaviours, investigating the internal autonomic and endocrinological processes, which accompany behavioural output, may provide important additional information about the value and quality of interactions and relationships. The assessment of non-invasive physiological stress markers has long traditions in animal behaviour research, in particular correlates of the hypothalamus-pituitary-adrenal (HPA) axis through measures of cortisol metabolites in faeces and saliva, and, more recently, through heart rate variability (HRV) (Minton, 1994; von Borell et al., 2007a). HRV measures are derived from differences between interbeat-intervals of heart rate and image the balance between the sympathetic and parasympathetic branch of the autonomic nervous system (ANS) (Shaffer and Venner, 2013). The ANS in turn is known to control homeostasis and vital functions, and to interact with physiological and emotional responses to significant events by adapting the organism to an appropriate level of agitation ("fight or flight") or relaxation ("rest and digest"). Most advantageously, HRV reflects the very current response to a situation or event, and at the same time allows conclusions about the general (cardiac) health status (von Borell et al., 2007a; Kovács et al., 2014).

2.7 Connections to animal welfare science

The present work was conducted in the broader framework of evaluation and improvement of animal husbandry systems and innovations in animal welfare assessment. While it is now generally accepted that welfare assessment should focus on animal-based measures, the absence of negative indicators, which additionally refer mostly to measures of the physical state of the animals, is still in the foreground of the approaches used (Winckler, 2018). Modern holistic models of animal welfare include ethical concerns related to affective states, or concepts such as integrity or naturalness (Fraser, 2003). The scientific search for positive indicators of welfare is ongoing (Lawrence et al., 2019; Rault et al., 2020), and positive social relationships and affiliative interactions are promising candidates (Napolitano et al., 209; Laister et al., 2011). Findings related to form, function and impact of specific in-depth aspects

of the social environment such as valuable relationships or social buffering on animal welfare may therefore forward dairy cow health and welfare assessment and improvement.

3 General aim of the dissertation

The present doctoral project aims at examining the characteristics, determinants and relevance of social relationships among dairy cows kept under commercial conditions in a dynamic group. Theoretical basis are assumptions from behavioural ecology, valuing individual social relationships as an essential part of gregarious beings in an evolutionary framework. In this sense, establishing and maintaining social relationships belongs to the behavioural needs of an animal and are therefore likely to be connected to emotional experience, health and welfare. The studies want to add knowledge of the form, function and significance of social relationships among dairy cows that can help to adapt the farm environment and management in a way that individualised, functionable relations are enabled and promoted and therefore can unfold to a win-win optimum.

First attempt was to characterise dimensions of social relationships among dairy cows and to search for determinants of differing qualities. Associations between spatial proximity and different types of social interactions were analysed and related to specific characteristics of the two participants such as breed, age difference or varying levels of familiarity. Associated dyadic patterns would open up possibilities to easily estimate the social potential of a given group.

The second approach focused on lying behaviour and synchrony at the dyadic as well as group level as a proxy for social connectedness, integration and group cohesion. The particular relevance of this approach lies in the fact that lying behaviour could be collected automatically, therefore it is predestined for animal welfare research and on-farm assessment in terms of feasibility and practicability.

The third analysis presented here virtually attempts to take a look inside the cows. Variations in the interbeat-intervals of heart rate provide an image of the sympathetic and parasympathetic nervous system's activity, which serves to control homeostasis, vital functions, and to adapt the organism to an appropriate level of agitation ("fight or flight") or relaxation ("rest and digest"). An analysis of the so-called heart rate variability therefore allows conclusions about the inner state, even when animals show the seemingly same behaviour. With this study we attempted to learn more about the influence of social company on relaxation.

The following chapter describes the general study design and research site.

4 General methods

4.1 General design

The project design was developed around certain premises. First, we aimed at investigating dairy cows in their normal, undisturbed, 'natural' environment, preferably representative for a large part of dairy cow population. Second, variation among animals should be maximised to yield a most comprehensive sample of forms of social behaviours, interactions and relationships. Third, observations should be at an individual level, considering the identity of all involved animals, and at a detailed and descriptive level including fine scale behaviours. Fourth, as we focused on stress relieving, beneficial effects of social relations, a presumably rather stressed sample should be compared with a presumably rather relaxed control or baseline sample. Those factors led to the present basic study design. We chose one group of dairy cows at one farm representative in size and management practices for a large part of European dairy farms and continuously observed focal animals over several days. Focal animals were chosen based on the farm's management rhythm and therefore covered a random and representative sample of its cows. We observed all cows that entered the group after calving as the "stressed sample", and in parallel cows that were already present in the group as the "control sample" matched by breed and lactation number. With the aim to maximize variation and to control for period-specific influencing factors, we decided to pseudo-replicate our study and repeated data collection four times.

4.2 Research site and data collection

The project was conducted in cooperation with the Výzkumný ústav živočišné výroby, v. v. i. (Institute of Animal Science) in Prague, Czech Republic, at the associated research farm 'Netluky'. The farm keeps a variety of farm animals from rabbits over red deer and pigs to different cattle breeds in breeding and fattening units, and about 230 dairy cows plus female followers and male offspring for fattening.
Dairy cows are kept in three groups consisting of early and high lactating, mid to late and lower lactating, and dry standing cows, respectively (Figure 4). The cows are kept in a cubicle system and fed with a Total Mixed Ration (TMR) that is available ad libitum at a feed bunk with neck rail. Milking occurs twice a day at 3:30 a.m. and 3:30 p.m. in a 2 x 4 tandem milking parlour by alternating two teams of two milkers each.

Figure 4: Research farm "Netluky". Marked in red the dairy units with (1) the investigated group of early and high lactating cows, (2) the group of mid to low lactating cows, (3) the milk house, and (4) the group of drystanders, where also the calving pens and pens for freshly calved cows are located.

Data were collected in the group of early and high lactating cows with a group size between 46 and 54 animals. Fresh cows were introduced there in groups of 2-3 animals about one week after calving, and cows getting into lower productive state left the herd in groups of about 10, resulting in a dynamic herd composition with more or less marked changes every 2-4 days.

Data were collected during four observation periods of about eight weeks each in 2010 and 2011 (Table 1). A timespan of at least 100 days in between was chosen as only occasionally cows would spend a longer time in the high producing group. Therefore, with the beginning of a new observation period most individuals the group would be replaced and the achieved data sets would be as independent from each other as possible and serve as pseudo-replicate.

Table 1: Observation periods.

No.	Time period	Regroupings	Focal cows freshly introduced + matched resdient
0	30.05.-09.07.2010	8	19 + 19 – pilot phase, not in analysis
1	28.10.-08.12.2010	9	21 + 21
2	29.03.-19.05.2011	10	21 + 21
3	31.08.-08.10.2011	8	18 + 18

Figure 5: Impressions of the video recording and player software.

Before an observation period started, the barn and cows were prepared. To enable parallel observations of several cows, we used video recordings. The whole barn was video recorded during daylight periods using 9 IP cameras (Sanyo VCC-HD 2300P with YV2.8x2.8SASA2 lenses) that were installed at about 3-4 m height all around the barn. Videos were recorded on a digital hybrid video recorder (NUUO ® NDVR-16-1TB) in colour without sound at a high spatial resolution of 1024 x 768 pixels and 12.5 frames per second (Figure 5). The high video quality allowed individual recognition of the cows, further facilitated by large markings with hair dye (Figure 6), and included features such as zooming, rewinding, slow motion playback etc. to assess even fine scale behaviours.

The farm management provided routinely collected individual data of all cows including date of birth, parents, regrouping events, veterinary treatments, inseminations, pregnancies, calvings, milk yield, weight and more. Each cow of the group was equipped with a HOBO ® accelerometer to automatically collect basic activity data (lying vs. not lying, Figure 7). Initial equipping and follow-up replacements took place in the milking parlour during routine milking times with the help of two technicians of the VUZV. Focal animals (i.e. all incoming cows as well as their matched resident control cows (same lactation and breed, at about 5th week of lactation), see Table 1) were additionally equipped with polar ® heart rate monitors and electrode belts to record cardiac activity as interbeat-interval length (Figure 7). Data download as well as checkings and corrections of the equipment were carried out twice daily by the author after milking.

More detailed and explicit description of data collection and processing are provided in the respective research articles in the chapters 6 to 8.

Figure 6: Hair-dying procedure and result.

Figure 7: The transparent cow. Girth to hold the eletrodes and monitor of the polar ® heart rate measurement system, HOBO ® accelereometer for the automated collection of activity data, pedometer, collar with transponder connecting cow identity with her milk, pedometer and stored life history data.

5 Related Master Theses

In the course of the dissertation project, some methodological aspects were outsourced Specific issues concerning heart rate variability (HRV) were investigated in the course of their Master theses by Kira Ledochowski and Angelika Latschbacher. They added valuable guiding information for the analytical procedure of heart rate variability data of dairy cows. Kira Ledochowski (2012) evaluated whether breed, age or activity influence HR and HRV and showed that not only basic activity in terms of lying vs standing vs walking affected cardiac activity, but partly also more subtle movements such as ruminating. In particular, her study underlined the higher sensitivity and informational value of HRV as compared to heart rate (HR) alone.

In studies using HR and HRV, measurements are often taken during resting periods to avoid interferences due to physical activity. Angelika.Latschbacher (2013) evaluated circadian variations of HR and HRV during resting and in relation to parity and breed, and addressed the important question what time span is needed to reach stable resting values after lying down and before standing up, i.e. how long HR and HRV values need to be no longer or not yet influenced by (preparing) physical movements. She found out that it is important to consider breed, parity as well as time of the day, and to apply appropriate waiting times, as latency to stable resting values was related to parity.

Another conceptual approach was investigated by Anna Herzog (2014) who conducted a pilot study about the use of social network analysis (SNA). She correlated interaction and proximity networks related to different functional areas to describe basic network features of a dairy cow group. Her results show that adjacency patterns differed depending of functional areas, and that SNA captures social relationships differently depending on whether based on spatial distance or forms of interacting.

6 Article 1: Long-term familiarity creates preferred social partners in dairy cows

6.1 Introductory words

The first sub-set of data was assessed from video recordings with the aim to describe forms and find determinants of social relationships among dairy cows. If we would find strong associations between specific aspects of social relationships such as type, frequency, intensity or severity of interactions, or frequency, duration or location of encounters and spatial proximity, this could help describing social relationships at an aggregated qualitative level and to adjust and reduce data collection to the main relevant aspects. If relationships at distinct quality levels would be related to some assignable factors such as age, breed, or shared experience, the first step for the possibility to estimate a "social potential" in a given group could be reached. Given the main limiting factor in the observation and assessment of farm animal behaviour and its relation to welfare is time, such an approach could allow consideration of the social environment in a time-saving way.

6.2 Published article
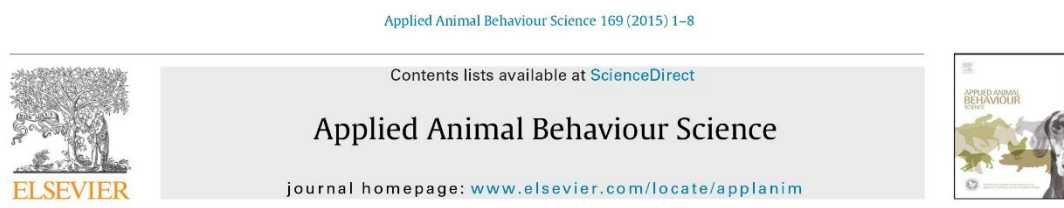
Long-term familiarity creates preferred social partners in dairy cows

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LONG-TERM FAMILIARITY CREATES PREFERRED SOCIAL PARTNERS IN DAIRY COWS

Running title: Familiarity creates preferred partners in dairy cows

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ABSTRACT

The group is an essential resource for gregarious animals. Dairy cows are however frequently (re-)grouped according to productivity and reproductive state leading to an unstable social environment for the animals. The present study aimed at investigating whether cows maintain social relationships in a dynamic group. Therefore we analysed whether more familiar cows spend more time in close proximity, and interact more often in an affiliative way. Social interactions and direct neighbours during feeding and resting of 12 Holstein cows (1st to 3rd lactation) in a dynamic dairy cow group of 50 animals were assessed continuously over four days using focal animal sampling. A principal component analysis over the twelve assessed social behaviour variables per pair revealed four main components: social relationships may be characterised by time spent as direct neighbours when feeding and interacting affiliative as well as agonistically (excluding displacements), by displacement success, allogrooming interactions, and time spent as direct neighbours when resting. Long-term (shared youth experience, shared adult experience) and short-term (shared dry-period, synchronised group entry) familiarity was associated with higher scores for interacting and being direct neighbours when feeding ($p < 0.05$ for shared youth experience, shared adult experience, and shared dry-period), allogrooming ($p < 0.1$ for shared adult experience * shared dry-period), and being direct neighbours when resting ($p < 0.05$ for shared youth experience * shared adult experience). Long-term familiarity had a stronger effect on the intensity of social relationships, i.e. regarding investment of time and energy, than very recent shared experience. These results support the notion that dairy cows actively maintain valuable dyadic relationships. In practical terms, keeping well-acquainted cows together may contribute to a stable inner structure of a dairy herd and thus promote dairy cow welfare.

1. INTRODUCTION

The group is an essential resource for gregarious animals, and as such potentially related to biological fitness, health and welfare (Mendl and Held, 2001; Špinka, 2012). Isolation, the risk of social exclusion, or threat to social bonds elicit physiological and behavioural stress responses (Aureli and Smucny, 2000). Proximity, security, or assurance of social bonds on the other hand elicit responses that facilitate and reinforce to maintain the situation. In the long-term, such a physiological and emotional state has 'stress-buffering' effects, i.e. it promotes coping with and recovery from non-social as well as social stress (Cohen and Wills, 1985; Kikusui et al., 2006). A secure and positive social environment therefore has beneficial short-term as well as long-term effects both on psychological and physiological health and resilience (Hennessy et al., 2009; Karelina and DeVries, 2011). There is growing interest in considering this so-called 'social buffering' in farm animal health and welfare (Rault, 2012). The importance and complexity of individual dyadic relationships within a group and their potential social buffering effectiveness differ between and also within species depending on various factors including ecological aspects, social structure, strength of bonding or relationship quality (Hennessy et al., 2009; Pollard and Blumstein, 2012). Either way, social interactions form the basis: social structure can be devided into patterns of individual social relationships differing in nature and quality, that are in turn defined by content, quality and patterning of social interactions (Hinde, 1976). Depending on the physical appearance of the involved animals, e.g. muscle tension and strain, and depending on the outcome of an interaction, i.e. roughly said whether the distance between the animals is reduced, maintained, or increased, interactions are distinguished into affiliative, neutral and agonistic ones to describe animal social relationships.

Based on observations of extensively kept or semi-wild domestic cattle groups (Schloeth, 1961; Hall, 1983; Reinhardt et al., 1986), cattle ancestors lived in stable groups of largely related females and their young offspring, and probably experienced fission-fusion dynamics, i.e. temporarily joining with and splitting from other groups for foraging, resting, or migrating (Cornélis et al., 2014; van Vuure, 2014). Such a social environment is supposed to promote individualised long-lasting, complex, and valuable relationships (Hamilton, 1964; de Waal and Tyack, 2003; Aureli et al., 2008). Cases of 'friendship' have been reported among adult cattle (Sambraus, 1976) and are frequently mentioned in standard textbooks on cattle social behaviour (Bouissou et al., 2001) even though systematic and hypothesis-driven studies on relationship quality in cattle are lacking.

For dairy cows, loose group housing is nowadays widely implemented, facilitating choice of social partners and expression of social behaviour. However, the social environment in large-scale dairy herds of hundreds of animals is fragile and demanding: It is well documented that husbandry routines, namely regrouping, large group-size, and high stocking density, lead to

an increase of agonistic interactions combined with a decrease in feeding and lying time (Raussi et al., 2005; DeVries and von Keyserlingk, 2006; Huzzey et al., 2006; Gupta et al., 2008; von Keyserlingk et al., 2008a; Hill et al., 2009), have effects on fertility (Dobson et al., 2001), weight gain and health in calves (Pedersen et al., 2009), and productivity (Arave and Albright, 1976; Brakel and Leis, 1976; Von Keyserlingk et al., 2008b). On the other hand, results of regrouping experiments comparing single versus pair- or groupwise integration into a herd provide evidence that the presence of familiar peers positively influences behaviour and helps to alleviate stress (Bøe and Færevik, 2003; for calves: Færevik et al., 2007; O'Connell et al., 2008; Gygax et al., 2009b). In large intensive systems, social adaptability could conceivably be overstrained and dairy cows may no longer be able to maintain individualised relationships. The possible consequences of living in an unstable and rather anonymous social environment on emotional and physiological wellbeing, health and resistance, and on the ability to benefit from the group through social buffering of stressful events, or positive emotional experiences, have rarely been considered yet (Jóhannesson and Sørensen, 2000; DeVries et al., 2003a; Rault, 2012).

The present study therefore aimed at investigating whether indicators of preferential social relationships differ between pairs of dairy cows depending on their familiarity. Our hypothesis was that cows seek to maintain stable relationships and therefore spend more time and interact more often in an affiliative way with their most familiar herd mates. Social relationships among cows were assessed on the basis of time they spent in close proximity and frequencies of social interactions, which are common means in the study of non-human animals' social relations (Whitehead, 2008).

2. ANIMALS, MATERIAL AND METHODS

The study was designed according to European and Czech laws and current guidelines for ethical use of animals in research (ASAB, 2006). The study was approved by the Institutional Animal Care and Use Committee of the Institute of Animal Science (Permit Number 09/2010).

2.1 Study site, animals and management

The data presented here were collected from videos that had been recorded in April and May 2011 at the research farm 'Netluky' of the Institute of Animal Science, Prague, Czech Republic. The study was carried out in the group of early lactating and high yielding cows comprising ± 50 dehorned animals of 70% Holstein breed (9,900 kg average 305 day milk yield) and 30% Czech Spotted breed (7,800 kg yield). Introduction into this group after calving occurred about twice per week (2-5 animals together). Prior to calving, dry cows and pregnant heifers (last three months before calving) were kept together in a separate barn. A

few days before calving cows were individually moved into an adjacent part of this barn which allowed full visual and olfactorial, and partly physical contact to the group of dry cows and pregnant heifers. Calving took place in an adjacent single calving pen, and the first three to ten days after calving the cows were kept separated from the other groups in single stalls in a separate building. The early lactating group was housed in a 30 x 15 m stable with curtain ventilation system that contained a 30 m feeding face with neckrail, 54 cubicles with straw bedding (1.35 x 2.4 m) in two rows facing each other and one row facing the wall, concrete corridors littered with small amounts of straw, two water troughs, two salt licks and an automatic brush. The animals were fed ad libitum (fresh feed twice daily at about 06:00 and 14:00 h) with a total mixed ration (TMR). Milking was carried out by alternating two pairs of milkers twice daily at 03:30 and 15:30 h in a 2 x 5 automatic tandem milking parlour. The corridors were cleaned twice daily during milking with a skid loader.

2.2 Focal animals and behavioural observations

For the video recordings, nine IP-cameras (Sanyo VCC-HD 2300P with YV2.8x2.8SASA2 lenses) were installed at about 3-4 m height all around the barn. Videos were recorded on a digital hybrid video recorder (NUUO ® NDVR-16-1TB) in colour without sound at a resolution of 1024 x 768 pixels and 12.5 frames per second.

For individual recognition, all animals in the group were marked with large symbols (about 50 cm in diameter) on both flanks using hair-dye ('Kurcreme Oxid' (9% hydrogen peroxide) and 'Bleaching Powder dust-free' by ROMA Friseurbedarf, Robert Maurer GmbH, Laxenburger Straße 165-171, 2331 Vösendorf, AUSTRIA).

