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The effects of mother-bonded rearing in dairy farming on animals' social traits

Master thesis

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1 Introduction

Calves in common dairy production are separated from their mothers within 24 h after birth (EFSA 2009). Flower and Weary (2003) pointed out that early separation is argued to be economically beneficial; e.g. calf milk replacer is cheaper than cow milk and higher milk sales are possible. Further arguments are the allowance of a better supervision of calves' milk intake, the reduced risk of disease transfer (Flower & Weary 2003), higher feed intake after weaning (Roth et al. 2009) and faster rumen development through earlier solid food consumption, which consequently allows earlier weaning (Khan et al. 2011).

A strong bond characterizes the relationship between a mother cow and her calf (von Keyserlingk & Weary 2007), which is one reason why cow-calf separation is problematic from a welfare perspective. This bond is established in a sensitive period after calving through licking, already 5 min of contact is enough to create a bond that lasted even after 12 h of separation (Hudson & Mullord 1977). Under natural circumstances (free ranging) the maternal care is long-lasting. During a period of six months the amount cows lick and allow their calves to suckle does not change with calves age (Lidfors & Jensen 1988) and natural weaning occurs at 8-12 month after birth (Waiblinger et al. 2004, pp. 125). Mothers are able to give the best individual needed care to their calves by adopting their maternal care to the calf's condition (Stěhulová et al. 2013) and move protectively in front of their calves in response to a threat (Flörcke et al. 2012).

Early separation can lead to disadvantageous consequences for calves' health and behaviour, while allowing mother contact at young age affects the animal's welfare positively (for review see Johnsen et al. 2016, Meagher et al. 2019). Calves reared with mother contact gain more weight, have less diarrhoea (Weary & Chua 2000, Grøndahl et al. 2007, Roth et al. 2009, Valnickova et al. 2015) and exhibit less abnormal oral behaviour in form of cross-sucking (Margerison 2003, Roth et al. 2009, Fröberg & Lidfors 2009) compared to artificially reared calves. Already contact with the mother of two weeks is advantageous for calves' weight gain and social competence (Flower & Weary 2001). Furthermore, in contrast to concerns about economic losses, at least production systems of dual-purpose milk and beef farms, that allow longer suckling, were rated to be even more economical beneficial than common practices (Asheim et al. 2016).

In various species, social experience at a young age is crucial for social competence and coping behaviour later in life (Taborsky et al. 2012, Ruploh et al. 2014, Langenhof & Komdeur 2018). Research suggest that social experiences influence brain development through epigenetic modification and that also maternal traits can be passed on to the next generation (Champagne & Curley 2005). The authors suppose that especially maternal grooming and licking affect social behaviour of next generations. Since cattle are known to be capable of various emotions and have complex cognitive and social behaviours (Marino & Allen 2017), also in dairy cows and calves, social competence as well as social and maternal behaviour is affected by rearing conditions (for review see Cantor et al. 2019).

Mother contact has a positive effect on social competence e.g. demonstrated by displaying more appropriate social behaviour in mother-bonded reared compared to artificially reared calves (Buchli et al. 2017). Moreover, calves who have contact with their mothers within the first 12 weeks of life showed a higher social motivation while coping better, as well as trying to escape more actively when being isolated and are more attentive towards unfamiliar conspecifics (Wagner et al. 2013). The impact rearing seems to have on calves were also found in heifers, who lived under same rearing conditions. Mother-bonded reared heifers show also more appropriate social behaviour (subordinate gestures) while being integrated into the dairy cow herd (Wagner et al. 2012), and cope more actively (i.e. performed more exploration and walking), when being isolated (Wagner et al. 2015). The obviously long-lasting impacts rearing has on social motivation, led to the authors' assumption that animals reared with mother contact might have a higher sociality (Wagner et al. 2015).