Data were collected during three periods of four consecutive observation days. Per period, four Holstein cows each were observed using continuous focal observation for 5.5 hours per day, alternating from 13:00 to 19:30 h (including a break of ± 1 hour during afternoon milking), and from 07:30 to 13:00 h. Focal cows were chosen in order to cover a broad range of individual experiences with farm life, with the group, and single group mates. The focal animal group thus consisted of 12 cows, six of them freshly introduced after calving (start of observation) in 1st, 2nd and 3rd lactation, respectively, and six age-matched resident cows (4th to 5th week of lactation) observed in parallel. During the four-day observation periods no further cows joined or left the group except one occasion were a cow had to be removed due to health problems.

Data were collected by one person continuously from the videos using Mangold INTERACT® (programme version 9.6.4.375) video analysis software and comprised start and end time of basic activity of the focal animals (feeding, standing and lying in a cubicle, and standing at or walking through the corridor), start and end time and identitiy of direct neighbours during feeding and cubicle periods, and start and end time, type of, and identity of the partner in

social interactions (for definitions see Table 2). As a basis to calculate frequencies of independent repeated encounters between cows, meal and non-meal bouts were extracted from the raw data. Following DeVries et al. (2003b), meal criterion was set to 30 minutes, i.e. a meal bout started with the first feeding activity after 30 minutes without feeding or after leaving a cubicle, a non-meal bout accordingly when entering a cubicle or after 30 minutes without feeding. Frequency of repeated encounters was defined as being neighbour and/or interacting during independent meal and non-meal bouts.

Social interactions were defined and collected at a behavioural level for both the focal and its partner cow and then summarised into eight directed categories: the focal animal could either be 'actor' or the 'recipient' in a 'displacing', 'agonistic', 'non-agonistic' or 'licking/soliciting' interaction. Table 2 gives an overview over definitions of social behaviours and interaction categories.

Table 2 (Article 1, Table 1):Definitions of basic behaviours, neighbourhood, social behaviour and interaction categories.

basic behaviour	definitions
feeding	putting mouth into the feed (start), overstepping the (imagined) line with the forelegs where the hind quarters of feeding cows (would) end (end)
standing in a cubicle	first foot entering (start) and the last foot leaving (end) the cubicle, start (end) and end (start) of lying
lying in a cubicle	going down on the carpal joint (start),standing on all four feet (end)
standing at or walking through the corridor	end of feeding or cubicle periods (start), start of feeding or cubicle periods (end)

neighbours	definitions
feeding neighbour	standing next to a focal cow at the feeding face at a maximum of three cow-widths distance
	one animal overstepping the line with the forelegs where the hind quarters of feeding cows end (start, end)
cubicle neighbour	standing or lying in an adjacent cubicle
	one animal entering (start) and the last foot leaving (end) the cubicle

social behaviour	definitions
butting	forceful and/or repeated butts with the forehead towards any body region of the recipient, actor may swing its head or step sidewards or backwards to continue with more power
pushing	softly butting with the forehead or pushing with other parts of the head towards any body region of the recipient, actor does not swing its head, and actor's legs stand still
rubbing	repeated up- and down movements with forehead or other parts of the head in contact to any body region of the recipient, actor's legs stand still
threatening	shown when two cows can see each other, typical display with hunched neck and shoulder, and (presenting) lowered forehead while standing still or approaching, also swinging head movements towards recipient, no body contact
nose-pushing	softly pushing with the nose towards any body region of the recipient, actor does not swing its head, and actor's legs stand still
contact-feeding	feeding with mouth or head contact, or with the head stretched under the neck of the recipient, no pushing movements
sniffing	actor stretches its head and neck towards any part of the body of the recipient until its nostrils have nearly contact, and holds its position for at least 2 seconds
licking	tongue in contact with the body surface (except ano-genital region, udder, teats or claws) of the recipient, repeated up- and down head movements
soliciting	shown when two cows are close and can see each other, typical display with lowered head, more or less downwards stretched neck, ears often in a flat and/or backward posture. If not answered with licking by the recipient, soft touches with forehead or nose may occur and may change over to rubbing or pushing.
interaction categories (focal animal as actor or recipient)	**included social behaviour**
displacing	butting, pushing, rubbing, threatening or approaching that results in the recipient leaving (i.e. moving side- or backwards for at least two body widths or one body length)
agonistic	butting, pushing, rubbing or threatening without any obvious consequence for the recipient
non-agonistic	sniffing, nose-pushing, contact- feeding, irrespective of the reaction of the recipient
licking/soliciting	licking, soliciting

2.3 Statistical analysis

All statistical analyses were calculated in IBM SPSS statistics (programme version 21.0.0.2). The analytical unit was the pair. Frequencies of interactions and repeated encounters per meal/non-meal bout, and time spent as direct neighbours during feeding and cubicle periods were summed up per pair over the four observation days for further analyses. Pairs that were observed only once over all meal and non-meal bouts were excluded.

Since interactions and spatial proximity between two focal animals were recorded twice, i.e. from each focal perspective, this subset of data was used to determine intraclass correlation coefficents (two-way mixed, single measures, absolute agreement) as a measure of intra-observer reliability for interaction categories and time spent as direct neighbours during feeding and resting (N = 36 (3 observation periods * 4 cows * 3 parallel observed partners)). Of the 596 possible pairs, only 27 (4.5 %) were genetically related with r ≥ 0.25, including one mother-daughter pair, no full sisters, and 26 half-sisters. A preliminary analysis showed that the binary factor 'genetically related' did not have an influence on the response variables and therefore this factor was omitted from the statistical analysis.

2.3.1 Principal component analysis on social interactions and spatial proximity

To reduce the number of variables and to extract statistically independent aspects of social relationships among cows, relations between frequencies of the eight categories of social interactions (Table 1), frequencies of repeated encounters, and time spent as direct neighbours when feeding and during cubicle periods were evaluated using a principle component analysis (PCA) based on correlation matrices without rotation. Extracted components with Eigenvalues greater than one and explaining at least 10% of variance were further considered. Suitability of data for calculating a PCA was evaluated using the Kaiser-Meyer-Olkin criterion, and measures of sampling adequacy (MSA) as well as communalities for the single variables.

Due to the repeated occurence of focal animals and their partners in the data set – at different proportions – the assumption of independence of cases was violated. To better estimate the influence of individual focal cows or partners on the pattern of different types of social interactions and time spent as direct neighbours, we repeated the PCA on ten sub-samples comprising a randomly chosen approximate half of the cases (random PCA 1-10, average N = 227 (214 – 250)). We compared the extracted main components qualitatively, and quantitatively using Spearman's correlations between loadings of the variables on the main components of the main and random PCA 1-10.

Table 3 (Article 1, Table 2):Definitions of familiarity variables.

familiarity variable	levels	definition
shared youth experience (SYE)	yes	difference between birthdays ≤ 90 days

	no	difference between birthdays > 90 days
shared youth experience (three levels, for Component 2 only)	yes	difference between birthdays ≤ 90 days
	no-focal older	difference between birthdays > 90 days, focal's birthday earlier than partner's birthday
	no-partner older	difference between birthdays > 90 days, focal's birthday later than partner's birthday
shared adult experience (SAE)	yes	both animals are multiparous
	no	at least one animal is primiparous
shared dry-period (SDP)	yes	time spent together is at least 1/3 of the maximum possible time
	no	time spent together less than 1/3 of the maximum possible time
synchronised group entry (SGE)	yes	time since integration is ≤ 14 or > 14 days for both animals
	no	time since integration is ≤ 14 for one animal, and > 14 days for the other animal
synchronised group entry (three levels, for Component 2 only)	yes	time since integration is ≤ 14 or > 14 days for both animals
	no-focal resident	time since integration is > 14 for focal, and ≤ 14 days for partner
	no-partner resident	time since integration is ≤ 14 for focal, and >14 days for partner

2.3.2 Familiarity as determinant of social relationships

The regression factor scores per pair obtained from the main PCA were used as dependent variables in general linear mixed models (GLMM) to evaluate whether different levels of familiarity would account for differences in relationships between cows. Variables supposed to indicate familiarity were 'shared youth experience' (SYE), 'shared adult experience' (SAE), 'shared dry-period' (SDP), and 'synchronised group entry' (SGE) (**Fehler! Verweisquelle konnte nicht gefunden werden.**). All these familiarity variables were binary. However, for PCA components in which the loadings differentiated between the roles of the focal and the partner animals, the 'shared youth experience' and 'synchronised group entry' familiarity variables were modified to three-level categorical variables, thus distinguishing which of the two animals was older and entered the herd earlier, respectively. The identity of focal cows and partners were included as random factors in the model, and 'shared youth experience', 'shared adult experience', 'shared dry-period', and 'synchronised group entry' as well as their two-way interactions as fixed factors. After removing non-significant interactions the model was re-run, considering Bayes Information Criterion (BIC) for the decision on the final model.

Post-hoc pairwise comparisons were carried out using Bonferroni correction for mutiple testing. Residuals were graphically checked for normal distribution.

3. RESULTS

Average total observation time per cow was 18.6 hours ranging from 17.7 to 20.3 hours, which was due to the different time cows could spent out of view, e.g. during milking. Out of 596 possible pairs, data of 454 pairs that had repeated encounters during independent meal/non-meal bouts were further analysed. The median number of social interactions per focal cow was 296 (range 140-498), distributed over a median of 37 (26-45) different partners (53-97% of the herd members). The number of interactions per partner ranged from two to 48. Repeated encounters ranged between two and nine. Focal cows spent between two and 68 minutes (1-20 %) of their feeding time next to specific direct neighbours, distributed over one to six meal bouts (8-71 % of meal bouts), and between zero and 215 minutes (0-45%) of their cubicle time, distributed over zero to six non-meals bouts (0-63%). Intra-observer reliability was satisfactory as measured by the intraclass correlation coefficients between observations made from each focal animal's perspective in a given dyad. The correlation coefficients were $R = 0.689$ for 'displacing', $R = 0.752$ for 'agonistic interactions', $R = 0.850$ for 'non-agonistic interactions', $R = 0.626$ for 'licking/soliciting', and $R = 0.994$ for duration of being direct neighbours.

3.1 Principal component analysis on social interactions and spatial proximity

Data were suitable for computing a PCA as based on Kaiser-Meyer-Olkin criterion (0.658) and as based on measure of adequacy (MSA) for the single variables (ranging from 0.367 to 0.765). All communalities were greater than 0.5 except for 'focal recipient – non-agonistic' (0.474). PCA revealed four main components with Eigenvalues greater than one. The components explained 25.8%, 13.7%, 12.0%, and 10.7% of variance, respectively, thus resulting in a total variance explained of 62.2%. Variables with loadings ≥ |0.5| were used to interpret the meaning of the main components (Table 3). Component 1 represented duration of being direct neighbours during feeding and frequencies of non-agonistic as well as agonistic interactions and was labelled 'Feeding/Social Interactions'. Component 2 reflected displacements and was special in that it was directional within the pair, i.e. the focal animal displacing loaded positively, and the focal animal being displaced loaded negatively on Component 2. This component was labelled 'Displacement Direction'. Component 3 represented licking and soliciting interactions and got the label 'Allogrooming'. Finally Component 4 described duration of being direct neighbours during resting and was labelled 'Resting'.

Table 4 (Article 1, Table 3):Loadings of social behavior variables on the main components 1-4 (variance explained given in paratheses) revealed by Principal Component Analysis. Loadings ≥ |0.5| were considered to interpret the meaning of the main components and are highlighted in bold.

component → variable ↓	1 – Feeding/Social Interactions (25.8%)	2 – Displacement Direction (13.7%)	3 – Allogrooming (12%)	4 – Resting (10.7%)
repeated encounters (meals)	**0.706**	-0.270	-0.135	-0.039
repeated encounters (non-meal)	-0.112	**0.527**	0.236	**0.581**
feeding NB [min]	**0.841**	-0.133	-0.081	0.043
cubicle NB [min]	-0.110	0.333	0.282	**0.610**
focal actor – non-agonistic	**0.647**	0.149	-0.008	0.255
focal recipient – non-agonistic	**0.611**	0.086	-0.152	0.265
focal actor – displacing	0.327	**0.536**	-0.419	-0.210
focal recipient – displacing	0.224	**-0.564**	0.290	0.242
focal actor – licking/soliciting	0.375	0.364	**0.637**	-0.361
focal recipient – licking/soliciting	0.442	0.230	**0.624**	-0.386
focal actor – agonistic	**0.514**	0.404	-0.417	-0.020
focal recipient – agonistic	**0.566**	-0.433	0.161	0.226

The ten random samples analysed in order to test reliability of the components obtained were all suitable for computing a PCA (Kaiser-Meyer-Olkin criterion 0.599 – 0.691). All random PCAs 1-10 revealed four main components that were qualitatively similar to the four components of the main PCA. and PCAs. Spearman correlation coefficients between variable loadings on the components of the main PCA as compared to the variable loadings on the components of the random PCAs 1-10 for Component 1 ranged from 0.955 to 0.994, for Component 2 from 0.799 to 0.986, for Component 3 from 0.437 to 0.950, and for Component 4 from 0.337 to 0.972. The high correlations thus confirmed the reliability of the main PCA despite the non-independence of the data collected at the pair level.

3.2 Familiarity as determinant of social relationships

Results of GLMM on PCA scores of the four main components as dependent variables, and the four familiarity variables as main effects are given in Table 4. Pairs with shared youth experience, with shared adult experience and with shared dry period all scored higher on the Feeding/Social Interactions component than pairs that lacked the respective shared experience. Whether the animals of a pair had a synchronised group entry did not affect scores on the Feeding/Social Interactions component. Moreover, there were three significant interaction effects (Table 5). Pairs with shared adult experience that spent their last dry

period together had by far the highest scores (SAE*SDP interaction). Pairs with shared adult experience had higher scores when their group entry was not synchronised, and pairs that did not have shared adult experience got higher scores when their group entry was synchronised (SAE*SGE interaction). A similar pattern was revealed for the SDP*SGE interaction.

There was no significant main effect on scores of the Allogrooming and Resting components but there were two interaction effects (Table 5). For scores on the Allogrooming component, pairs with shared adult experience that shared their last dry-period gained highest scores. For scores on the Resting component, animals with shared adult experience that had been born in the same birth cohort (shared youth experience) gained highest scores.

The Displacement Direction component differentiated roles within a pair, i.e. positive scores indicated that the focal animal was displacing the partner and negative scores indicated the opposite. For this component, one main effect was found. Estimated mean scores were significantly lower in pairs where the focal and the partner animal have been born within 90 days (same youth experience) as compared to pairs where the focal animal was either the younger or the older animal, indicating that in pairs from the same birth cohort the focal animals were dominated by their partners. In pairs with a directed age difference, estimated mean scores around zero indicate a more balanced displacement success independent of age relation.

Table 5 (Article 1, Table 4): Results of GLMM. Main effects of familiarity variables on pair-wise scores of the PCA main components. F = focal animal, P = partner animal. Significant differences are highlighted in bold (p<0.05) and italic (p<0.1).

PCA main components	PCA scores on factor level (LSmeans ± SE)				
factors	**yes**	**no**		**F**	**p**
Feeding/Social Interactions					
shared youth experience	**0.41 ± 0.16**	**0.10 ± 0.12**		**6.59**	**0.011**
(N)	(74)	(380)			
shared adult experience	**0.50 ± 0.14**	**0.01 ± 0.14**		**13.60**	**<0.001**
(N)	(250)	(204)			
shared dry period	**0.43 ± 0.16**	**0.09 ± 0.12**		**6.93**	**0.009**
(N)	(99)	(355)			
synchronised group entry	0.21 ± 0.13	0.31 ± 0.16		0.54	0.464
(N)	(215)	(239)			
Displacement Direction					
shared youth experience	**-0.32^a ± 0.17**	**0.04^b ± 0.16**	**-0.02^b ± 0.15**	**3.25**	**0.041**
(N) [yes/F older/P older]	(74)	(136)	(244)		
shared adult experience	-0.11 ± 0.15	-0.09 ± 0.15		0.10	0.921
shared dry period	-0.16 ± 0.16	-0.03 ± 0.13		1.10	0.294
synchronised group entry	-0.03 ± 0.14	-0.16 ± 0.22	-0.11 ± 0.17	0.31	0.736
(N) [yes/F earlier/P earlier]	(215)	(32)	(207)		
Allogrooming					
shared youth experience	0.13 ± 0.13	0.02 ± 0.08		0.66	0.417
shared adult experience	0.16 ± 0.11	0.02 ± 0.11		2.04	0.156
shared dry period	0.11 ± 0.12	0.04 ± 0.09		0.41	0.525
synchronised group entry	0.08 ± 0.10	0.07 ± 0.11		0.03	0.875
Resting					
shared youth experience	0.13 ± 0.13	-0.05 ± 0.08		1.82	0.178
shared adult experience	0.13 ± 0.11	-0.05 ± 0.12		1.44	0.235
shared dry period	0.01 ± 0.13	0.07 ± 0.09		0.18	0.671
synchronised group entry	0.04 ± 0.10	0.04 ± 0.11		0.01	0.944

Table 6 (Article 1, Table 5): Results of GLMM. Significant interaction effects of familiarity variables on pair-wise scores of the PCA main components. Long-term experience: SYE = shared youth experience, SAE = shared adult experience; SDP = shared dry period; short-term experience: SDP = shared dry-period, SGE = synchronised group entry. Probability levels are highlighted in bold (p<0.05) and italic (p<0.1). Different row-wise superscript letters indicate pairwise differences at p<0.05 level (Bonferroni adjustment).

PCA main component interaction	PCA scores on factor level interaction (Lsmeans ± SE)				F	p
	yes-yes	**yes-no**	**no-yes**	**no-no**	**F**	**p**
Feeding/Social Interactions						
SAE*SDP	**0.86^a ± 0.20**	**0.14^{ab} ± 0.13**	**-0.01^b ± 0.18**	**0.03^b ± 0.14**	**11.40**	**0.001**
(N)	(46)	(204)	(53)	(151)		
SAE*SGE	**0.32^{ab} ± 0.15**	**0.69^a ± 0.19**	**0.10^b ± 0.16**	**-0.07^b ± 0.17**	**6.69**	**0.010**
(N)	(117)	(133)	(98)	(106)		
SDP*SGE	**0.13^{ab} ± 0.15**	**0.73^a ± 0.24**	**0.28^a ± 0.15**	**-0.11^b ± 0.13**	**13.12**	**<0.001**
(N)	(81)	(18)	(134)	(221)		
Allogrooming						
SAE*SDP	*0.31 ± 0.16*	*0.02 ± 0.10*	*-0.09 ± 0.15*	*0.06 ± 0.11*	*3.80*	*0.052*
Resting						
SYE *SAE	**0.36^a ± 0.17**	**-0.10^b ± 0.20**	**-0.09^{ab} ± 0.10**	**-0.01^{ab} ± 0.10**	**4.19**	**0.041**
(N)	(42)	(32)	(208)	(172)		

4. DISCUSSION

To our knowledge this is the first study investigating interactions and spatial proximity in the non-experimental setting of a commercial dynamic dairy cow herd using detailed continuous observation at the dyadic level. Increased levels of previous familiarity in a pair of cows were associated with higher intensity of their social relationship, i.e. regarding investment of time and energy. Long-term familiarity originating from shared experience that happened weeks to years before had stronger effects on the intensity of social relationships than a very recent one.

There was considerable within- and between-animal variability in frequencies of interacting, repeated encounters, and time spent in close proximity with specific partners when feeding or resting. On average, focal animals met 20% of their herd mates only once during four days of observation, in contrast to a maximum of nine repeated encounters with specific single animals. This distribution indicates non-random and individual choices of partners. A pattern of non-random preferential and avoidance relationships has been reported before for dairy cattle groups (Gygax et al., 2010).

The PCA showed that choice of feeding and resting neighbours was not associated. Similarly, Gygax et al. (2010) found no relation between the median distance of pairs of dairy

cows during lying and during feeding. This suggests that preference for social partners is context-dependent. A clear relation between time spent in close proximity and socio-positive interactions could not be confirmed, and single interaction categories were differently related to each other on different components. All behavioural variables were positively related to each other on the Feeding/Social Interactions component, whereas allogrooming/soliciting was positively related to active agonistic behaviours on the Displacement Direction component but negatively on the Allogrooming component. This indicates that the same behaviour might have a different meaning depending on context and relationship.

Results on the Feeding/Social Interactions component supported the hypothesis that more familiar cows, especially those with a shared long-term experience, were preferential partners for feeding proximity and social behaviours. Shared adult experience and shared dry period promoted more frequent feeding and social interactions in the pair. Moreover, shared adult experience strongly interacted with shared dry period in that pairs of multiparous cows that spent the last dry period together were by far most likely to stay next to each other during feeding and interact socially. In line with evidence that long-lasting affiliative relationships in cattle may be built early in life (Bouissou and Andrieu, 1978a), pairs of animals born within 90 days also interacted more, indicating that individuals with shared youth experience maintain a relationship for years to come. Similarly, Gygax et al. (2010) found animals that grew up together and shared their last dry-period fed more synchronously in smaller groups, and animals that grew up together fed at smaller distances in larger groups (group size ranged from 23-43).

The short-term factor of synchronised group entry had influence only through its interaction with shared adult experience and shared dry period. One possible explanation is that when one of a pair of well-acquainted animals was already a resident in the current group, the other used the proximity of this "friend" as a secure point after its introduction into the group and interacted with it intensely.

It is questionable whether we can equate preferential partners during feeding and in social interactions, as quantified through the Component 1, with the traditional notion of affiliative bonding. The concept of spatial proximity as a measure of preferential affiliative relationships is widely accepted (Whitehead, 2008). However, high loadings of spatial proximity at feeding, non-agonistic behaviours (except grooming) as well as agonistic behaviours (except those associated with displacements) on the Feeding/Social Interactions component indicate a more general tendency to interact preferentially with familiar animals, both in seemingly agonistic and seemingly non-agonistic ways. One possible explanation is that some of the 'non-agonistic interactions' such as the contact-feeding and nose-pushing may reflect low-level competition rather than friendliness or a positive social relationship. A preferential relationship thus may not reflect an affiliative, but rather a 'preferred competitor' relationship.

Along a similar line, Val-Laillet et al. (2009) found that animals which showed more agonistic interactions under enhanced feeding competition also groomed each other more often. On the other hand, head-butts and threats, especially when not leading to a withdrawal of the recipient, may form part of communicating a valuable and positive relationship between (dehorned) cows, for example through assuring tolerance, or more subtly confirming a well-accepted dominance relationship. Perhaps the simplest explanation is that cows with high degree of familiarity are keen to stay close to each other and due to the proximity, they also frequently interact socially in a number of different ways. Conversely, unfamiliar animals may avoid each other and consequently, all types of social interactions (feeding, affiliative and agonistic) may be reduced among them. The current results thus open the intriguing possibility that the opportunity to interact with familiar associates may be more important for dairy cows than the nuances in the nature and valence of these interactions. Doubts about a clear-cut distinction between agonistic ('bad') and affiliative ('good') behaviours are not new. The concept of dominance was questioned before, highlighting the complexity of agonistic interactions in dairy cows (Val-Laillet et al., 2008), and the idea of including positive social interactions as a positive indicator of good welfare repeatedly failed because of lack of clear interpretation, rare occurences and/or poor repeatability (Boissy et al., 2007; Knierim and Winckler, 2009).

Mutual grooming and proximity during resting were positively influenced by shared long-term experience in our study. The ambiguous relation between agonistic and allogrooming behaviour as revealed by PCA supports the view that allogrooming serves multiple functions. Allogrooming has been interpreted in a context of bonding, reconciliation, and appeasement (Sambraus, 1969; Aureli et al., 2002), while resting together has been interpreted as an indicator of affiliation (Neisen et al., 2009a; Coulon et al., 2010; Gygax et al., 2010; Patison et al., 2010). Specifically, in this study, multiparous pairs with a synchronous reproductive cycle as indicated by a shared dry period groomed each other frequently, while multiparous pairs that had grown up together rested near each other frequently. The low number of pairs in these specific categories suggests that for grooming and resting partnerships, dairy cows may selectively prefer a small subset of herd mates that they know particularly well. However, the low F values indicate that most of the variability in the choice of grooming and resting partners remains unexplained by the familiarity factors. Resting in spatial proximity is considered a relaxed and content situation, and might therefore be an especially suitable indicator for the quality of social relationships. Thus, one could assume to find the most pronounced effects here. On the other hand, in a cubicle system the choice of resting neighbours might be less meaningful as compared to e.g. free lying areas or pasture. Finally, methodological constraints such as the time (daylight period) or the duration of observation have to be considered.

The ability to displace the partner by one animal in the pair on the first view appeared to reflect dominance relationship. However, there were unexpected effects of age relation: focal animals were more often attacked and displaced by same-aged partners, otherwise age difference in the pair was unrelated to displacement success. This may indicate that the concept of dominance as measured by displacement success might not be appropriate for dairy cow herds under intensive conditions. Whereas extensively kept beef suckler cows build very stable, age-dependent linear dominance hierarchies as measured by displacement success (Šárová et al., 2013), only low levels of transitivity and consistency of displacement direction have been observed in experimental dairy herds (Val-Laillet et al., 2008; Gibbons et al., 2009). One explanation could be that the outcome of feeding related agonistic interactions might rather be related to hunger or motivation to feed than to dominance (Beilharz and Zeeb, 1982; Val-Laillet et al., 2008).

In conclusion, long-term familiarity between pairs of cows makes them spend time in proximity and interact socially in a number of ways. This supports the notion that dairy cows actively maintain valuable dyadic relationships. In practical terms, the results indicate that keeping well-acquainted cows together contributes to stable inner structure of a dairy herd and thus promotes dairy cow welfare.

5. ACKNOWLEDGEMENTS

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7 Article 2: Does familiarity facilitate integration into the milking group after calving in dairy cows?

7.1 Introductory words

The results of the first analysis and the availability of activity data from the whole group led to the second research article. Familiarity, specifically long(er)-term and continuous familiarity, proved to predict more intensive social relationships. We were therefore interested whether incoming cows would synchronise their lying behaviour with familiar peers and consequently would lie longer and more synchronous with increasing number of familiar group mates present, indicating facilitated integration. Supportive results would underline the importance of social affiliation and at the same time forward the use and interpretation of automatically collectable behaviour.

7.2 Published article

Do familiar group mates facilitate integration into the milking group after calving in dairy cows?

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DO FAMILIAR GROUP MATES FACILITATE INTEGRATION INTO THE MILKING GROUP AFTER CALVING IN DAIRY COWS?

Running title: Do familiar group mates facilitate integration?

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ABSTRACT

As dairy cows' needs and demands change over the different phases of their reproductive cycle, regrouping is common practice in dairy farming to facilitate management and handling. However, social instability associated with regrouping is known to have negative effects on the cows, including disturbances in their lying behaviour. In this study, we examined the effect of familiar group mates on lying time and lying synchrony in a dynamic group of approximately 50 early lactating dairy cows during 23 regrouping events. We focussed on 13 primiparous and 33 multiparous post partum cows during 24 hours after their introduction to the group as compared to a matched control sample of resident cows. We hypothesised that freshly introduced cows would lie shorter and behave less synchronously with the group as compared to resident cows. Further, we hypothesised that lying duration and lying synchrony will increase with the number of familiar animals present and that these effects may depend on whether the familiarity was acquired early in life or only recently.

As predicted, primiparous fresh cows lied less and behaved less synchronous at the dyadic level than their matched residents. However, no such effect was present in multiparous cows. The presence of recently familiar animals had no influence on either primiparous or multiparous cows' behaviour. In contrast, early familiar animals affected the cows' behaviour in several aspects, yet differently in primiparas and multiparas. In fresh primiparas, an increasing number of early familiar animals present had a negative effect on lying duration. Among both fresh and resident primiparas, early familiar dyads were more synchronized than other pairs of animals. In multiparous cows, a higher number of early familiar cows present led to more synchronous behaviour with the group.

We conclude that primiparous and multiparous cows are differently affected when introduced to a lactating group after calving. Duration and synchronization of lying behaviour indicated that primiparas are strongly challenged by their entrance to the group while multiparas cope well with it. In both primiparas and multiparas, lying behaviour was affected, albeit differently, by the presence of early familiar individuals, but not by recently familiar animals. The relations between familiarity, group dynamics, behavioural synchrony and lying behaviour are complex and need deeper investigation.

1. INTRODUCTION

Regrouping is common practice in dairy farming to facilitate management and handling according to the changing demands over the reproductive cycle of the animals. One regularly occurring regrouping is the introduction of recently calved cows into a group of lactating cows. This regrouping is especially challenging because it is imposed in the middle of the critical 'transition phase' when the animals have to cope with pronounced endocrinological, physiological, metabolic, and immunological changes (Sundrum, 2015; Bruckmaier and Gross, 2017; Wankhade et al., 2017) that may negatively affect their health and production. In medium-sized dairy herds, this regrouping usually takes the form of introducing a few cows (hitherto referred to as "fresh" cows) into an already established group of "resident" cows. For dairy cows, regrouping is a stressful experience (Johannesson and Sorensen, 2000; Dobson et al., 2001; Fukasawa and Tsukada, 2010; Schirmann et al., 2011; Wagner et al., 2012; Pošćić et al., 2017) that challenges their allostasis (McEwen and Wingfield, 2003; Sundrum, 2015) and also induces several types of behavioural changes. Over periods of hours to several days, those changes may comprise any of the following: reduced feeding and lying times (Cooper et al., 2007; Von Keyserlingk et al., 2008b), increased time spent active (Reith and Hoy, 2012), an increase in the number of lying bouts indicating more restless behaviour (Schirmann et al., 2011), higher frequency of agonistic encounters (Von Keyserlingk et al., 2008b; Kucevic et al., 2010; Lobeck-Luchterhand et al., 2014) and lower occurrence of socio-positive behaviour (Von Keyserlingk et al., 2008b).

During regrouping, social facilitation (Bailey et al., 2000; Costa et al., 2016; Acevedo-Triana et al., 2017) or social buffering (Hennessy et al., 2009; Rault, 2012; Kiyokawa, 2018) may enhance adaptation and reduce the stress originating from the new physical and social environment. In cattle, pairwise integrated heifers adapt faster as compared to singly integrated ones (O'Connell et al., 2008; Gygax et al., 2009a; Neisen et al., 2009b). For the effectiveness of social buffering, familiarity might be a critical factor in different species including cattle (Takeda et al., 2003; Færevik et al., 2006; Rault, 2012; McLennan, 2013; Kiyokawa and Hennessy, 2018). Our study therefore aimed at investigating possible effects of familiarity on behaviour after regrouping, taking into account that early familiarity and recent familiarity may influence the animals differently (Raussi et al., 2010; Gutmann et al., 2015).

One easily measurable proxy for the functioning of a cattle group is lying behaviour. Lactating dairy cows stand under high metabolic pressure due to the synthesis of large amounts of milk. As ruminants, they therefore need sufficient times of rest, preferentially in lying position, to maintain biological functioning. Lying has proven to be a highly motivated behaviour (Jensen et al., 2004; Jensen et al., 2005; Munksgaard et al., 2005) and is used as a valid indicator of social stress and welfare (Von Keyserlingk et al., 2008b; Talebi et al.,

2014; Krawczel and Lee, 2019). Resting phases of cattle are highly synchronised under semi-natural conditions (Šárová et al., 2007; Stoye et al., 2012). In intensive farming systems, behavioural synchrony is counteracted by factors such as reduced space or high stocking density (Raussi et al., 2011; Winckler et al., 2015; Flury and Gygax, 2016). Behavioural synchrony therefore has been used as a measure of undisturbed behaviour, sufficient resources and welfare (Fregonesi and Leaver, 2002; Faerevik et al., 2008; O'Driscoll et al., 2008; Napolitano et al., 2009). When focusing on individual animals, behavioural synchrony could be quantified either as individual-with-group synchrony (i.e., performing the same behaviour as the majority of the group, Michelena et al. (2006)) or as dyadic synchrony (i.e. performing the same behaviour as another specific individual, e.g., a familiar partner, Šárová et al. (2007)). To the knowledge of the authors, the relation between synchrony and post-calving regrouping in dairy cows has not been directly investigated yet. We investigated whether the number of familiar animals would influence the cow lying behaviour, including its synchrony, in a dynamic commercial herd after introduction of new group members. As the re-grouping effects are most acute during the first day (Von Keyserlingk et al., 2008b), we focused on the lying behaviour during 24 hours after mixing. We hypothesised that because transfer to a novel social and physical environment is stressful, freshly introduced cows would lie shorter and behave less synchronously with the group as compared to resident cows. Further, we hypothesised that because of the putative effects of familiarity on social facilitation and social buffering, lying duration and lying synchrony will increase with the number of familiar animals present. Based on our previous results (Gutmann et al., 2015) and on studies showing that stable social relationships in cattle are built during early ontogeny (Bouissou and Andrieu, 1978b; Wagner et al., 2012), we hypothesised that these effects would be more pronounced for early acquired familiarity than for recently established familiarity.

Primiparous cows undergo parturition, separation from the calf and introduction into the group of lactating cows for the first time in their life. Their behavioural and stress reaction to the process was shown to be different from multiparous animals (González et al., 2003; Calderon and Cook, 2011; Boyle et al., 2013; Sepulveda-Varas et al., 2014; Tienken et al., 2015). Therefore, we tested the above hypotheses separately for primiparous and multiparous cows, in line with some previous studies (Sepulveda-Varas et al., 2014).

2. ANIMALS, MATERIAL AND METHODS

The study was designed and performed according to European and Czech laws and current guidelines for ethical use of animals in research. The study was approved by the Institutional Animal Care and Use Committee of the Institute of Animal Science (Permit Number: 15155/2010-17210).

2.1 Study site, animals and management

The study was carried out at the research farm Netluky of the Institute of Animal Science, Prague, Czechia, in a group of early and high lactating cows kept together during their approximately first third of lactation, comprising about 50 dehorned animals of 70% Holstein (9,900 kg average 305 day milk yield) and 30% Czech Fleckvieh breed (7,800 kg yield). The group was housed in a 30 x 15 m stable with curtain ventilation system that contained a 30 m feeding face with neckrail, 54 cubicles with straw bedding (1.35 x 2.4 m) in two rows facing each other and one row facing a wall, concrete corridors littered with small amounts of straw, two water troughs, two salt licks and an automatic brush. The animals were fed ad libitum (fresh feed twice daily at about 6 a.m. and 2 p.m.) with a total mixed ration. Farm management aimed for a maximum stocking density of 1 cow per cubicle, which resulted in a minimum feeding space of 0.56 m per cow (30 m/54 cows); for exact cubicle stocking densities see also Table 1 in the supplementary materials. Milking was carried out by two alternating pairs of milkers twice daily starting at 3:30 a.m. and 3:30 p.m. in a 2 x 5 automatic tandem milking parlour. The corridors were cleaned twice daily during milking with a skid loader. Introduction into this group after calving occurred about twice per week in groups of 2-4 cows (see also Table 2 in the supplementary materials). Prior to calving, dry cows and pregnant heifers (last three months before calving) were kept together in a smaller, but similarly structured barn. A few days before calving they were moved into an adjacent compartment of the barn which allows full visual and olfactorial, and partly physical contact to their former group mates. Calving took place in a single calving pen located next to this compartment. The first days after calving the cows were kept separated from the other groups in single stalls in a separate building (median of 5 days (min-max 3-14) in the analysed data set; see also Table 2 in the supplementary materials).

2.2 Focal animals, data collection and data processing

The data presented here were recorded during three observation periods between October 2010 and October 2011, each lasting about 6 to 8 weeks (43, 61 and 50 days). To maximise independence of data between observation periods, a time span of at least 100 days was imposed between the periods. Consequently, most of the cows of the observed early lactating group had been replaced when the next observation period started.
To gain information about the lying behaviour within the group, all cows present at the beginning, and all cows entering the group during the observation period were equipped with HOBO Pendant® accelerometers that recorded their activity in terms of 'lying' vs. 'not lying'. The loggers were set to record the acceleration of the y- and z-axis every 60 seconds. We attached them during normal milking times in the milking parlour to the cows' left or right hind

leg using specifically developed silicon moulds and self-adhesive Co-Flex® bandages. Memory capacity required exchange of the loggers and data downloading every 20 days. The resulting text data files were merged, resulting in a list containing information about date, time, cow identity and behaviour (lying vs. not lying). For a detailed description and corresponding codes see Schröder (2012).

During observation periods 1 to 3, fresh cows were introduced into the group at 37 days in units of 2-4 animals (median of 2) at 4-16 (median 6.5) days after parturition (see Table 2 in the supplementary materials). Accelerometer data from 24 hours following each introduction (after the afternoon milking) were extracted. Cows for which data from less than 23 hours were available were excluded, and only those days were considered for which data from at least 90% of all cows were available. After this cleaning step, the data finally available comprised 23 'integration days' with lying data from 1–3 fresh cows (median of 2, resulting in 13 primiparous and 33 multiparous freshly introduced cows; see Table 2 in the supplementary materials) and from 90–98% (mean of 96%) from the cows already present in the group were (see Table 1 in the supplementary materials). We omitted the milking periods, i.e. time intervals between 3:00-5:00 and 15:00-17:00, for morning and afternoon milking, respectively, resulting in 1200 data points per cow and day (20 hours x 60 recordings per hour). These lists were summarised per integration day into several descriptive measures at group level (see Table 1 in the supplementary materials), into individual lying duration per day and into an association coefficient with regard to lying behaviour at individual-with-group and dyadic level, calculated following Michelena et al. (2006) as

$$r_\phi = \frac{A \times D - B \times C}{\sqrt{(A + B) \times (C + D) \times (A + C) \times (B + D)}}$$

with A, B, C and D defined as given in Table 7. This association coefficient may range from -1 (never synchronous) through 0 (independent) to 1 (always synchronous). 'Majority' was defined as 'more than half of the true group size' (i.e based on all animals present including those without data, avoiding false positives). Individual attributional cow data available from the research farm's management software (AfiFarm TM 3.03 © 2006) comprising dates of birth, dry-off, calving, and introduction, as well as breed and lactation number were added at individual level and further processed into the dyadic, i.e. relational level.

Table 7 (Article 2, Table 1): Defintion of elements used for calculation of the association coefficients.

	individual-withgroup synchrony	dyadic synchrony
A	animal i active while majority of group is active	both animals i, j active

B	animal i active while majority of group is lying	animal i active while j lying
C	animal i lying while majority of group is active	animal i lying while j active
D	animal i lying while majority of group is lying	both animals i, j lying

For the analysis, the lying duration, and the individual-with-group as well as the dyadic association coefficents of 13 primiparous and 33 multiparous freshly introduced cows plus data from matching resident cows were extracted. Matching resident cows had been in the group for on average about 6–7 weeks (see Table 2 in the supplementary materials) and had been allocated by breed and as best as possible by lactation number, never mixing primi- and multiparous cows. At the dyadic level, in total, both primiparous fresh and resident focal cows formed 628 dyads each, and multiparous fresh and resident focal cows both formed 1545 dyads each with the other cows present in the group at the respective integration days. While the sample of freshly introduced focal cows consisted of 13 plus 33 different individuals, some resident focal cows and most other group mates contributed to the data set repeatedly (e.g. for the analysis of synchrony of / with the group). Table 1 and 2 of the supplementary materials contain a detailed description of group composition as well as behaviour and of the introduced units and focal animals, respectively.