Sociality (the extent individual animals need social companionship), sociability (the motivation to stay close to other group members) as well as the frequency of social interaction are elements of social behaviour, that show a degree of variation between individual animals (Erhard & Schouten 2001, pp. 338). Among these elements, also social competence e.g. the avoidance and performance of aggressive behaviour in appropriate situations is from importance. The definitions of these terms sometimes differ among studies and overlaps are possible.

However, the manifestations of these elements in a certain individual seem to reflect animals' personal properties (personality traits), rather than merely states (Erhard & Schouten 2001, pp. 335). Also, Marino and Allen (2017) outlined that there is evidence that cow's sociality is a personality trait and personality traits are "traits that differ across individuals and are consistent over time" (pp. 485). Further, so far, it is widely accepted that sociability is part of the extraversion dimension of the Five-Factor Model of personality, a model that describes all personality dimension and which seem also applicable to nonhuman animals (Gosling & John 1999).

Further research is needed to detect whether the observed effects in cows from different rearing treatments are due to actual differences in personality (social) traits or if they are merely situation-based. General intra-situational behavioural consistency in cows was detected during social separation (Müller & Schrader 2005), while inter-situational behavioural consistency was found with respect to undisturbed social interactions and social competition (Mülleder et al. 2003). It is particularly interesting to examine to what extent the rearing condition on a long-term basis, affects personality traits and especially sociality as a personality trait due to the assumption that socio-positive relationships can enhance well-being (Rault 2012). Cows in dairy industry who develop distinct social competence are advantageous for modern farming, they may cope better with social challenging (e.g. regrouping) situations (Gibbons et al. 2010). However, it is unknown to what extent maternal contact and maternal behaviour during rearing, especially through the frequency and duration of the socio-positive interactions the calf experience at young age, affects the development of personality traits.

The aim of the study was to investigate in dairy cattle the influence of early social experiences on social behaviour later in life by focusing on sociality, social competence and the propensity to engage in socio-positive relationships. The effects of rearing conditions (mother-bonded reared vs. artificially reared) and maternal behaviour on social traits were examined.

It was hypothesised

(1) that spontaneous socio-positive behaviour and social competence are enhanced in mother-bonded reared animals compared to artificially reared animals,

(2) that animals reared with mother contact show a higher sociality (motivation to end isolation) compared to artificially reared animals and

(3) that animals' spontaneous socio-positive behaviour correlates positively with received duration of maternal social contact after calving.

For this purpose, the social behaviour of heifers and calves from both treatments were observed in the group's home pen and a social isolation and reinstatement test was used to investigate the motivation of animals to re-join its peers, as well as their reaction pattern to the situation in general. The social isolation is a common instrument to investigate manifestations of behaviours in cattle related to fear and nervousness (Müller & Schrader 2005) and the latency until they walk towards its peers after isolation is a verified method to analyse social motivation in dairy cows (Gibbon et al. 2010). Furthermore, the maternal behaviour animals of the mother-bonded reared group received after calving was analysed to test if they correlate with animals' propensity to engage in socio-positive relationships later in life. We expected that animals reared with mother contact show more social-positive, more adequate socio-specific behaviours, are more motivated to re-join its peers and show less stress related behaviours in isolation than artificially reared animals. Further, we expected that animals from the mother-bonded treatment, who received high amounts of maternal contact in the first hours after birth also engage often in socio-positive interactions (and *vice versa*) in the herd later in life.

2 Methods

2.1 Farm and Animals

The study was conducted between April and June 2019 at the Johann Heinrich von Thünen-Institut (VTI, Federal Research Institute for Rural Areas, Forestry and Fisheries) in Trenthorst, Germany. Cows in this facility were kept in two dairy herds, separated by horn status (genetically hornless/with horns). No animals were dehorned. Each dairy cow herd had a space allowance of 785 m^2 and both were kept in a cubicle loose housing system (see Wagner et al. 2012 for more details). The barns were subdivided into a feeding area (total 43 m x 4 m), a cubicle equipped lying area (39 m x 3 m) and a walking area (43 m x 3 m) with transponder-controlled concentrate feeders. Cows of the two dairy herds were milked twice daily.