Familiarity of the group members with the focal animals was defined at two levels: a) early familiar (EF) with a focal cow, defined as both cows born within 90 days (as they then grew up together for about one and a half years in the same or in adjacent pens allowing physical contact), and b) recently familiar (RF), i.e. a shared dry period (overlap of at least 1/3 of maximum possible overlap). Over all dyads including a focal animal, 12,1% fulfilled EF, and 45,3% fulfilled RF criteria (for a detailed description see Table 3 in the supplementary materials).

2.3 Statistical analysis

The activity patterns at group level were analysed using descriptive measures and Spearman's correlation to check for associations with group size (i.e. stocking density). At individual level, the dependent variables were lying duration in hours and the individual-with-group association coefficient, while at the dyadic level the dyadic association coefficient was the dependent variable. Data were analysed separately for primiparous and multiparous cows as their behavioural and stress reaction to the process of regrouping was shown to be different (González et al., 2003; Calderon and Cook, 2011; Boyle et al., 2013; Sepulveda-Varas et al., 2014; Tienken et al., 2015).

For analyses at individual level, we included the residence status, i.e. whether a cow was freshly introduced or resident, and breed, i.e. Holstein or Czech Fleckvieh, as fixed factors, and as covariates the number of cows present in the herd at the day of integration which

were a) early familiar (EF) with a focal cow, and b) recently familiar (RF). EF and RF were z-transformed, i.e. standardised to $\aleph(0, 1)$, to account for their empirical distribution where zeros (almost) never occur, and to allow for a more intuitive interpretation of the intercepts. A detailed description of the distribution of familiar group mates at individual level can be found in the supplementary materials (Table 3). Interaction effects between the covariates and residence status were also included. To account for the fact that data from the same integration day and within observation period were not independent from each other but likely to be correlated, we modelled random intercepts for 'integration day' nested in 'observation period', and in case of repeatedly observed individuals we added a crossed random factor for 'cow ID'.

Models set up to analyse effects on dyadic association coefficients included residence status (fresh vs. resident), breed relation (both Holstein Friesian (HF), both Czech Fleckvieh (CF), mixed (mixed)), and two binary factors EF and RF describing the familiarity relation of a pair as fixed factors. A detailed description of the distribution of familiarity levels among dyads can be found in the supplementary materials (Table 3). To account for non-independency of residuals due to multiple measurements of focal cows (within integration day over all group mates, and partly over integration days, see Table 2) and of partner cows (within integration day over all focal cows, and largely over integration days), crossed random intercepts for 'focal cow ID' and 'partner cow ID' were included. As before, 'integration day' nested in 'observation period' was considered an additional crossed random intercept. Residuals were checked graphically for normal distribution. Statistical software packages used for data processing and statistical analyses were of R (RCoreTeam, 2011), Microsoft Excel 2010, IBM SPSS Statistics 24 and SAS 9.4.

3. RESULTS

Residence status effects

For primiparous cows, the models confirmed the hypothesis that fresh cows would lie shorter than resident ones (LSmeans ± SE: 4.49 ± 1.39 < 11.16 ± 1.51, $F_{1, 17.2}$=11.10, p=.004, Fig. 1). Concerning synchrony, freshly introduced cows yielded slightly lower dyadic, but similar individual-with-group association coefficients as compared to their matched residents (fresh vs. resident dyadic LSmeans ± SE: 0.05 ± 0.02 < 0.09 ± 0.02, $F_{1, 33.6}$=3.95, p=.055; individual-with-group LSmeans ± SE: 0.34 ± 0.11 vs. 0.28 ± 0.10, $F_{1, 18.5}$=0.15, p=.704). For multiparous cows, these hypotheses were not supported. Fresh cows tended to lie longer than resident ones (11.25 ± 1.01 > 9.34 ± 0.72, $F=2.95_{1, 58.3}$, p=.091, Fig. 1), and synchrony did not differ at the individual-with-group or dyadic level between freshly introduced and resident animals (individual-with-group LSmeans ± SE:0.26 ± 0.06 vs. 0.24 ± 0.04, $F=0.10_{1, 56.9}$, p=.759; dyadic LSmeans ± SE: 0.06 ± 0.01 vs. 0.05 ± 0.01, $F=1.12_{1, 196.4}$, p=.292).

Familiarity effects

Recent familiarity (RF) did not affect any of the outcome variables (lying duration, individual-with-group synchrony, dyadic synchrony) in either primi- or multiparous cows. In contrast, early familiarity (EF) affected the cows' behaviour in several ways. Figures 2 and 3 illustrate the relations between number of EF group mates present and lying duration and individual-with-group synchrony, and the relations between dyadic familiarity status and dyadic synchrony, separately for both parity and residence status. There were three apparent effects. First, for primiparous fresh cows, lying duration was negatively related to the number of EF group mates present (interaction effect residence status x EF: $F=3.81_{1, 17.5}$, p=.067, with a slope for fresh cows: -3.19 ± 0.90 hours/additional EF ($t_{17.6}=-3.53$, p=0.002) as compared to the slope for resident cows: 0.22 ± 1.27 hours/additional EF ($t_{17.1}=-0.18$, p=.863), see Figure 2, top graphs).

Second, independent of the residence status, primiparous EF dyads behaved more synchronously than other dyads (LS means $\pm$ SE $0.08 \pm 0.02 > 0.05 \pm 0.02$, $F_{1, 529.5}=4.45$, p=.035, Fig. 2 bottom graphs). Third, in multiparous cows independent of their residence status, the number of EF group mates present was positively related to the individual-with-group synchrony (slope of 0.07 ± 0.02, $F_{1, 50.3}=9.35$, p=.004, Fig. 3, middle graphs). An overview of the full statistical results of the models as well as descriptive data statistics can be found in the supplementary materials (Tables 4-7).
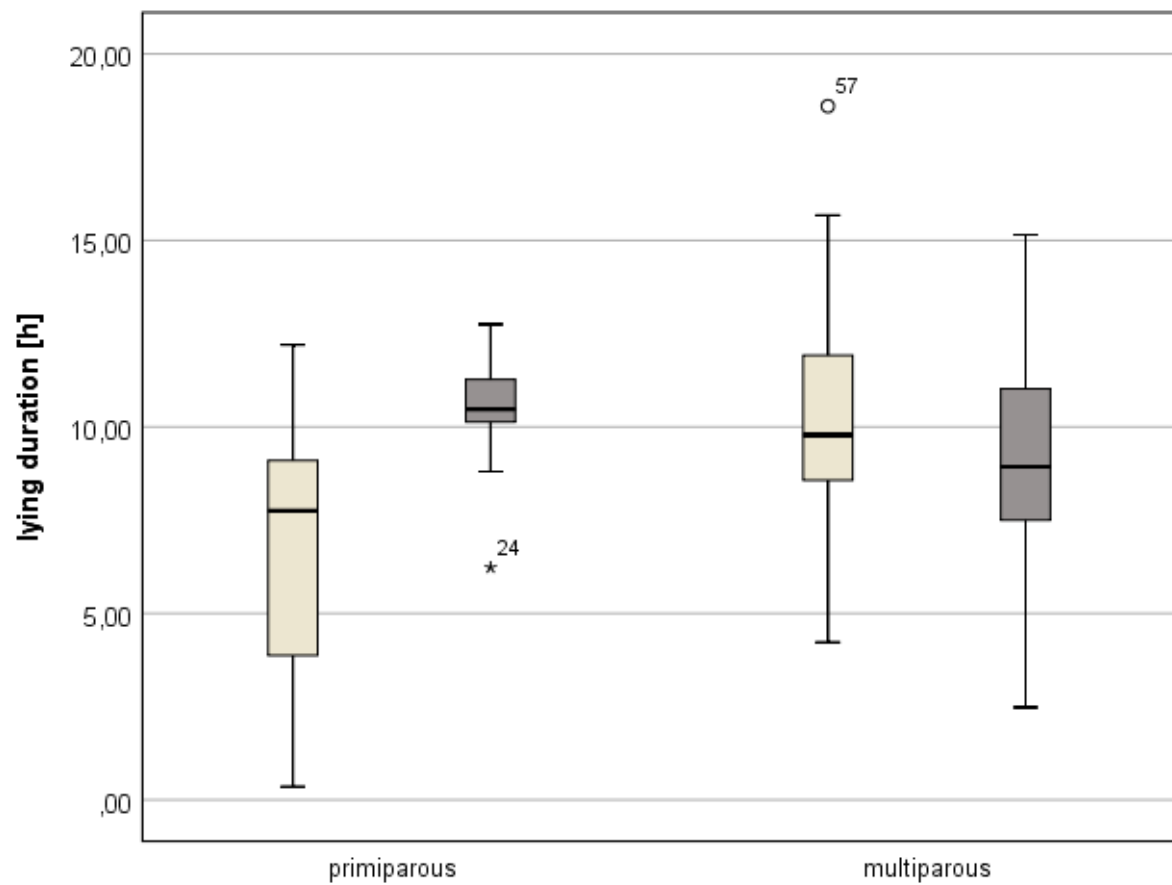
Figure 8: Lying duration (Article 2, Figure 1): Box plots of lying durations of primiparous and multiparous freshly introduced (light grey) and resident (dark grey) cows.
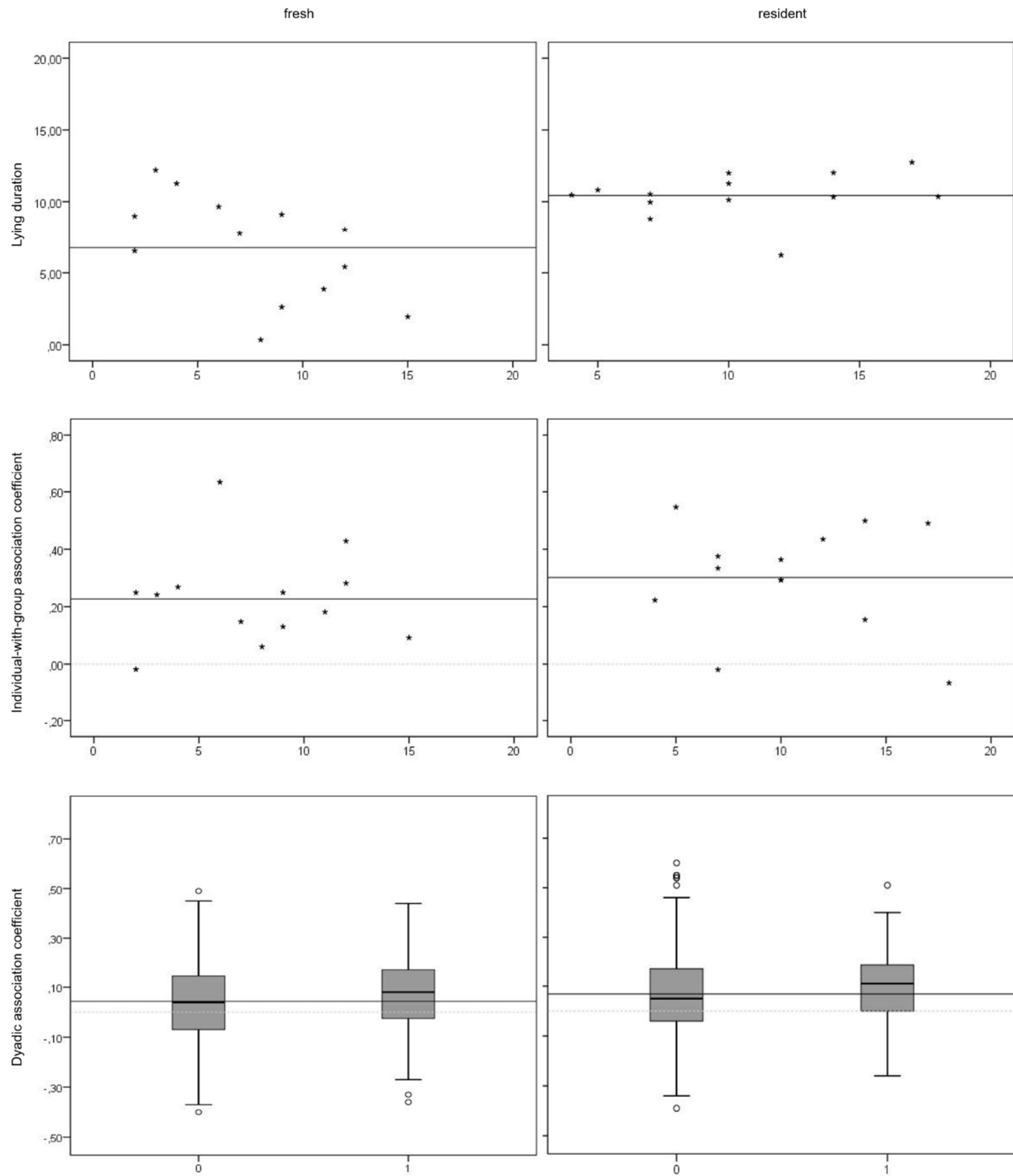
Figure 9: Primiparas' behaviour as related to number of early familiar cows present (Article 2, Figure 2) Relation between number of early-familiar animals present (E-F) and lying duration (top graphs), individual-with-group association coefficient (middle graphs) and dyadic association coefficient (bottom graphs) for primiparous fresh (left column) and resident (right column) cows. Black lines indicate the arithmetic mean, light grey dashed lines (middle and bottom graphs) the zero line.
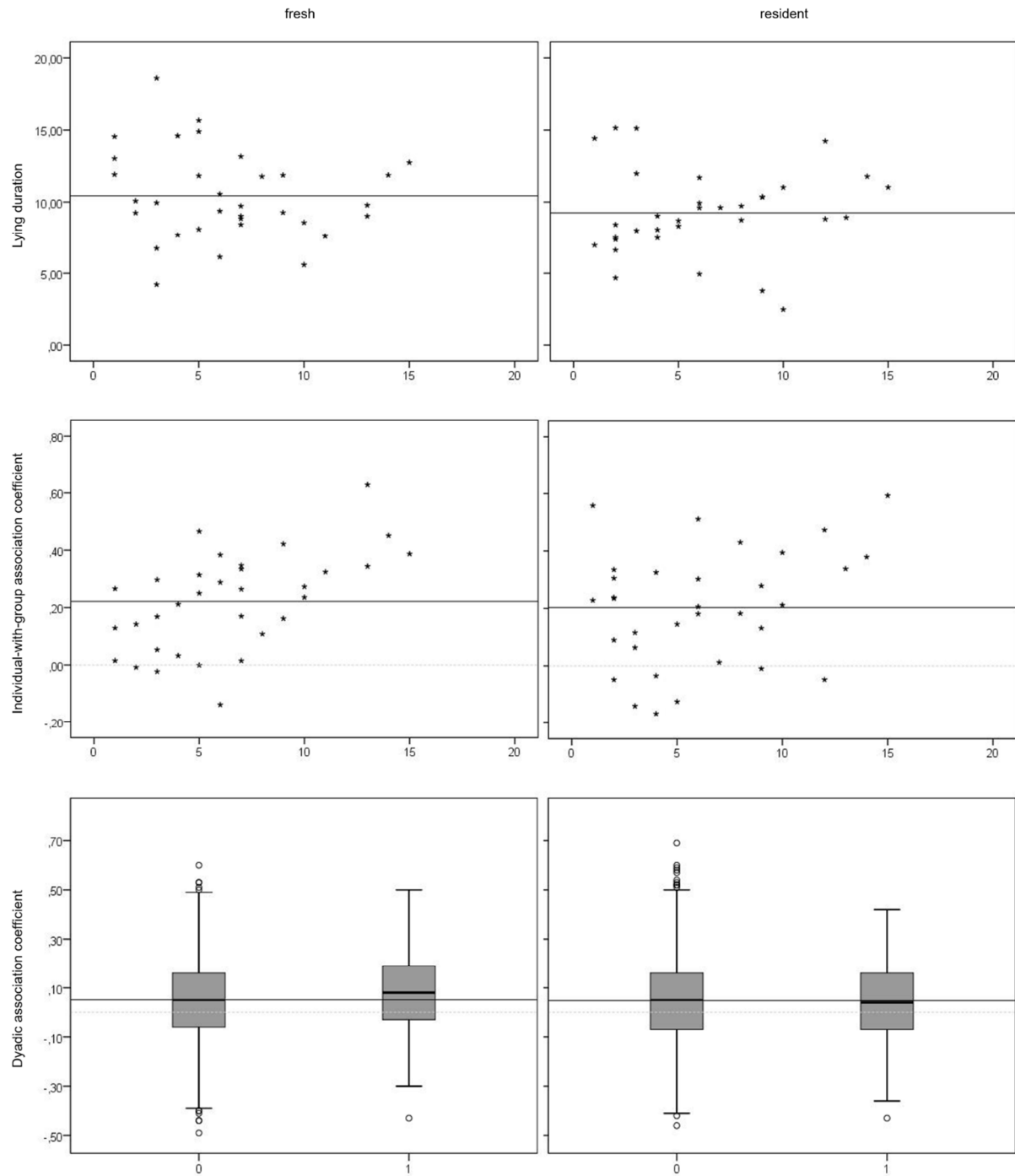
Figure 10: Multiparas' behaviour as related to the number of early familiar cows present (Article 2, Figure 3) Relation between number of early-familiar animals present (E-F) and lying duration (top graphs), individual-with-group association coefficient (middle graphs) and dyadic association coefficient (bottom graphs) for multiparous fresh (left column) and resident (right column) cows. Black lines indicate the arithmetic mean.

4. DISCUSSION

This is the first study to examine the effect of familiarity between dairy cows on their adjustment to the post-calving transfer into the milking group. Specifically, we investigated the combined effects of residence status and familiarity on cow lying behaviour during 24 hours after fresh 2-4 cows were introduced into a milking group. We hypothesized that fresh cows would show reduced lying duration and diminished lying synchrony and that the presence of familiar animals would facilitate integration into the group as reflected by longer lying times and higher group or dyadic synchrony of the introduced cows. The picture emerging from our results is complex. In line with previous findings (Calderon and Cook (2011); Sepulveda-Varas et al. (2014); Duncan and Meyer (2018), but see Bewley et al. (2010)) we found that lying behaviour of primiparous and multiparous cows was shaped differently by the examined factors. Below we discuss the effects of residence status and familiarity in primiparous and in multiparous cows.

Residence status effects

Primiparous freshly introduced cows lied shorter than the resident primiparas. Short lying times may reflect an aroused state caused by the very recent experiences of the first calving, translocation into an unfamiliar environment, meeting mainly unfamiliar animals and being milked. Lying durations recorded in our study are in accordance with the range found by others (O'Connell et al., 2008; Bewley et al., 2010; Boyle et al., 2012; 2013; Hendriks et al., 2019). Furthermore, the dyadic synchrony was depressed in fresh primiparas in comparison to their matched residents. These results confirm, in agreement with previous findings (Wagner et al., 2012), that the introduction into the milking group is challenging for primiparous cows as they do not manage to achieve sufficient rest and have difficulties to maintain behavioural coordination with their group mates.

In multiparous cows, neither lying duration nor lying synchrony was negatively affected by the introduction into the milking group. In fact, fresh multiparas lied slightly longer than their resident group mates, indicating that they 3 able to cope well with the entry to the milking group and find sufficient rest. Several authors concluded that experienced cows may tolerate regrouping well according to physiological indicators of stress, e.g., immunological and metabolic responses, as well as reproductive and productive parameters (Coonen et al., 2011; Silva et al., 2013; Walker et al., 2015; Chebel et al., 2016). In terms of immediate effects of regrouping on lying behaviour, Schirmann et al. (2011) found no change in dry multiparous cows, while several other studies worked with groups containing both multiparous and primiparous cows and report a slight decrease in lying times (Hasegawa et al., 1997; Huzzey et al., 2005; Von Keyserlingk et al., 2008b; Campler et al., 2019). Three of these studies did not take into account parity in their analyses while Campler et al. (2019) do

not report the result for primiparas and multiparas separately. In the light of the present results, the reported drop in lying times after regrouping might have been solely due to the primiparous unexperienced animals. In future studies on behavioural effects of cow regrouping, parity needs to be taken into account as experienced multiparous cows may react quite differently from the naïve primiparas.