Offspring of cows from the dairy herds were included in the study, in total eighteen female Black-and-White-German-Holstein cattle, half of them polled (genetically hornless, n = 9), the other half had horns (no disbudding is performed on the farm). The experimental animals had been reared in the first 12 weeks of life according to two different treatments either with contact to their mother (*Contact*, n = 9) or without (*Automat*, n = 9, for details see 2.1.2.). For calving, mothers of the experimental animals were brought to an individual calving pen, which additionally had an outdoor run. All calves were bottle-fed with 2 l colostrum in the first hours after birth.

One to eight days after calving (depending on treatment), calves of both treatments were brought to the calf area. The calf area was subdivided in a deep litter lying area (13 m^2) and a running area (54 m^2) (see also Wagner et al. 2013). Dependent on the rearing condition (see 2.2), calves had transponder-controlled access either to a selection gate that led to the dairy herd in the cow barn where they could suckle their mother or to a milk feeder (FA Förster-Technik GmbH, Engen, Germany). Water, hay and silage were accessible *ad libitum* and concentrate portions were provided by an automatic feeder.

After weaning on day 90 calves were kept in a distinct building for youngstock (Fig. 1) until they were 16-month-old heifers. The barn was in total subdivided into six pens and one breeding bull pen. Experimental animals were housed in four different groups according to their age, the total herd size varied between the groups (Table 1).

Table 1 Overview of group composition and pen size (according to floor plan) during barn-housing of the four groups. Number of experimental animals of the different rearing treatments in each group, total group size, average group age, average group weight and allocated pasture side

	Calves 1	Calves 2	Heifers 1	Heifers 2
N Experimental animals (Automat/Contact)	4/2	1/1	3/4	1/2
Total group size [n]	8	16	13	8
Average group age [months]	5	8.1	13.6	16.7
Average group weight [kg]	139	197	311	392
Total space pen [m^2]	152.4	304.8	304.8	304.8
Feeding alley [m^2]	30.30	60.6	60.6	60.6
Walking alleys [length m x width m]	-	-	5 x 2.5	5 x 2.5
			5 x 1.5	5 x 1.5
			12 x 3	12 x 3
Lying area [m^2]	47.10	91.45	41.75	41.75
Outdoor area [m^2]	72.09	145.80	145.80	145.80
Pasture side	Pasture 1	Pasture 1	Pasture 2	Pasture 2

The two heifer pens and calf pens were located next to each other, animals of these similar aged groups could have had visual and tactile contact. Heifers were housed on one side and pens were divided into four areas: (a) a feeding alley with feeding racks, (b) a lying area with two rows of cubicles (facing each other) bedded with a straw mattress,(c) three walking alleys with concrete floor and (d) a concrete outdoor area. Younger animals were housed on the opposite side of the barn. These pens had (a) a feeding alley with feeding racks, (b) a deep litter bedded lying area and (d) a concrete outdoor area. Each pen provided *ad libitum* access to water, minerals, as well as a swinging brush and pens for younger animals additionally were equipped with hay racks, which were refilled on a regular basis.