Familiarity effects

Our second hypothesis was that familiar animals facilitate the integration of the cows into the group of lactating cows. We distinguished between recent familiarity (RF), defined as having been in the same group of dry cows before the last parturition, and early familiarity (EF), delineated as being born within 3 months of each other and thus having the opportunity to interact during early ontogeny. Recent familiarity had no effect on lying duration or lying synchronicity in either primiparas or multiparas. In contrast, early familiarity influenced lying behaviour in several ways.

In primiparous cows, the presence of EF animals had two seemingly contradictory effects. On the one hand, the dyads of EF primiparous cows behaved more synchronously than other dyads, demonstrating that cows that had early contact recognise each other and align each other's lying behaviour. This result complements previous findings that early familiar female dairy cattle tend to affiliate with each other in spatial and social behaviour (Bouissou and Andrieu, 1978b; Raussi et al., 2010; Wagner et al., 2012; Gutmann et al., 2015). On the other hand, the presence of EF animals diminished the lying time in fresh primiparas. One possible explanation for this seemingly paradoxical combination of effects is that within the 24 hours post introduction when behaviour was recorded in this study, the newcomer primiparas attempted to be active whenever familiar animals had been active, thus reducing lying time.

For multiparous cows, an increasing number of EF animals was associated with higher synchrony with the group. This seems to support the idea that for experienced animals, the presence of EF group mates makes it easier to align individual behaviour with the functioning of the whole group. However, the mechanism of this effect is not clear, as on the dyadic level, EF pairs of multiparous cows did not behave more synchronously than other dyads. This negative results is surprising as a former study about the same dairy herd showed that early familiarity predicts the intensity of social relationships (Gutmann et al., 2015). Similarly, Boyland et al. (2016) found that cows with similar lactation numbers, i.e. familiar with each other for longer times, were more often spatially associated above chance level in a dynamic dairy herd. Future studies encompassing all of familiarity, behavioural synchrony, spatial association and both affiliative and agonistic social interactions are needed. While many studies assessed the relationships between two or three of those aspects, often on moderate

numbers of animals (e.g., this study, Kondo and Hurnik (1990); Hasegawa et al. (1997); Huzzey et al. (2005); Von Keyserlingk et al. (2008b); Gibbons et al. (2010); Schirmann et al. (2011); Wagner et al. (2012); Gutmann et al. (2015); Sosa et al. (2019)), more comprehensive research projects investigating all or at least most of those aspects on a sufficiently large sample would substantially improve our understanding of the behavioural functioning of dynamic dairy cattle herds.

5. CONCLUSIONS

We conclude that effects of postpartum regrouping and familiarity of the present group members on lying behaviour were differently present in primiparous and multiparous cows. Primiparous cows in our study had lower lying times and lower dyadic synchrony when they were freshly introduced into a group of lactating cows. These results confirm that the post-partum period is socially a very challenging period for primiparous dairy cows. In contrast, multiparous freshly introduced cows had moderately greater lying times, indicating that they were coping well with the transition. As for the familiarity effects, cows in our study were affected in their lying behaviour by the presence of early familiar individuals, but not by the recently familiar animals. The relations between familiarity, group dynamics, behavioural synchrony and lying behaviour are complex and need deeper investigation.

6. ACKNOWLEDGEMENTS

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8 Article 3: The challenge of regrouping: cardiac activity of dairy cows during resting and the effect of familiar group mates

8.1 Introductory words

Externally observable behaviour provides comprehensive information, however additional data about the internal state may reveal refining or even unexpected details. Referring to the preceding article, not only the quantity of resting behaviour, but the quality and level of relaxation are probably related to the stress experience. Using heart rate variability as an image of regulatory processes of the autonomous nervous system therefore allows a more accurate evaluation of the stress response following regrouping in dairy cows, and of possible beneficial effects through social company.

8.2 Manuscript

THE CHALLENGE OF REGROUPING: CARDIAC ACTIVITY OF DAIRY COWS DURING RESTING AND

THE EFFECT OF FAMILIAR GROUP MATES

Running title: HRV of dairy cows after regrouping

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KEYWORDS: heart rate variability; HRV; familiarity; social relationship; dairy cattle; regrouping; lying behaviour; integration

ABSTRACT

Changes in group composition and mixing of more or less unfamiliar animals are potential sources of stress to farmed animals. To evaluate the stress response of dairy cows to regrouping, we analysed heart rate (HR) and heart rate variability (HRV) measures during resting in cows that have been freshly introduced into the milking group after calving as compared to resident cows matched in breed and lactation number. Further, we analysed whether the number of early familiar (EF) or recently familiar (RF) group mates present would affect HR and HRV measures, suggesting a stress-relieving effect of social support. HR and HRV patterns only partly confirmed our hypotheses. Primiparous and multiparous cows were differently affected. HR and HRV patterns indicate an aroused physiological state in freshly introduced primiparous cows as compared to a more adaptable and relaxed physiological state in resident primiparas, and in contrast a more relaxed state in freshly introduced multiparous cows as compared to resident multiparas. The presence of familiar animals did not affect HR and HRV measurers indicative of stress in freshly introduced primiparas. EF group mates however seemed to positively influence resident primiparas, therefore highlighting the possible relevance of continuous long-term relationships. One HRV metric (SDNN) of multiparas decreased with increasing number of EF group mates present, otherwise no effects were detectable. Multiparas' HRV values however were generally low, probably due to metabolic effort. Our result confirm findings that regrouping is bearable for experienced cows, however all the more challenging for unexperienced primiparous animals. The factor parity should therefore always be considered were applicable. HR and HRV measures provide important additional information about dairy cows' stress response, however more basic research in healthy non-stressed (physiologically as well as psychologically) cattle would be needed to allow more confident conclusions from applied settings.

1. INTRODUCTION

Different approaches are used to evaluate the stress response of farmed animals to specific aspects of husbandry systems, management or handling procedures. One important subject concerns changes in group composition and mixing of more or less unfamiliar animals. Regrouping is common practice in dairy farming to facilitate optimal management of cows with differing and changing demands during their (re-)productive cycle. The accompanying social instability and changes of the environment are likely to be stressful to the animals. Several studies have reported behavioural changes indicative of stress in regrouped cows. These include reduced feeding and lying times (Von Keyserlingk et al., 2008b), increased time spent restless (Reith and Hoy, 2012), an increase of lying bouts indicating more restless behaviour (Schirmann et al., 2011), an increase of agonistic encounters (Von Keyserlingk et al., 2008b; Kucevic et al., 2010; Lobeck-Luchterhand et al., 2014), and a decrease of socio-positive behaviour (Von Keyserlingk et al., 2008b).

Beneath behaviour, physiological markers can be indicative of stress. For example, milk cortisol levels as a correlate of the hypothalamic-pituitary-adrenal (HPA) axis' activity were found to be increased after regrouping (Fukasawa and Tsukada, 2010; Pošćić et al., 2017). Another basic regulatory system of bodily functions is the autonomic nervous system (ANS) with its sympathetic and parasympathetic branch, adapting an organisms' physiology to its current situation. The ANS directly influences heart rate by modulating the rhythm of its two internal pacemakers, the sinoatrial and the atrioventricular node (Shaffer and Venner, 2013). This leads to irregularity in the intervals between heartbeats, described by the term heart rate variability (HRV). In brief, the parasympathetic system slows down, is responsible for the reduction of the sinus rhythm to appropriate resting values and stands for relaxation ("rest and digest"), while the sympathetic system speeds up and strengthens the contractility of the atria and ventricles, preparing the organism to physical effort ("fight or flight"). Therefore, HRV is considered a promising non-invasive tool to assess current stress reactions in farm animals (von Borell et al., 2007b).

In cattle, the assessment and analysis of HRV have been applied to evaluate stress deriving from various sources. In their review about HRV research in dairy cattle, Kovács et al. (2014) categorised related research into four main topics: (1) disease-induced pathological loads; (2) stress caused by husbandry and veterinary procedures; (3) animal's behavioural and physiological stress reactions during milking and (4) short-term cardiac responses to fearful situations (novelty, weaning and isolation). They conclude that HRV analysis is a useful and sensitive measure of acute responses to environmental challenges. However, effects of regrouping on HRV in dairy cattle have not been investigated yet.

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Regrouping management strategies instead gained some attention and it has repeatedly been shown that regrouping pairs or groups of animals is preferable over single cows, as they seem to integrate and adapt faster (O'Connell et al., 2008; Gygax et al., 2009a; Neisen et al., 2009b; Mazer et al., 2020). Social amplifiers such as social facilitation or social buffering effects probably play a role in this context (Hennessy et al., 2009; Kiyokawa, 2018). It has been shown that the familiarity or relationship between conspecifics is an important aspect of such effects (Takeda et al., 2003; Færevik et al., 2006; McLennan, 2013; Acevedo-Triana et al., 2017), thus dairy cows might as well benefit when they are familiar to at least parts of the resident group.

In our study we were interested whether HRV measures during resting indicate higher stress in dairy cows freshly introduced into the milking group after calving, and whether the presence of familiar animals may alleviate such effects. We hypothesised that freshly introduced cows would have lower HRV measures during resting as compared to resident cows. Further, we hypothesised that with a higher number of familiar cows already present in the resident group HRV measures would increase, but dependent of the degree of familiarity (long-term more than short-term).

2. ANIMALS, MATERIALS AND METHODS

The study was designed according to European and Czech laws and current guidelines for ethical use of animals in research. The study was approved by the Institutional Animal Care and Use Committee of the Institute of Animal Science (Permit Number 15155/2010-17210).

2.1 Study site, animals and management

The study was carried out at the research farm 'Netluky' of the Institute of Animal Science, Prague, Czech Republic. Data were collected in the group of early lactating and high yielding cows comprising ± 50 dehorned animals of 70% Holstein breed (9,900 kg average 305 day milk yield) and 30% Czech Spotted breed (7,800 kg yield). The group was housed in a 30 x 15 m pen with curtain ventilation system that contained 54 cubicles with straw bedding (1.35 x 2.4 m) in two rows facing each other and one row facing the wall, a 30 m feeding face with neckrail, concrete corridors littered with small amounts of straw, two water troughs, two salt licks and an automatic brush. The animals were fed ad libitum with a total mixed ration (TMR). Fresh feed was delivered twice daily at about 06:00 and 14:00 h, and pushed up by a feed pusher or manually every hour. Milking took place in a 2 x 5 automatic tandem milking parlour and was carried out by alternating two pairs of milkers twice daily at 03:30 and 15:30 h. The corridors were cleaned twice daily during milking with a skid loader.

Prior to calving, dry cows and pregnant heifers (last three months before calving) were kept together in a separate barn. The first days after calving, until stable increases in milk yield and a reduced risk of complications was achieved, the cows spent in individual pens in a separate building. Cows were then introduced into the group of early lactating and high yielding cows in small groups of mostly two to three, which occurred about twice per week.

2.2 Focal animals, data collection and data processing

The data presented here were recorded during three observation periods between October 2010 and October 2011, each lasting about 8 weeks. A time span of at least 100 days was imposed between the periods. As consequently most of the cows of the observed early lactating group had been replaced when the next observation period started, independence of data between observation periods was maximised.

Animals

Each cow that entered the group after calving and a resident cow already present in the group for about 5 weeks (median 35 days, range 13-97 days) and matched by breed and lactation served as focal animals. For each focal animal, all other cows present in the group were assigned in terms of familiarity and counted. As lasting social relationships in cattle are built early in life (Raussi et al., 2010; Wagner et al., 2012) and are likely to be disturbed by separation (McLennan, 2013), we differentiated between early and recent familiarity. Group mates were defined as early familiar (EF) if two cows had been born within 90 days, and as recently familiar (RF) if two cows had spent at least one third of their dry period (or, in case of heifers, late pregnancy) together. By means of youngstock management at the farm EF pairs had grown up in the same or in adjacent stables allowing physical contact during their first years. Therefore, they did not only have a long shared experience, but a shared experience in the most important period for establishing durable social relationships. RF pairs spent a very recent time period together and were never separated for longer than twice as long the period they spent together.

Lying phases

For the analysis of cardiac activity during resting we had to identify lying phases. To this end, all cows present at the beginning of an observation period, and all cows entering the group later were equipped with HOBO Pendant® accelerometers. The loggers were set to record the acceleration of the y- and z-axis every 60 seconds. They were attached during normal milking times in the milking parlour to the cows' left or right hind leg using specifically developed silicon moulds and self-adhesive Co-Flex® bandages. Memory capacity allowed recording periods of 20 days, after which loggers were exchanged and data downloaded. The resulting text data files were merged and processed in R (RCoreTeam, 2011), resulting

in a list containing information about date, time, cow identity and behaviour (lying vs. not lying). For a detailed description and corresponding codes see Schröder (2012).

HR and HRV data

Cardiac activity was recorded with the help of polar® monitors (type RS800CX) as inter-beat-intervals. To equip the cows, they left the milking parlour via a separate alley (usually used for ultrasonic pregnancy checks) where they were not fixated, but prevented from moving more than two meters forward or backward. Here, cows were equipped with a standard elastic surcingle for horses which hold the two electrodes, and a second specifically developed broader elastic tape with pocket (orthopaedic material for waistbands) for cover, protection and to hold the monitor. A wet sponge optimised the contact between skin and electrodes. Cows were firstly equipped 24 hours before regrouping to allow habituation. After every milking, cows were brought into the alley to check the equipment and the position of the electrodes, to renew the wet sponges and to download data.

To extract robust and representative HRV resting values per cow we considered and defined several criteria. Firstly, as cardiac activity strongly underlies diurnal rhythms (Latschbacher, 2013), we divided the first 24 hours after regrouping into four time windows 1 – evening (18:00-21:00; post milking, no more routines, staff leaving), 2 – night (23:00-02:00; deep night, lights off), 3 – morning (06:00-09:00; post milking and fresh feed delivery) and 4 – midday (11:00-14:00; rather undisturbed interim). Although time windows do mirror actual pacemakers and other aspects of diurnal rhythm, they remain artificial categories. By choosing long breaks of two hours between time windows, we therefore aimed at maximising separation between them. The time span of morning milking (03:00-05:00) plus a buffer of one hour was excluded. Secondly, within time windows 1 – 4, we identified all lying periods longer than 40 minutes. The corresponding heart rate recordings were checked and processed in Kubios HRV®. Artefact correction was only allowed for single missing beats and to a maximum of 5% of beats per 5-minute-bout. The threshold was set to "medium" (Niskanen et al., 2004). As a general rule, we extracted four equally distant 5-minute-bouts per lying period: bout 1 from the beginning (starting 10 minutes after lying down), bouts 2 and 3 with equal distances from in between, and bout 4 from the end of the lying period (ending 10 minutes before standing up). However, in case of artefact correction > 5%, undetected artefacts (such as singly occurring zigzag deviations from the curve), or successive missing beats, the position of one or more 5-minute-bout(s) was manually moved to the closest analysable section (except the first and last 10 minutes to avoid effects of (preparing) physical activity (Gutmann et al., 2013)). Bouts were analysed in terms of heart rate (HR), root mean square of successive differences (RMSSD), and standard deviation of inter-beat-intervals (SDNN) and averaged per lying phase.

2.3 Data set and statistical analysis

The analysed data set comprised data from three observation periods and 17 integrated groups (2 – 4 (mean = 2.3) cows per group), resulting in heart rate variability (HRV) measures from 50 individual cows during in total 181 lying phases, distributed over factor levels as summarised in Table 1. Occasionally, the quality of heart rate recordings did not allow to extract 4 x 5-minutes-bouts, thus the number of analysed bouts per lying phase varied between three and four (overall mean = 3.8).

We examined whether resting HRV reflects the stress of regrouping and whether familiar group mates may alleviate such effects. We therefore aimed at comparing freshly introduced cows with the sample of matched residents as a control. There was confounding between explanatory variables that prohibited the combination of both factors in one analysis, as residence status (freshly introduced vs. resident) was likely to be related to milk yield (early lactation and peak lactation) and both were likely to affect cardiac activity. Therefore, we ran separate analyses for freshly introduced and resident animals and compared their results graphically and at a descriptive level.

The models comprised parity, time window, number of early familiar (EF) as well as recently familiar (RF) animals present, and milk yield as well as all parity-interaction terms as fixed factors, and cow ID as random factor (see Table 2). To account for their empirical distribution and to allow a more intuitive interpretation of the intercepts, we z-standardised the number of EF and RF animals present as well as milk yield to "א" (0,1) separately between data subsets (residence status) and individually for parity within data subsets.

Initially, observation period was as well included as random factor, but removed as it did not account for considerable variance. We ran models for three HRV time domain measures as dependent variables, i.e. HR (heart rate, beats per minute [bpm]), RMSSD (root mean square of successive differences, milliseconds [ms]), and SDNN (standard deviation of interbeat intervals [ms]).

Table 8 (Article 3, Table 1): Descriptives of the analysed sample over factor levels. Time windows: 1 – Evening (1800-2100), 2 – Night (2300-0200), (0300-0500 morning milking), 3 – Morning (0600-0900), 4 – Midday (1100-1400). Breed: CF – Czech Fleckvieh, HF – Holstein Friesian.

	Freshly introduced		Resident	
	primiparous	multiparous	primiparous	multiparous
Focal cows (CF/HF)	7 (2/5)	17 (8/9)	9 (5/4)	17 (7/10)
Lying phases (CF/HF)	15 (5/10)	70 (29/41)	34 (20/14)	62 (28/34)
Lying phase duration [min] mean per cow (min-max)	87 (38-163)	82 (40-342)	85 (40-179)	95 (40-184)
Lying phases per time window 1/2/3/4	2/2/6/5	18/19/16/17	9/4/11/10	14/14/21/13
Mean cows per time window (min-max)	3.25 (2-5)	11.5 (10-17)	6 (3-8)	12 (10-17)
Mean time windows per cow (min-max)	1.9 (1-4)	2.7 (1-4)	2.7 (2-4)	2.8 (1-4)
Lying phase duration [min] mean per time window 1/2/3/4	71/39/112/83	79/73/103/75	85/99/95/69	101/79/104/88
DIM	6.7 ± 3.6 (5-14)	7 ± 2.7 (4-12)	55.8 ± 20.4 (24-93)	42.2 ± 29.4 (18-123)
Milk yield 24h after regrouping mean ± std (min-max)*	21.9 ± 5.7 (14.7-29.3)	30.9 ± 5.3 (20.0-47.2)	31.2 ±4.7 (25.1-41.2)	38.2 ± 7.0 24.2-50.7)
Number of early familiar group mates mean ± std (min-max)*	5.8 ± 3.6 (2-12)	5.4 ± 3.9 (1-14)	9.6 ±4.6 (4-18)	6 ± 4.8 (1-15)
Number of recently familiar group mates mean ± std (min-max)*	25.8 ± 2.8 (20-29)	18.2 ± 2.9 (14-23)	36.0 ± 6.1 (24-44)	25.6 ± 4.0 (18-36)

* equivalent to z-standardised mean ± std (0 ± 1) in the model

3. RESULTS

Figures 1 to 3 visualise our main findings. Figure 1 shows the effect of the time of day (time window) on heart rate (HR) and heart rate variability (HRV) in relation to parity and residence status. Figures 2 and 3 illustrate HR and HRV values in relation to the number of early familiar group mates present for primi- and multiparous freshly introduced (Figure 2) and resident (Figure 3) cows, respectively.

The pattern of HR and HRV values as function of parity, time window, and the number of early familiar (EF) or recently familiar (RF) group mates present differed between freshly introduced and resident cows. Independent from parity (parity x time window: $F = 0.23$, $p = .877$), HR of resident cows showed a clear diurnal pattern (time window: $F = 16.41$, $p < .001$), with highest values in the evening and a rather stable lower level until midday. Figure 1 suggests that, in contrast, freshly introduced primiparous cows had an overall higher HR than multiparous fresh cows, and that only the latter showed a diurnal pattern comparable to that of resident cows; however, this has not been fully statistically proven (HR (LSmean ± SE);

freshly introduced primiparas vs. multiparas: 84.2 ± 2.6 vs. 78.6 ± 1.4, F = 3.40, p = .083; time window: F = 4.67, p = .005; parity x time window: F = 0.66, p = .581). HR of resident animals was not affected by any other of the explanatory variables, whereas in freshly introduced animals milk yield was positively related to HR at a level close to statistical significance (F = 3.97, p = .063).

HRV of freshly introduced animals was not affected by parity, time window, milk yield or number of EF or RF group mates present. In resident animals however, both RMSSD (primiparas vs. multiparas 10.18 ± 0.80 vs. 5.58 ± 0.57, F = 21.96, p < .001) and SDNN (primiparas vs. multiparas: 27.0 ± 2.3 vs. 20.8 ± 1.6, F = 5.07, p = .038) were higher in primiparous than multiparous cows. Coherently, resident primiparas showed a diurnal RMSSD pattern with lowest values in the evening and rather stable values at a higher level until midday (parity x time window: F = 4.85, p = .004).

Effects of the number of familiar animals present were only evident in resident cows and depended on parity. With increasing number of EF group mates HRV (RMSSD as well as SDNN) increased in primiparas, while it was rather unaffected (RMSSD) or decreased (SDNN) in multiparas (estimated slope ± SE in primiparas (unit ≙ 4.6 group mates) vs. multiparas (unit ≙ 4.8 group mates): RMSSD 4.35 ± 1.32 vs. -0.99 ± 0.64, F = 13.32, p = .002; SDNN 7.33 ± 3.70 vs. -4.28 ± 1.80, F = 7.97, p = .012). Concerning RMSSD of resident cows, the statistically significant main effects 'time window' and 'number of EF group mates' (see Table 2 (B)) obviously reflected the strong effects within primiparous cows and were therefore not interpretable as such, but only as interaction effects with parity.
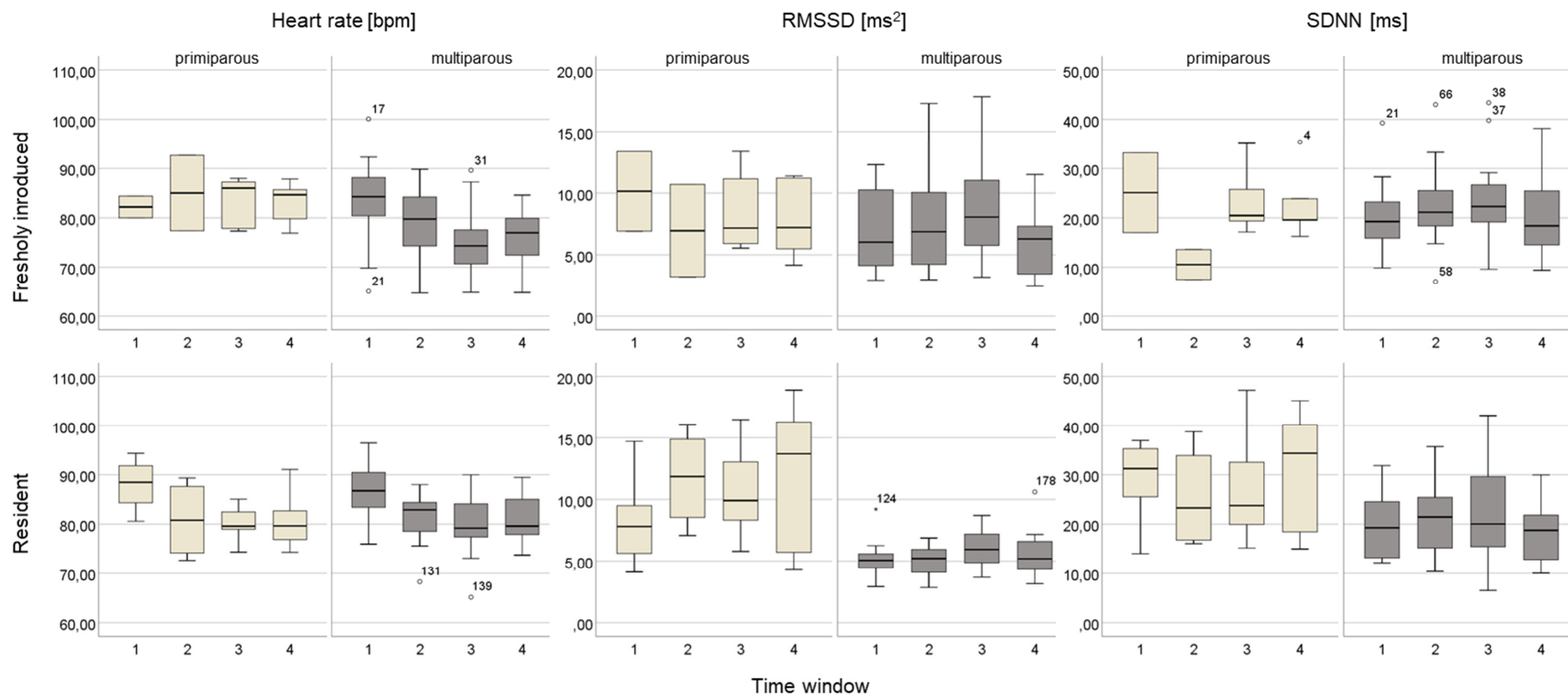
Figure 11 (Article 3, Figure 1): HRV measures HR, RMSSD and SDNN as related tp parity and time window. Comparison of arithmetic medians of primiparous (light grey) and multiparous (dark grey) freshly introduced (upper row) and resident (lower row) cows over time windows: 1 – Evening (1800-2100), 2 – Night (2300-0200), (0300-0500 morning milking), 3 – Morning (0600-0900), 4 – Midday (1100-1400).
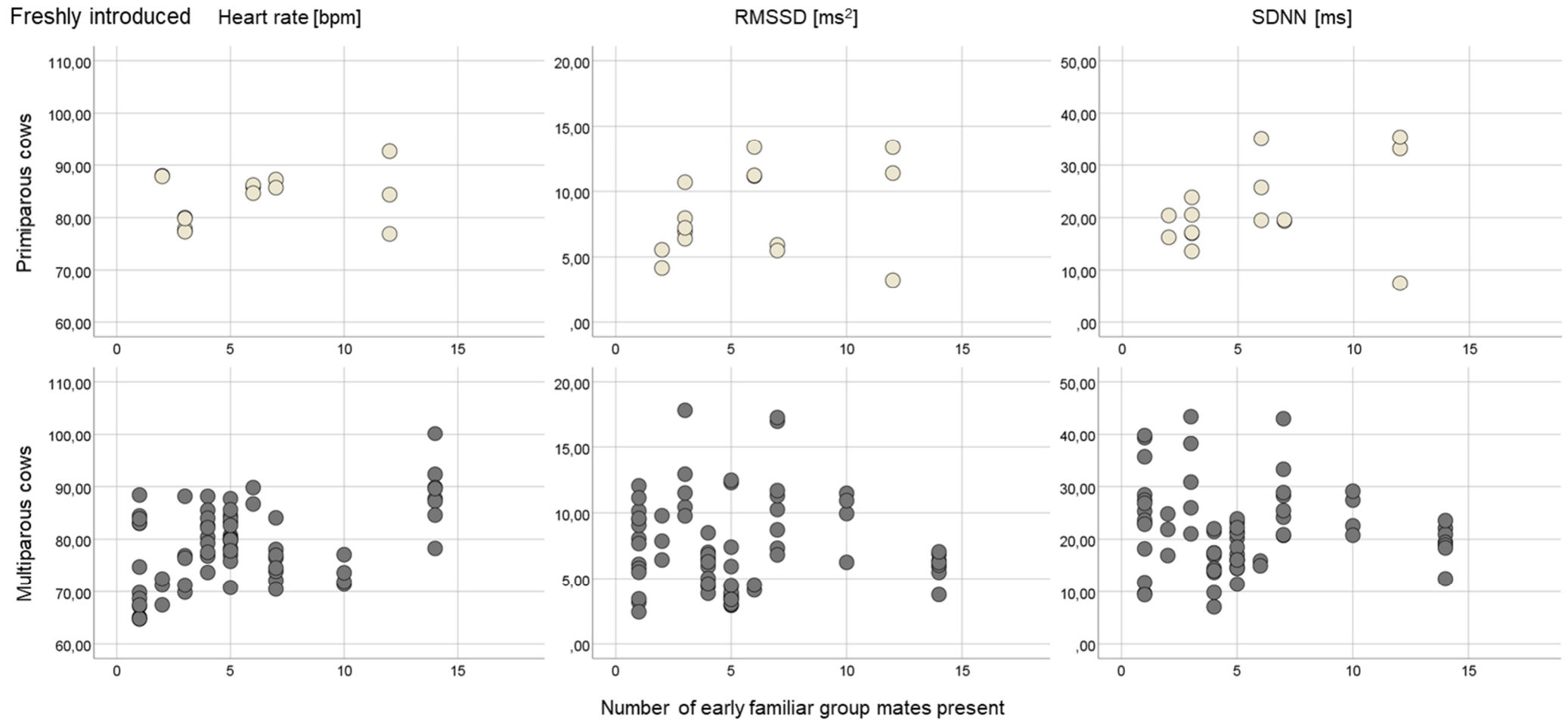
Figure 12 (Article 3, Figure 2):Freshly introduced cows' HRV measures heart rate, RMSSD and SDNN in relation to the number of early familiar group mates present. Upper row: primiparas ($N_{Animals}$ = 7, $N_{Lying phases}$ = 15). Lower row: multiparas ($N_{Animals}$ = 17, $N_{Lying phases}$ = 70).
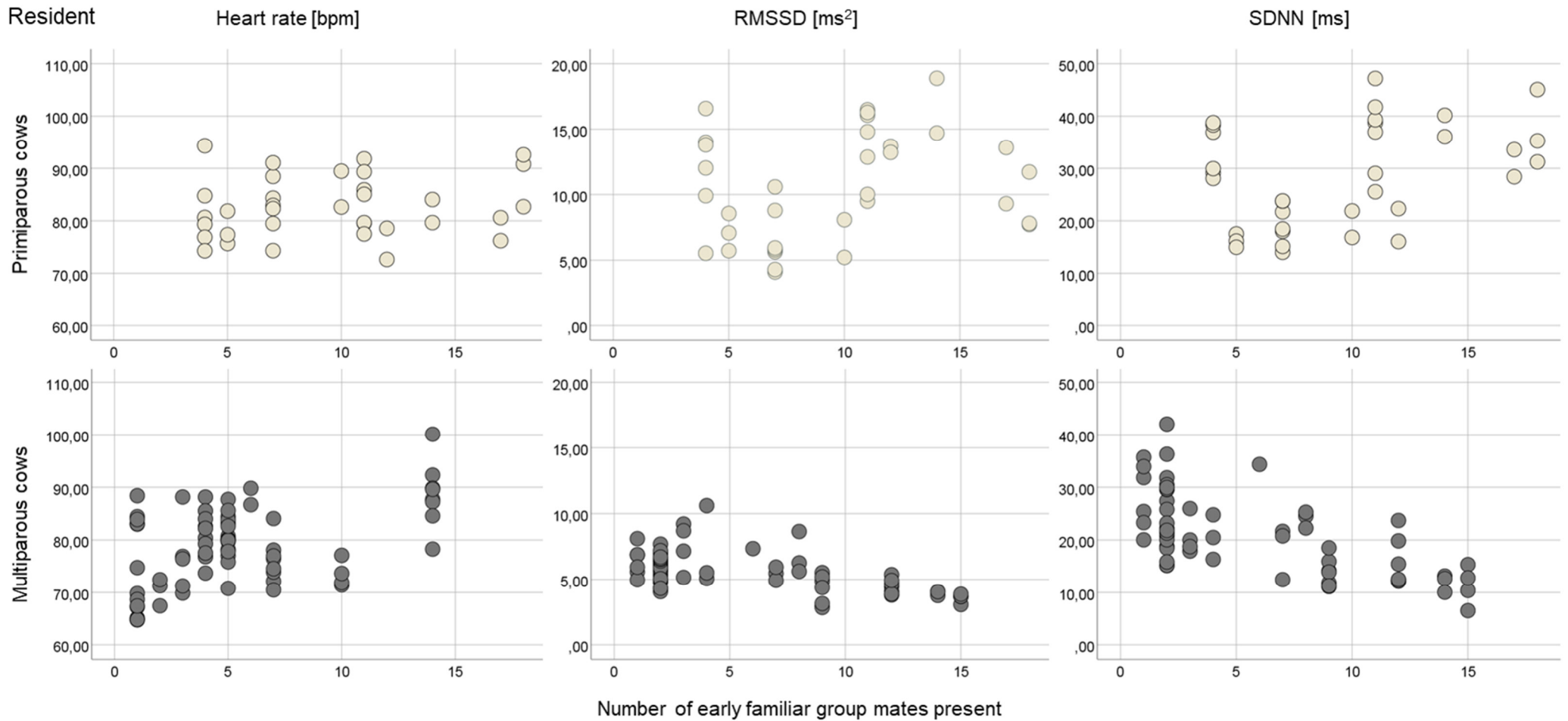
Figure 13 (Article 3, Figure 3):Resident cows' HRV measures heart rate, RMSSD and SDNN in relation to the number of early familiar group mates present. Upper row: primiparas ($N_{Animals}$ = 9, $N_{Lying\ phases}$ = 34). Lower row: multiparas ($N_{Animals}$ = 17, $N_{Lying\ phases}$ = 62).

Table 9 (Article3, Table 2):Full model results. Models were run separately for freshly introduced and resident animals, and for (A) heart rate, (B) RMSSD and (C) SDNN. Parity 1 = primiparous, 2 = multiparous; NoEF = number of early familiar group mates; NoRF = number of recently familiar group mates; Time window 1 = Evening (1800-2100), 2 = Night (2300-0200), (0300-0500 morning milking), 3 = Morning (0600-0900), 4 = Midday (1100-1400).

(**A**) Heart rate							
		Freshly introduced			Resident		
		LSmean / slope	F	p	LSmean	F	p
Parity	1	84.2 ± 2.6	3.40	.083	82.5 ± 1.5	0.01	.947
	2	78.6 ± 1.4			82.6 ±1.1		
Time window	1	84.7 ± 1.9	4.67	.005	87.9 ± 1.2	16.41	.000
	2	81.6 ± 2.0			82.3 ± 1.5		
	3	79.6 ± 1.6			79.8 ± 1.1		
	4	79.3 ± 1.6			80.4 ± 1.2		
NoEF		0.59 ± 1.43	0.17	.684	0.38 ± 1.34	0.08	.782
NoRF		-1.42 ± 1.83	0.60	.449	-0.17 ± 1.31	0.02	.896
Milk yield		3.27 ± 1.64	3.97	.063	0.08 ± 0.95	0.01	.935
Parity * time window	1-1	86.4 ± 3.4	0.66	.581	88.0 ± 1.9	0.23	.877
	1-2	83.9 ± 3.7			81.4 ± 2.6		
	1-3	83.3 ± 2.8			80.1 ± 1.8		
	1-4	82.5 ± 2.8			80.6 ± 1.8		
	2-1	83.1 ± 1.6			87.7 ± 1.5		
	2-2	79.4 ± 1.5			83.2 ± 1.5		
	2-3	75.8 ± 1.6			79.5 ± 1.3		
	2-4	76.2 ± 1.6			80.1 ± 1.5		
Parity * NoEF	1	-0.81 ± 2.33	0.96	.338	-0.56 ± 2.41	0.49	.497
	2	1.99 ± 1.65			1.32 ± 1.18		
Parity * NoRF	1	-3.93 ± 3.24	1.87	.189	0.68 ± 2.45	0.43	.522
	2	1.09 ± 1.70			-1.03 ± 0.90		
Parity * milk yield	1	4.89 ± 3.00	0.98	.337	-1.48 ± 1.55	2.68	.120
	2	1.65 ± 1.33			1.63 ± 1.10		

(**B**) RMSSD							
		Freshly introduced			Resident		
		LSmean / slope	F	P	LSmean	F	p
Parity	1	8.44 ± 1.78	0.06	0.805	10.18 ± 0.80	21.96	< .001
	2	7.93 ± 0.98			5.58 ± 0.57		
Time window	1	7.87 ± 1.23	1.40	0.251	6.15 ± 0.58	10.85	< .001
	2	9.24 ± 1.31			8.06 ± 0.68		
	3	8.37 ± 1.09			8.63 ± 0.55		
	4	7.26 ± 1.09			8.67 ± 0.57		
NoEF		0.58 ± 0.98	0.35	0.563	1.68 ±0.73	5.30	.036
NoRF		1.08 ± 1.27	0.72	0.409	1.11 ± 0.70	2.55	.128
Milk yield		-1.38 ± 1.13	1.48	0.243	0.21 ± 0.51	0.17	.682
Parity * time window	1-1	8.36 ± 2.23	0.44	0.722	7.22 ± 0.92	4.85	.004
	1-2	9.92 ± 2.40			10.94 ± 1.17		
	1-3	8.03 ± 1.90			11.14 ± 0.90		
	1-4	7.46 ± 1.91			11.43 ± 0.89		
	2-1	7.37 ± 1.06			5.09 ± 0.70		
	2-2	8.56 ± 1.05			5.18 ± 0.70		
	2-3	8.72 ± 1.06			6.12 ± 0.64		
	2-4	7.07 ± 1.07			5.92 ± 0.70		
Parity * NoEF	1	1.11 ± 1.59	0.29	0.596	4.35 ± 1.32	13.32	.002
	2	0.05 ± 1.15			-0.99 ± 0.64		
Parity * NoRF	1	2.51 ± 2.25	1.27	0.278	2.37 ± 1.32	3.27	.087
	2	-0.35 ± 1.18			-0.15 ± 0.42		
Parity * milk yield	1	-1.63 ± 2.07	0.05	0.826	0.31 ± 0.83	0.04	.851
	2	-1.13 ± 0.93			0.11 ± 0.57		

(C) SDNN							
		Freshly introduced			Resident		
		LSmean / slope	F	p	LSmean	F	p
Parity	1	19.8 ± 3.1	0.44	.516	27.0 ± 2.3	5.07	.038
	2	22.1 ± 1.7			20.8 ± 1.6		
Time window	1	20.6 ± 2.6	1.99	.124	22.6 ± 1.7	2.10	.109
	2	17.1 ± 2.8			22.6 ± 2.0		
	3	23.8 ± 2.1			26.1 ± 1.6		
	4	22.3 ± 2.1			24.4 ± 1.7		
NoEF		2.15 ± 1.78	1.46	.241	1.52 ± 2.06	0.55	.470
NoRF		1.08 ± 2.20	0.24	.631	2.56 ± 1.98	1.68	.211
Milk yield		-2.14 ± 1.99	1.16	.296	-1.89 ± 1.43	1.74	.203
Parity * time window	1-1	22.8 ± 4.8	1.58	.202	25.1 ± 2.7	0.32	.813
	1-2	11.8 ± 5.2			25.4 ± 3.5		
	1-3	24.1 ± 3.6			29.2 ± 2.6		
	1-4	22.5 ± 3.6			28.5 ± 2.6		
	2-1	20.4 ± 2.0			20.0 ± 2.1		
	2-2	22.5 ± 2.0			19.8 ± 2.1		
	2-3	23.5 ± 2.0			22.9 ± 1.8		
	2-4	22.2 ± 2.0			20.4 ± 2.1		
Parity * NoEF	1	4.22 ± 2.99	1.35	.259	7.33 ± 3.70	7.97	.012
	2	0.08 ± 1.94			-4.28 ± 1.80		
Parity * NoRF	1	2.94 ± 3.93	0.72	.401	4.10 ± 3.74	0.61	.447
	2	-0.79 ± 2.00			1.02 ± 1.27		
Parity * milk yield	1	-1.36 ± 3.65	0.16	.697	-1.63 ± 2.35	0.03	.855
	2	-2.93 ± 1.59			-2.16 ± 1.64		

4. DISCUSSION

In this study, we analysed whether heart rate (HR) and heart rate variability (HRV) measures during resting would indicate a stressful experience in cows that had been freshly introduced into the milking group after calving as compared to resident cows matched in breed and lactation number. Further, we analysed whether the number of early familiar (EF) or recently familiar (RF) group mates present would affect HR and HRV measures, suggesting a stress-relieving effect of social support. HR and HRV patterns only partly confirmed our hypotheses.