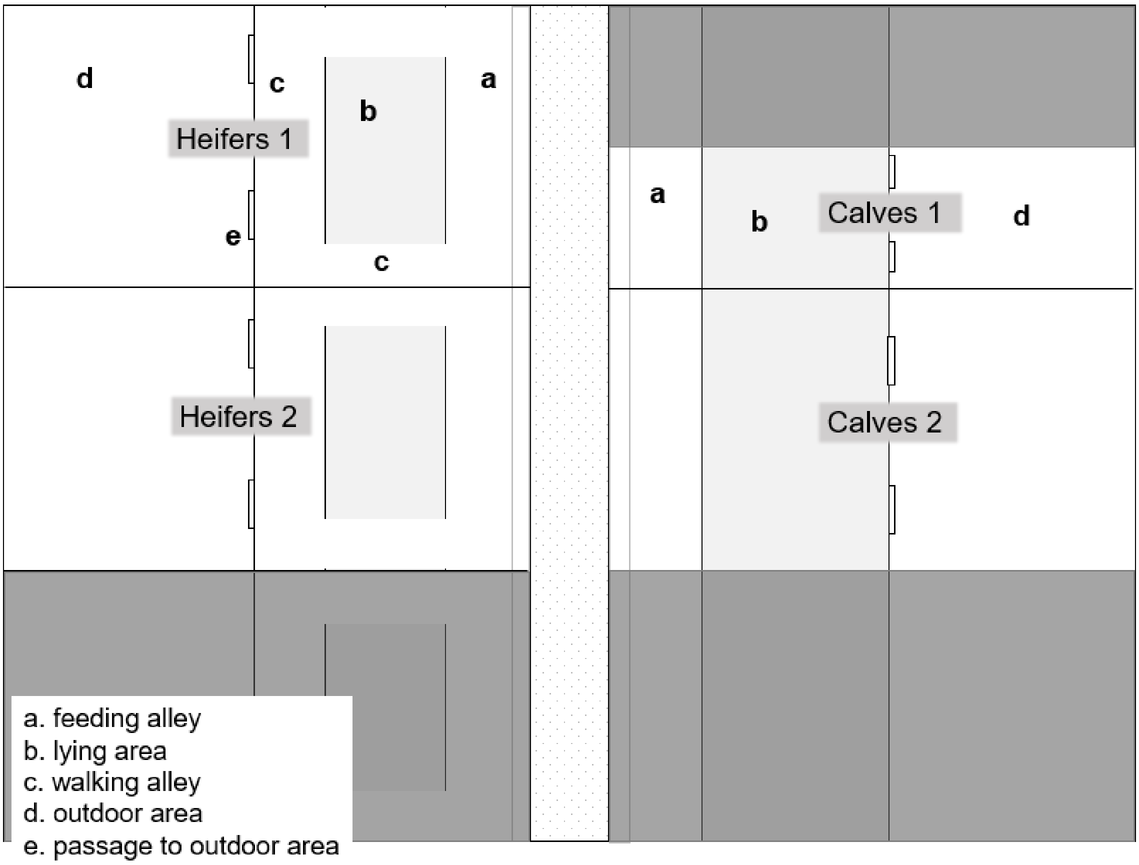

Fig. 1. Sketch of the heifer and calf barn with the four different groups that include experimental animals. Letters define the different areas. Dark areas indicate pens of non-experimental animal groups that were not part of the study.

During summer all animals were pastured. From May on heifer groups with experimental animals were pastured together (Pasture 1, 3.47 ha). Calves groups participated in another study (Mix-Enable) and were pastured together (Pasture 2) from June on in a rotational grazing system, switching to a new pasture section every week (6 sections in total, each 0.31 ha). As mentioned before, animals of the joined groups had visually and tactile contact to each other before to minimize possible negative effects of regrouping. Animals on pasture got concentrate in feeding troughs (Heifers: 1 kg/day, Calves: 0.5 kg/day) as well as *ad libitum* water and mineral access. Pastures were limited by electric strands.

The project was discussed and approved by the institutional ethics committee (University of Veterinary Medicine) and conducted in accordance with GSP guidelines and national legislation.

2.2 Rearing Treatments and animal selection

Animals were selected to participate in the study as follows: For *Contact* animals, all female youngstock between weaning and until first insemination; *Automat* animals were then selected according to a balanced distribution of age and group between treatments.

Artificial reared (Automat, n = 9): Within 24 h after birth, the calves were separated from their mothers and were moved to single calf igloos. In there, calves were fed with colostrum milk four times daily at least for six days depended on the health status. At day eight after birth, under precondition of a good health status, they were brought to the calf area and were habituated to an automatic feeder. The provided milk portions increased gradually to a maximum of 12 kg per day/animal at the age of 17 days and remained constant until the 75th day of life. Then portions were continuously reduced until weaning on day 90.

Mother-bonded reared (Contact, n = 9): Within the first 7 days, calf and mother stayed together in the calving pen and the calf was suckled by its mother. The cow was milked in the milking parlour twice daily. At day 8, each calf was moved to the calf area. Mother-bonded reared calves were trained to use the selection gate to have free access to the cow barn and, thus, to its mother and herd. The calves were weaned on day 90 by nose flap and totally separated from the mother one or two weeks later. The nose flap cause that calves cannot suckle their mothers while contact is still possible.

2.3 Test procedures and data recordings

2.3.1 Social Behaviour Observations

Social behaviours were recorded in April until the beginning of May 2019, while the animals were housed in the barn. For identification, animals got collars with a distinct combination of two numbers. Herd conspecifics who did not participate in the study and had no collar for identification were recorded as “0”. The same person, who marked all experimental animals, also conducted all observations (author of this thesis). Because she also had to select the participating animals from a list of all animals, she was only partly blinded. She did not use any treatment marker throughout data recording, but she remembered treatment in some (about one third) of the animals. Initiated and received social interactions as well as some non-social behaviours of the experimental animals were recorded using focal animal sampling and continuous recording of behaviour (Martin & Bateson 2011). Behaviour parameters included socio-positive behaviours, aggressive behaviours and subordinate behaviours as well as non-social behaviours indicative of good or poor welfare (Table 2, 3). Furthermore, distances (in meters) to the first and second nearest neighbouring animal and their identity were recorded per instantaneous scan sampling, but the data was not analysed within the scope of this thesis.

Observations were conducted as follows: One experimental animal was observed for a period of 10 min, then for all experimental animals in the herd the two nearest neighbours were assessed and afterwards continuous recording for the next focal experimental animal started, until each experimental animal of a group had been observed for a total of 5 h. Then the next group was observed. Observations of one group took several days, depending on the number of experimental animals in the group. The observation order of the animals within each group remained stable over each day but was balanced between days, so that every observation day started with a different animal. Most of the time the person who conducted observations was standing indoors. Animals being outside were visible from indoors due to transparent panelling and if not, time was stopped until the person followed the animal by walking behind the building to the outdoor area. The missing time was added to the end of the observation interval. All recordings were handwritten and transferred to an excel sheet afterwards.

The observation time span included periods of high competition (feeding time) and typical periods for socio-positive interactions. Usually observations started right after concentrate feeding in the morning between 7:30 and 8:00 am and lasted until 16.00 pm in the afternoon with an approximate 1 h midday break in between. Sometimes the observation had to be cancelled earlier or had longer interruption in between due to general stable and herd management. Additionally, observations were interrupted as soon as 10 % of the animals in the group laid down, because cattle’s natural ruminating and resting phases reduced chances of the appearance of social interactions. A regular observation included two periods of high competition phases per day, triggered in the morning by concentrate feeding as well as moving remaining food into position and silage feeding in the afternoon.

Table 2 Recorded behaviours and their definitions. I, Initiator; R, receiver; D, duration; F, frequency