Signs of stress in regrouped cows

Freshly introduced primiparous cows showed signs of an aroused physiological state, indicating a stressful experience: firstly, their HR remained consistently high over time, while the other groups showed the expected diurnal pattern (Latschbacher, 2013). Secondly, their HRV did not differ from freshly introduced multiparas' HRV, while it did in the resident group, where primiparas had higher HRV values than multiparas. These results are in line with former studies reporting behavioural signs of stress such as reduced feeding and lying times or more restless behaviour during the first hours up to days after regrouping (von Keyserlingk et al., 2008a; Schirmann et al., 2011; Reith and Hoy, 2012). Explicitly focussing on primiparous cows after regrouping, Wagner et al. (2012) reported decreased total lying times and Soonberg et al. (2021) an increased number of lying bouts and higher activity. Regrouping likely is a stressful experience to primiparas, who do not achieve a physiological state indicating relaxation comparable to their matched residents even during night's rest. It is unlikely that other factors such as calving or onset of milk production would explain these differences in HR/HRV pattern of primiparous cows, as there is no such difference between freshly introduced and resident multiparous cows.

No evidence for enhanced arousal was found in freshly introduced multiparous cows. In contrast, their HR followed a diurnal pattern and yielded lower estimated values compared to their matched residents, while their RMSSD was more variant and higher. Based on the analysis of lying behaviour, a relaxing effect of regrouping in multiparous and therefore experienced cows has been proposed before, which may be explained by the fact that regrouping into a (partly) familiar group in a familiar environment is experienced as an improvement compared to being kept in individual pens during the first days after calving (Gutmann et al. 2020). Our results concerning multiparas are in line with several studies concluding that the stress responses to regrouping are well bearable when focussing on the physiological effects of stress, i.e. immunity, metabolic responses, and reproductive and productive parameters (Coonen et al., 2011; Silva et al., 2013; Chebel et al., 2016). Coonen et al. (2011) and Silva et al. (2013) indeed found no treatment effects at all when comparing stable and dynamic groups of cows around parturition. However, the authors emphasise the

crucial role of optimal management in terms of sufficient feed and space provided as a necessary condition, and restrict their conclusions to multiparous (Jersey) cows. Several studies addressing regrouping did not consider or report the factor parity in their analyses (von Keyserlingk et al., 2008; Huzzey et al. 2005; Campler et al 2019), rendering differing outcomes difficult to interpret. However, our current and previous results (Gutmann et al. 2015; Gutmann et al. 2020) underline the need to differentiate between primi- and multiparous animals.

Stress-relieving effects of familiar group mates

In a review, Bøe and Færevik (2003) supposed that, based on the literature about social relationships in cattle, the presence of familiar or related conspecifics would make social integration easier. In our study, the presence of only recently familiar group mates did not seem to affect arousal or relaxation in freshly introduced animals during resting as measured by HR and HRV. Concurrently, earlier studies did not find effects of short-term familiarity as related to relationship intensity (Gutmann et al., 2015) or synchrony (Gutmann et al., 2020). However, in the present study we found higher HRV in resident primiparas with an increasing number of early familiar group mates present. This might be evidence for social support and a positive effect of experienced social stability on health and welfare and add to the notion that early social bonds are the important ones in cattle (Bouissou et al., 2001). The fact that the effect is absent in multiparous cows may even support this interpretation, as only primiparous pairs' relationship is characterised by a continuous shared experience, and it has been shown that separation can have a disrupting effect (McLennan, 2013). However, a possible relationship would need further evaluation. Experienced cows may as well just be less dependent on their early relationships.

Our results bear relevance for research subjects that may implicitly deal with familiarity. For example, studies focussing on the regrouping strategy, i.e. comparing integration of single vs. pairs or groups of animals, reported somewhat inconsistent results concerning effects on behaviour: No differences were found (Mazer et al., 2020) as well as desirable (Knierim, 1999; Gygax et al., 2009a; Neisen et al., 2009b) and undesirable effects (Menke et al., 2000) when introducing more than one cow at once. This may be partly explained by individual differences such as dominance or coping strategy (Hasegawa et al. 1997, Nogues et al. 2021), but may also implicitly reflect effects of familiarity, as varying familiarity among the regrouped animals was not addressed in the mentioned studies, but ranged from three days (Mazer et al. 2020) up to life-long (Gygax et al., 2009a). Moreover, the (varying) familiarity with the members of the main group was only rarely considered yet (Wagner et al., 2012; McLennan, 2013).

General evaluation of HR and HRV values

Generally, HR and HRV values derived from our analysis are within the range reported in the respective literature for lactating cows (Mohr et al., 2002; Kovács et al., 2015a). As mentioned above, differences in cardiac activity due to parity were primarily found in resident cows. This contrast may on the one hand reflect differences in arousal under less productive effort – i.e. milk yield – in the case of freshly introduced cows, and differences in physiological fitness under less arousal, but more productive effort, in the case of resident cows on the other hand. Especially RMSSD was low and rather invariant in resident multiparas, indicating a reduced vagal tone and therefore loss of regulatory capacity and cardiovascular adaptability (Shaffer and Venner, 2013; Erdmann et al., 2018). In line with this, only in resident primiparas the diurnal RMSSD pattern reflected the HR pattern, i.e. a higher HRV with lower HR, indicating a dynamic balance of the sympathetic and parasympathetic branch of the autonomous nervous system as expected. Therefore, at least in terms of the ANS' regulatory actitvity, all but primiparous resident cows seemed to be seriously psychologically and/or metabolically challenged. More basic research on HRV in healthy non-stressed (physiologically as well as psychologically) cattle would be needed to allow more confident conclusions from applied settings.

5. CONCLUSIONS

Our results show that regrouping is challenging and stressful to primiparous cows at least during the first 24 hours, and that the presence of familiar conspecifics is not sufficient to alleviate the situation. Multiparous cows instead can be able to tolerate regrouping well. Our results therefore highlight the need to include parity as a factor in respective research designs. Positive effects of more early familiar peers present as observed in resident primiparas indicate possible beneficial effects of a stable and continuous social environment and deserve further investigation. HRV measures contain important information about dairy cows' inner state. However more basic research on HRV in healthy non-stressed (physiologically as well as psychologically) cattle as well as a comprehensive methodological consensus are needed to allow more confident conclusions from applied settings.

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9 Summary of results

The three scientific articles presented here aim to add to the understanding of form, function and relevance of social relationships among dairy cows in a dynamic group. Taken together, the main results may be described as followed.

The first analysis characterised social relationships among dairy cows and resulted in four main components that: (1) time spent as direct neighbours when feeding and interacting affiliative as well as agonistically (excluding displacements), (2) displacement success, (3) allogrooming interactions, and (4) time spent as direct neighbours when resting. Familiarity was associated with higher scores on components 1, 3 and 4. Long-term familiarity and continuity, i.e. either from birth on or due to repeated shared periods of adulthood, had stronger effects on the intensity of social relationships, i.e.regarding investment of time and energy, than very recent shared experience (shared preceding dry period only).

The second analysis showed that in the first 24 hours after integration, primiparous fresh cows lied less and behaved less synchronous at the dyadic level than their matched residents. No such effects were found in multiparous cows. The presence of animals only recently familiar due to a shared dry period had no influence on either primiparous or multiparous cows' behaviour. In contrast, early familiar animals, i.e. known from birth on, affected the cows' behaviour in several aspects, yet differently in primiparas and multiparas. In fresh primiparas, an increasing number of early familiar animals present had a negative effect on lying duration. Among both fresh and resident primiparas, early familiar dyads were more synchronised than other pairs of animals. In multiparous cows, a higher number of early familiar cows present led to more synchronous behaviour with the group.

The third analysis revealed that primiparous and multiparous cows' heart rate (HR) and heart rate variability (HRV) pattern were differently affected by the factors 'residence status' and 'number of (early or recently) familiar group mates present'. In freshly introduced primiparous cows, HR and HRV patterns indicate an aroused physiological state as compared to a more adaptable and relaxed physiological state in resident primiparas. In contrast, freshly introduced multiparous cows showed a pattern indicative for a more relaxed state as compared to resident multiparas. The presence of familiar animals did not affect HR and HRV measurers in freshly introduced primiparas. Early familiar group mates however seemed to positively influence resident primiparas, therefore highlighting the possible relevance of continuous long-term relationships. Multiparas' SDNN (standard deviation of interbeat intervals) decreased with increasing number of early familiar group mates present, otherwise no effects were detectable. Multiparas' HRV values however were generally low.

10 General discussion & future research perspectives

10.1 Dairy cows' social relationships

Several authors have suggested that companionship and familiarity play an important and alleviating role in the context of stressful situations such as regrouping in cattle and especially dairy cows (Jóhannesson and Sørensen, 2000; Mounier et al., 2006; Rault, 2012; Sosa et al., 2018). The present project was based on these assumptions, extended by the hypothesis that the quality of a social relationship would be a critical factor. However, although we found indications that long(er)-term familiar cows recognise each other, interact with each other frequently and influence each other's behaviour, we could neither find distinct types of relationship quality, nor convincing evidence of positive integrating or guiding effects of social companionship in the context of regrouping. This was true for synchrony, lying behaviour or calmness during resting. The question remains whether these results are based on missing effects of social relationships, or on missing social relationships. Here, we can only approximate but not present the definite answer.

Some initial assumptions of the present work were repeatedly confirmed, while other results put doubt on widely accepted concepts and assumptions about cattle behaviour. As hypothesised, cows showed a non-random spatial and temporal distribution and individual choice of social partners (Article 1, pp.48). In accordance with former studies and biologically well explainable, long-term familiarity proved to be the main general factor associated with closer social relationships (Article 1, pp. 50 & 51). In wild or free-moving social groups, most often genetically related animals are found to be closely associated. Concerning the underlying mechanisms of kin recognition, disentangling genetic relatedness and familiarity often is not that easy in natural settings, but it has been shown that kin recognition is at least partly explained by familiarity (Murphey, 1990; Murphey and de Moura Duarte, 1990). In accordance, our results suggest that it is continuity that matters. No effects at all were found in relation to short-term familiarity. This indicates that a number of animals housed together is not automatically a social entity and bears relevance for the general definition of a "group". In addition, attempts to investigate effects of familiarity may fail when a chosen timespan of familiarisation is insufficient.

Other assumptions we made could not be proven or were even reversed, specifically concerning the distribution of social interactions of presumably different quality and content, and the distribution of displacement success (Article 1, pp.50). In (applied) ethology, spatial proximity serves as a proxy for social bonding and usually is interpreted as affiliative relationship (e.g. Whitehead, 2008). Regarding dairy cattle, Boyland et al. (2016) tested the correlation between spatial proximity and social interactions but found no relation to agonistic interactions, and only weak relations to allogrooming. Foris et al. (2019) did not find a

correlation between affiliative and agonistic interactions, but, in a follow-up study, a positive association between the time spent as feeding neighbours and affiliative interactions (Foris et al., 2021). In our case however, a clear relation between time spent in close proximity and socio-positive interactions could not be confirmed. In contrast, cows who spent much time in close proximity interacted with each other in all possible ways, with the only exception that displacement incidences formed an independent aspect of a relationship (Article 1, p. 48). Several aspects may explain these different results and should possibly attract more attention in or for future studies: The above mentioned studies differ in the complexity and variety of behaviours they are based on. In contrast to the present one, allogrooming was the only behaviour considered 'affiliative', and while Boyland et al. (2016) used a variety of 'agonistic' behaviours comparable to the present study, Foris et al. (2019) and Foris et al. (2021) limited their assessment to displacement incidences. Additionally, the studied cow groups considerably differed concerning group size, stability and space allowance, ranging from a stable group of 14 cows kept indoors in a cubicle system (Foris et al., 2019; Foris et al., 2021) over the present dynamic group of about 50 cows to a dynamic group of about 110 animals with pasture access (Boyland et al., 2016). The traditional concepts of "bad" agonistic and "good" affiliative interactions or relationships are neither consistently used, nor may they hold true for each dairy housing and management system. Additionally, it is unlikely as well as unsatisfactory that the rarely observable 'allogrooming' should be the only affiliative behaviour among cows. The specific way of interacting might not play a major role in the communication among (dehorned) cows under high metabolic pressure and confronted with limited resources, and seemingly "bad" agonistic interactions such as butting and rubbing still be a tool and sign of attachment between animals. On the other hand, focussing only on prominent and presumably unambiguous types of interactions, i.e. displacements and allogrooming, requires – at the dyadic level – long observation times, especially in large groups, and does still often result in low occurrences and unknown relationships due to missing data points. Additionally, even displacement incidences should be interpreted carefully. Especially concerning the identification of dominance relationships, measures of displacement success or approach-avoidance incidences might not be appropriate for dairy cow herds under intensive conditions. Low space allowance prevents stepwise escalation from threatening at a larger distance to interactions with physical contact. In crowded situations the identity of the acting animal might be even unknown to the recipient and e.g. a sudden head-butt from behind quite surprising, thus leading to displacement success irrespective of the dominance relationship of the pair (see also Val-Laillet et al. (2008)). The outcome of feeding related agonistic interactions might rather be related to hunger or motivation to feed than to dominance (Beilharz and Zeeb, 1982; Côté, 2000; Val-Laillet et al., 2008). Using displacement success to determine social rank hierarchies therefore should be

limited to defined situations, where alternative explanations of success can be excluded. Similar rules should probably be applied when focussing on other aspects of a social relationship and in relation to spatial proximity. Taken together, the interpretation of social interactions is not clear-cut and needs deeper investigation, including the meaning of absence of interactions.

Concerning synchrony, our tested variables did not well explain variation at pair level (Article 2, pp. 70 & 62). Synchrony defined as '(not) lying at the same time' may not be specific enough to capture truly joint activities. Also, at group level, the definition of synchrony as 'doing what the majority is doing' could be refined. In the context of welfare assessment using indices of lying behaviour such as the stall usage index or cow comfort index, it has been emphasised that data be assessed during appropriate times, i.e. when cows are expected to lie (O'Driscoll et al., 2008). Stricter definitions of synchrony, e.g. thresholds at higher proportions or a focus on periods of peak synchrony, are possibly needed to yield more significant results with respect to integration or adaptation.

A fairly common but rather 'by-product' outcome of the three studies shall be illustrated by some citations: "The picture emerging from our results is complex." (p. 63), "However, there were unexpected effects […]" (p. 45), "Partly supportive, results on the other hand were surprising […]" (earlier version of a manuscript of Article 1). The complexity, dependency and interrelatedness of the presented results show the multidimensional nature of cows' behaviour and social relationships, and possibly the corresponding potential that lies in a better understanding. On the other hand, the rather latent, weak patterns create the impression that their existence is covered by stronger determinative realities – the priorities are shifted, namely towards production-related behaviours, and socially motivated behaviours may not always be conducive in this context. The marked position of resident primiparous cows in the results adds to this reading as they might fall into a narrow window of feeling familiar while being fit and not metabolically stressed, i.e. have most free capacities.

While the complexity of our results complicates clear interpretation, it offers all the more future research questions and refined approaches. Closest related to the initial research goal of describing form, function and determinants of social relationships among dairy cows in a dynamic group, investigations of the distribution of social interactions over potential partners and preferences as a function of the level of stability/dynamic of a group could help clarify the found unexpected pattern of agonistic and affiliative interactions. How much are the clarity or unambiguousness of interactions related to the consistency of contact between the animals? How much to husbandry aspects such as space allowance or freedom to move? How strongly affected is the strength of association between spatial proximity and interaction quality by those factors?

To better understand quality and expression of dairy cows' social relationships, approaches in the assessment and analysis of social behaviour might be useful where the dyad rather than the individual or group is the analysed unit. Furthermore, statistics might be more appropriate which consider both individual and emergent dyadic effects, i.e. characteristics of a relationship which are not predictable on the basis of the behaviour and characteristics of the both individuals involved. Examples are correlational analyses not only within and between, but across partners and behaviours, or the below described social network analysis. In the majority of studies dealing with farm animal social behaviour, including the present one, behavioural categories differentiate between 'acting'/'actor' and 'receiving'/'receiver', ignoring the exchange, the inherent interdependencies of behaviour in social interactions. Furthermore, social interactions are frequently assessed on heterogeneous levels, i.e. in some cases initial behaviour and response are combined (as in 'displacement', or 'solicited licking'), in others the behaviour of one partner is disregarded, or assessed as a separate interaction (e.g. head-butt as response to solicitation, or displacement of an animal that initiated the interaction with a friendly sniffing). The present results might motivate further basic research on dairy cow social behaviour and relationship quality, to be better able to validly assess and to improve the social situation and well-being of dairy cows.

Another promising approach is Social Network Analysis (SNA), which offers new ways of quantifying associations and includes not only the individual, dyad and group level, but considers indirect, triadic and subgroup effects as well. Investigating key metrics of the functioning of cattle groups could offer approaches to search for critical factors, and to approve group management practices. For example, the traditional perspective on giraffes' social system describes a "loose amalgamation of non-bonded individuals that sometimes coalesce into a herd" (Bercovitch and Berry, 2013). However, recent studies using SNA draw a converse picture: giraffes live in a highly flexible fission-fusion system based upon long-term social associations often reflecting kinship, with subgroup dynamics probably regulated through sophisticated communication systems (Bercovitch and Berry, 2013). Giraffes' social connectedness is stronger when females have dependent offspring (Saito et al., 2020), during foraging (Muller et al., 2018), and during the wet season (in wild (Prehn et al., 2019) as well as in captive animals (Lewton and Rose, 2020)), indicating that context and priorities affect sociality. Connecting SNA metrics to group stability, feed availability, space allowance etc. in (dairy) cattle may reveal factors appropriate to promote strong social bonds. So far, the application of SNA to dairy cow groups draw a picture of dense but weakly connected groups rather unstable over time (Gygax et al., 2010; Herzog, 2014; Boyland et al., 2016), possibly reflecting management practices rather than cattle's social potential.

A feasible and time-saving method to assess relationships of differing quality among dairy cows could not be developed in the course of the present studies, however levels of long-term familiarity proved to be a promising proxy deserving deeper investigations. The strong association between spatial proximity and interacting could allow the reduction to spatial data, which can be collected automatically, facilitating replications over farms (Gygax et al., 2010; Boyland et al., 2016; Foris et al., 2019; Rocha et al., 2020). The data basis however should be critically defined, preferring times or situations of most freedom to choose, e.g. at pasture or during the nights or other undisturbed phases, and avoiding times of enhanced agitation and urgent needs during e.g. feed delivery or close to milking times. Apparently influential factors such as feed barrier design, stocking density or space allowance should also be considered. This would offer the opportunity to confirm long-term familiarity as predictor of close relationships and search for new ones, as well as testing supporting farm features and conditions.

10.2 External validity

The fact that all results originate from data collected at only one farm is the most obvious limitation of the studies' outcomes, as farm effect and transferability and therefore external validity of the results cannot be estimated. However, there is a trade-off between detailedness and practicability of on-farm study designs. Studies dealing with social aspects of dairy cow farming are mostly conducted either in smaller and/or experimental groups, or without individual recognition of the animals, and still by far not all of them replicate their observations at different farms. Concerning the design of the current study, we decided to stay at one farm. On the one hand it would not have been feasible at acceptable costs and effort at different farms, as it required complex technical equipment: To enable simultaneous continuous observation of several focal animals in their group of more than 50 potential partners, cameras had to be installed in sufficient number, temporal and spatial resolution to allow individual recognition of single animals and reliable assessment of fine-scale behaviour such as ruminating, ear-position etc. Merely installation of the video equipment and marking of the cows took several days before an observation period could start. Measurements of cardiac activity required specific logistics and habituation time. On the other hand, a reduction of complexity, e.g. reduction of group size or simplification of data assessment, would have counteracted the studies' subject and the aim to describe form and function of social relationships among dairy cows in their "natural" environment and at a fine-scale descriptive level. To enhance variation we pseudo-replicated our study with different cows and group compositions over time at the same farm. In many aspects, the farm is representative for standard commercial dairy farming in large parts of the EU, e.g.

concerning size, performance indicators, management practices, feeding regime, or breeds kept.

10.3 Effects of observation on behaviour

During my university lectures I learned not to begin data collection before my study subjects would act towards me "like I am a tree". Using video recordings, we avoided most effects of a physically present observer (Hawthorne effect: McCambridge et al., 2013). Still we might have affected cows' behaviour with our presence and work during preparation, with the handling procedures, short visits in the barn to adjust equipment or give back heart rate monitors, and in my experience even the cameras' eyes can make one feeling observed. The cows at Netluky research station and dairy cows in general are used to an intense contact with humans. They are driven and handled twice daily for milking, and periodically for insemination, gestation diagnosis, or claw trimming. Additionally close contact to humans happens during feed delivery or pushing-up of feed, and technical checks of the barn equipment, the water troughs etc. Also cows are often used to technical farm equipment such as feed pushing robots, automatic brushes, tractors and bigger vehicles. Compared to that, and based on the personal impression that cows did not behave obviously different or unusual, we estimate the influence of our observations insignificant.

10.4 Dealing with HRV data

Each step on the way to gain conclusive results from HRV analysis contains its own challenges. The intensive and repeated handling of the animals during equipping and data download, the wearing of the equipment and the equipment itself might affect both heart rate and animal behaviour, concerning the respective focal animals as well as their group mates. The latter for example might be scared or attracted by the appearance of their peer wearing strange things. Once the data are collected, artefacts have to be detected and removed, and influencing factors to be identified and considered in the analysis.

Concerning our collection, processing and analysis of HRV data we applied very strict rules and therefore achieved high quality data (Article 3, p. 76). During handling we carefully tried to ensure a relaxed state of the cows. To this end the animals were not fixated but only partly restricted in their freedom to move, they were allowed to take their time to calm down, and they were brushed for calming and rewarding. A habituation period wearing the equipment was set to 24 hours. These measures seemed to satisfy our demands, as the majority of the focal cows stood still during handling and did allow short handling procedures even while moving freely. Concerning the group, we could observe that the cows' initial interest in the

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belts disappeared during the first days and remained absent even in incoming cows, indicating a habituation effect at group level.

During data processing and analysis we never solely relied on implemented software correction algorithms and double-checked each analysed data bout. Additionally we took influencing factors into account which are often disregarded, such as time of the day, latency after change of physical activity or body posture. Generally, HR and HRV values derived from our analysis were within the range reported in the respective literature for lactating cows. However, there is considerable variation in resting values with ranges found for mean heart rate from about 65 to 81 bpm (Kovács et al., 2015b; Kovács et al., 2015c), and mean RMSSD from about 6 (this study and Mohr et al. (2002)) to 36 ms (Kovács et al., 2015c). Absolute differences may be due to differences in milk yield, age, breed, time of the day or season. Even if single factors do not have statistically significant influence within a given data set, combined effects may explain differing baseline values during lying. Most studies follow the recommendations as given by the Task Force of the European Society of Cardiology the North American Society of Pacing (1996), and by von Borell et al. (2007b) specifically for the study of HRV in farm animals. However, several aspects found to influence HRV are not regularly considered that might add to the observed differences, including time of the day or posture changes (Ledochowski, 2012; Latschbacher, 2013). Methodological aspects such as the level of accuracy during raw data correction might be another source of error. Therefore, a systematic evaluation and review of general recording and data editing methods, factors considered in the analysis and reported baseline values would facilitate comparison and therefore could lead to more generalisable conclusions. Standards for assessment and reporting should include explicitly defined and specified circumstances of baseline measures. Overall, the HRV values in our study were worryingly low (Article 3, p. 90 & 91), an outcome that deserves further systematic investigations as it bears methodological as well as welfare relevant implications. Although the focus often lies on the differences to baseline values and not on the absolute baseline levels as such, bottom effects could affect results. Transferring conclusions from human medicine, the cows seem to be close to their physiological limit. More basic research on HRV in healthy non-stressed (physiologically as well as psychologically), ideally extensively kept cattle as related to age, sex, breed and biorhythms (diurnal, season) would be needed to allow more confident conclusions from applied settings.

10.5 Implications concerning research on regrouping

In practical terms, our results include implications concerning the investigation of regrouping strategy and effects. As highlighted by others (Neave et al., 2017; Proudfoot et al., 2018; Soonberg et al., 2021), of most significant importance are the repeatedly found differences between primi- and multiparous cows. Our results add to the notion that experienced cows

are not affected by regrouping at a biologically significant level (Coonen et al., 2011; Silva et al., 2013; Walker et al., 2015; Chebel et al., 2016). Moreover, regrouping in many cases in fact is a release from isolation, as cows after calving might be kept separately for a couple of days. An even relieving effect therefore cannot be excluded. Primiparous cows instead appeared to be seriously challenged. After introduction they lay for less than 5 hours on average, and occasionally 24 hours were not long enough to calm down and find a place to rest. Unfortunately for them, alterations elicited by regrouping in many cases become manifest only in behaviour, but leave key production metrics such as milk yield or body weight unaffected (O'Connell et al., 2008). Depending on the particular focus, therefore, interpretations found in the literature differ substantially even while sometimes basing on similar results. Longer lying times can be interpreted as both negative (Piñeiro et al., 2019) and positive (von Keyserlingk et al., 2008a; Steensels et al., 2012; Talebi et al., 2014), higher activity as restlessness or reduced fearfulness (Gygax et al., 2009a; Boyle et al., 2013), more agonistic interactions as problematic or sign of confidence (Boyle et al., 2013). Our results from HRV analysis add important information about the physiological state of the animals. The inability to calm down physically as well as physiologically casts doubt on interpretations of restlessness as explorative behaviour and confidence (Boyle et al., 2013), and of longer lying times as sign of fear and suppression (Hasegawa et al., 1997; Boyle et al., 2013). Although the regrouping strategy was not subject of our studies, our results highlight the importance to carefully interpret behaviour, to not only compare treatment groups cross-sectional, but to consider within-treatment group differences over time, and to assess additionally for example physiological data.

11 Final words

After all I learned about and from the cows, my personal impression is that they are highly social and sensitive beings, able and willing to confidently communicate and cooperate not only amongst conspecifics, but also with humans. Companion animals, so to say. The data and results presented here do not always promote this view, in contrast, one could also emphasise the picture of a dairy cow group as a rather indifferent accumulation of competitive individuals. With my final words I would like to once more draw the attention to the fact that the cows that behaved as suggested when assuming a complex and differentiated social system, were the ones with free capacities. Personally, I conclude that many dairy husbandry systems suppress the social very nature of cows. About which by far not everything is known.

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15 Supplementaries

15.1 Supplementary materials of article 2

Table 10: Group-level descriptors and activity pattern. Percentage is related to true group size. R_P gives Pearson's correlation coefficient of the respective variable with 'true group size' over all observation periods. If not otherwise stated, values are given in [median (min-max)]. DIM = days after parturition ("days in milk").

observation period	1	2	3		
integration days analysed	5	9	9		
	median (min–max) over integration days				
				R_{Sp}	p
true group size	52 (49–55)	51 (47–53)	50 (45–55)		
stocking density (cows/cubicles)	0.96 (0.91–1.02)	0.94 (0.87–0.98)	0.93 (0.83–1.02)		
% primiparous cows (mean ± st. dev.)	41.4 ± 3.0	24.6 ± 3.5	28.4 ± 3.3		
DIM (mean ± st. dev. of medians)	83.9 ± 7.5	72.5 ± 4.0	65.8 ± 7.5		
length of stay [median/max (N cows leaving the group)]	93/293 (30)	94/173 (25)	65/271 (25)		
% of group measured	94.6 (92.3–96.2)	94.1 (89.8–96.2)	96.2 (90.7–97.9)		
mean % of cows lying	56 (54–57)	51 (41–53)	49 (39–54)	.432	.040
max. % of cows lying	83 (75–89)	73 (67–81)	76 (72–88)	.056	.801
% data points where majority of cows was lying	76 (68–84)	60 (15–68)	52 (28–68)	.393	.064

Table 11: Integrated units and description of the analysed sample of fresh and matched resident focal cows. CF = Czech Fleckvieh, HF = Holstein Friesian, DIM = day after parturition ("days in milk").

	observation period			
	1	2	3	overall
integration days observed	10	14	13	37
integration days analysed	5	9	9	23
cows per integrated unit observed [median (min-max)]	2 (2–3)	2 (2–4)	2 (2–3)	2 (2–4)
cows per integrated unit analysed [median (min-max)]	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)
primiparous				
no. of fresh cows	4	5	4	13
[HF, CF]	[1, 3]	[4, 1]	[4, 0]	[9, 4]
DIM [median (min-max)]	7 (5-14)	6 (5-10)	5 (5-16)	5 (4-16)
no. of resident cases	4	5	4	13
[HF, CF]	[1, 3]	[4, 1]	[4, 0]	[9, 4]
DIM [median (min-max)]	58.5 (24-72)	49 (29-93)	47 (44-51)	49 (24-93)
no. of additional cows	4	4	3	11
no. of former fresh cows	-	1	-	1
no. of repeated cows (cases)	-	-	1 (x2)	1 (x2)
days in group [mean ± st. dev.]	45 ± 20	49 ± 26	41 ± 4	45 ± 18
multiparous				
no. of fresh cows	6	12	15	33
lactation number [2/3/4/5/6]	[2/1/3/0/0]	[7/2/1/0/2]	[9/1/3/1/1]	[18/4/7/1/3]
[HF, CF]	[5, 1]	[7, 5]	[9, 6]	[21, 12]
DIM [median (min-max)]	6 (5-12)	8 (6-12)	5 (4-12)	7 (4-12)
no. of resident cases	6	12	15	33
lactation number [2/3/4/5/6]	[2/2/2/0/0]	[8/2/1/0/1]	[8/2/1/3/1]	[18/6/4/3/2]
[HF, CF]	[5, 1]	[7, 5]	[9, 6]	[21, 12]
DIM [median (min-max)]	65 (22-134)	44 (28-80)	37 (18-118)	39 (18-134)
no. of additional individuals	5	7	10	22
no. of former fresh cows	1	3	2	6
no. of repeated cows (cases)	-	1 (x3)	3 (x2)	3 (x2), 1 (x3)
days in group [mean ± st. dev.]	65 ± 53	41 ± 18	40 ± 30	45 ± 32
group mates (incl. focal cows)				
no. of individuals (once, twice, three times present over observation periods)	58	66	63	157 (129, 26, 2)
repeated over integration days [median ± avg. abs. dev. (min – max)]	5 ± 1 (1 – 5)	7 ± 2 (1 – 9)	9 ± 2 (1 – 9)	

Table 12: Distribution of familiarity levels for analyses at individual and dyadic level. CF = Czech Fleckvieh, HF = Holstein Friesian. EF = early familiar, RF = recently familiar.

parity	primiparous		multiparous	
group status	fresh	resident	fresh	resident
individual level				
no. of cows / cases	13	13	33	33
breed [HF / CF]	[9 / 4]	[9 / 4]	[21 / 12]	[21 / 12]
EF group mates	8 (2-15)	10 (4-18)	6 (1-15)	6 (1-15)
RF group mates	26 (21-32)	35 (24-44)	17 (12-24)	25 (17-36)
dyadic level				
no. of dyads	628	628	1545	1545
breed relation [HF / CF / mixed]	[350 / 32 / 246]	[350 / 32 / 246]	[782 / 117 / 646]	[782 / 117 / 646]
EF dyads	75	107	174	170
RF dyads	303	397	510	758

Table 13: Dependent variables – descriptive data statistics. Assoc.coef. = association coefficient.

parity	primiparous		multiparous	
	min – max (mean ± standard deviation)			
lying duration	0.35 – 12.75 (8.60 ± 3.37)		2.48 – 18.6 (9.84 ± 3.11)	
individual-with-group assoc.coef.	0.07 – 0.63 (0.26 ± 0.18)		-0.17 – 0.63 (0.21 ± 0.19)	
dyadic assoc.coef.	-0.40 – 0.60 (0.06 ± 0.16)		-0.49 – 0.69 (0.05 ± 0.17)	
	fresh	resident	fresh	resident
lying duration	0.35 – 12.20 (6.75 ± 3.68)	6.23 – 12.75 (10.44 ± 1.63)	4.22 – 18.6 (10.44 ± 3.12)	2.48 – 15.15 (9.24 ± 3.02)
individual-with-group assoc.coef.	-0.2 – 0.63 (0.22 ± 0.17)	-0.7 – 0.55 (0.30 ± 0.19)	-0.14 – 0.63 (0.22 ± 0.17)	-0.17 – 0.59 (0.20 ± 0.20)
dyadic assoc.coef.	-0.40 – 0.49 (0.04 ± 0.15)	-0.39 – 0.60 (0.07 ± 0.16)	-0.49 – 0.60 (0.05 ± 0.17)	-0.49 – 0.69 (0.05 ± 0.17)

Table 14: Lying duration – model results. Estimates ± standard error (SE) give LSmeans for categorical factors and slopes for covariates (number early-familiar (EF) and recently-familiar (RF) cows present). Random factors considered in the model for primiparas: Cow, IntegrationDay(ObservationPeriod); for multiparas: Cow, ObservationPeriod (Hessian matrix problems when including IntegrationDay). Covariates are set at zero (equals empirical mean due to standardisation). *tests difference between levels ** tests difference from zero => same p-value for covariates' main effect. CF = Czech Fleckvieh, HF = Holstein Friesian.

primiparous						
factor	F-test*		level	estimate ± SE	t-test**	
	F	p			t	p
breed	1.08	.314	HF	7.13 ± 1.09		
			CF	8.51 ± 1.39		
status	11.10	.004	new	4.49 ± 1.39		
			resident	11.16 ± 1.51		
EF	4.53	.048		-1.71 ± 0.80		
RF	1.20	.292		-1.23 ± 1.13		
status x EF	3.81	.067	new	-3.19 ± 0.90	-3.53	.002
			resident	0.22 ± 1.27	0.18	.863
status x RF	0.85	.370	new	-2.12 ± 1.64	-1.29	.215
			resident	-0.34 ± 1.30	-0.26	.796
multiparous						
factor	F-test*		level	estimate ± SE	t-test**	
	F	p			t	p
breed	0.05	.824	HF	10.24 ± 0.70		
			CF	10.35 ± 0.85		
status	2.95	.091	new	11.25 ± 1.01		
			resident	9.34 ± 0.72		
EF	0.00	.964		0.02 ± 0.42		
RF	1.65	.204		0.88 ± 0.68		
status x EF	0.45	.505	new	-0.21 ± 0.55	-0.38	.702
			resident	0.25 ± 0.54	0.46	.645
status x RF	2.14	.148	new	1.87 ± 1.20	1.56	.125
			resident	0.25 ± 0.54	-0.17	.862

Table 15: Group Association Coefficient – model results. Estimates ± standard error (SE) give LSmeans for categorical factors and slopes for covariates (number early-familiar (EF) and recently-familiar (RF) cows present). Random factors included in the model for primiparas: IntegrationDay(ObservationPeriod) (Hessian matrix problems when including Cow); for multiparas: Cow, IntegrationDay(ObservationPeriod). Covariates are set at zero (equals empirical mean due to standardisation). *tests difference between levels ** tests difference from zero => same p-value for covariates' main effect. CF = Czech Fleckvieh, HF = Holstein Friesian.

primiparous						
factor	F-test*		level	estimate ± SE	t-test**	
	F	p			t	p
breed	0.70	.416	HF	0.27 ± 0.08		
			CF	0.35 ± 0.10		
status	0.15	.704	new	0.34 ± 0.11		
			resident	0.28 ± 0.10		
EF	0.02	.890		0.01 ± 0.06		
RF	1.29	.277		0.09 ± 0.08		
status x EF	0.00	.977	new	0.01 ± 0.07	0.09	.926
			resident	0.01 ± 0.09	0.11	.914
status x RF	0.31	.587	new	0.14 ± 0.13	1.05	.306
			resident	0.05 ± 0.09	0.58	.572
multiparous						
factor	F-test*		level	estimate ± SE	t-test**	
	F	p			t	p
breed	1.65	.210	HF	0.22 ± 0.04		
			CF	0.28 ± 0.05		
status	0.10	.756	new	0.26 ± 0.06		
			resident	0.24 ± 0.04		
EF	9.35	.004		0.07 ± 0.02		
RF	0.00	.967		0.00 ± 0.04		
status x EF	0.31	.580	new	0.08 ± 0.03	2.61	.012
			resident	0.06 ± 0.03	2.03	.048
status x RF	1.36	.248	new	0.04 ± 0.07	0.67	.508
			resident	-0.04 ± 0.03	-1.18	.245

Table 16: Dyadic Association Coefficient – model results. Estimates ± standard error (SE) give LSmeans for categorical factors. Random factors included in the model: for primiparas: Cow_1, Cow_2, IntegrationDay(ObservationPeriod); for multiparas: Cow_1, Cow_2, IntegrationDay(ObservationPeriod). EF = early familiar, RF = recently familiar. HF = Holstein, CF = Czech Fleckvieh.

primiparous				
factor	F-test		level	estimate ± SE
	F	p		
breed relation	0.37	.692	HF	0.06 ± 0.02
			CF	0.07 ± 0.03
			mixed	0.07 ± 0.02
status	3.95	.055	new	0.05 ± 0.02
			resident	0.09 ± 0.02
EF	4.45	.035	yes	0.08 ± 0.02
			no	0.06 ± 0.02
RF	0.58	.449	yes	0.07 ± 0.02
			no	0.07 ± 0.02
status x EF	0.51	.476	new – yes	0.06 ± 0.03
			resident – yes	0.11 ± 0.02
			new – no	0.04 ± 0.02
			resident – no	0.07 ± 0.02
status x RF	0.39	.531	new – yes	0.06 ± 0.02
			resident – yes	0.09 ± 0.02
			new – no	0.05 ± 0.02
			resident – no	0.09 ± 0.02
multiparous				
factor	F-test		level	estimate ± SE
	F	p		
breed relation	0.58	.944	HF	0.05 ± 0.01
			CF	0.06 ± 0.02
			mixed	0.05 ± 0.01
status	1.12	.292	new	0.06 ± 0.01
			resident	0.05 ± 0.01
EF	0.25	.617	yes	0.06 ± 0.01
			no	0.05 ± 0.01
RF	0.06	.808	yes	0.05 ± 0.01
			no	0.05 ± 0.01
status x EF	1.40	.237	new – yes	0.07 ± 0.02
			resident – yes	0.05 ± 0.02
			new – no	0.05 ± 0.01
			resident – no	0.05 ± 0.01
status x RF	1.69	.194	new – yes	0.06 ± 0.01
			resident – yes	0.04 ± 0.01
			new – no	0.06 ± 0.01
			resident – no	0.05 ± 0.01

16 Curriculum Vitae

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Studium & Ausbildung …

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Okt 2005 – Jul 2008	Studium der Biologie auf Diplom mit erfolgreichem Abschluss an der Georg-August-Universität Göttingen
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