Non-social behaviours	
Tongue rolling (D, F)	Repeatedly contorting, rolling or stretching the tongue, without feeding or drinking, i.e. in the air
Bar biting (D, F)	Repeatedly chewing on rails with mouth
Cross sucking (D, F)	Abnormal oral behaviour, I non-nutritive sucking on any body part of R
Solitary Play (D, F)	
Locomotion play	Comprising locomotor play (i.e. gallop, leap, jump, buck, turn) according to Jensen et al. (2000)
Object play	Comprising object play (i.e. butting bars in a playful manner), according to Jensen et al. (2000)
Socio-positive behaviours	
Licking (D, F)	I licking at the body of R except the ano-genital region
Licking invitation (F)	I approaching R with stretched lowered head
Head play (D, F)	I leaning with forehead on forehead of R both rubbing forehead against each other, sometimes lateral rubbing and pressing head against the head or neck of the other animal.
Head rubbing (D, F)	I moving its head repeatedly along the body of R in a friendly manner (not exclusively with horns, not frontal with forehead, no pressure, apart from head and neck area to distinguish from head play) and no withdrawal reaction
Aggressive behaviours	
Pushing (F)	I butts R 1-2 times with head, resulting in R moving away or changing the position, also strong rubbing with forehead (horns) at any part of the body except the head leading to displacement
Pushing strong (F)	I butts R very strongly more than two times directly after one another with head or I pushing with running into R and R moving away or changing position.
Pushing gentle (F)	I butts or pushes R once gently with head, R moving away or changing position
Threatening (F)	I adopting a typical threat posture (presenting the forehead) front-nose-line builds a 90°angle or lower to the back line of the animal) without touching R, and R moving away or changing the position
Threat no success (F)	I using threat posture against R but R does not move away
Threat or Pushing (F)	Displacing but uncertain if body contact is involved (pushing) or not (threatening)
Butting (F)	I butting R with its head, but R staying in place
Shoving (F)	I displacing R by using its body (whole body or body parts)
Fighting (F)	I standing in front of R and both animals putting their foreheads against each other with forcefully mutual pushing, always ends with a winner and looser (often the winner is chasing the looser in the end over a short distance)
Chasing (F)	I displacing R and running after R for at least 2 m
Subordinate behaviours	
Submissive gesture (F)	I lowering head in an outstretched way in response to R's action (e.g. R moving to, threatening, pushing I)
Avoiding (F)	Evading another animal being closer than 2m away without visible precursory threat
Other social interactions	
Sniffing (F)	I muzzle is approaching and eventually touching the recipient's body
Naso-nasal contact (F)	Noses of both individuals are touching each other
Head lay against (F)	I laying forehead on R
Head lay on (F)	I laying head with mandible down on R
Mounting (F)	I jump up or try to jumps up on the backside of R with upper part of the body, forelegs holding the position by being pressed at body

Table 3 Recorded locations and their definitions. I, Initiator; R, receiver; D, duration; F, frequency

Location (modifier for aggressive social behaviour)	
Feed rack	R standing at feed rack (head through headlock) OR standing in front of the feed rack during attack and I going to same feeding place afterwards
Cubicle	R standing (with at least 2 legs) in the cubicle
Cubicle laying	R laying in the cubicle
Walking area between cubicles	R standing in walking area between the cubicles or outdoors
Walking area laying	R laying in walking area between the cubicles or outdoors
Drinker	R drinking or standing close to the drinker and I is drinking at same position after displacing R
Brush	R using the brushes outside or standing next to it
Mineral bucket	R standing at the mineral bucket or directly next to it
Out of sight	Focal animal is out of sight

2.3.2 Social isolation and social reinstatement test

On four different days in May and June 2019 all 18 experimental animals participated once in a test. During that time, animals were pastured in two different herds on two distinct pasture sites as mentioned above. The test setting was located close to pastures and comprised two asymmetrical pens connected with each other by a 12.65 m long alley (Fig. 2, 3). The left-sided pen was used as isolation pen and the right-sided pen as waiting pen. Both pens were fenced with metal horizontal bars, so that the animal could observe its surroundings. The top metal bar of fences in the isolation pen were marked with red tape in 1 m intervals to create a grid for data recording (Fig. 3). Between the isolation pen and the alley was a door, which could be opened with the help of a lever mechanism from outside of the alley. The alley comprised a scale and an examination sector, because usually the setting was used for medical examinations and weighting procedures during summer. Due to the combination of different sections, the alley ground was uneven. The ground was made of metal (scale) and concrete. Animals had to step over three small steps (e.g. scale platform) to reach the end of the alley. All animals were familiar with the test setting, but heifers had more experiences because they were pastured for the second year, while calves had gone through the scale at least once before the test. A familiar environment was chosen to test for behavioural reactions in animals that emerge only due the spatial isolation from its peer and not because of other factors.

The test comprises a 5 min isolation phase in the isolation pen (Fig. 2) that was followed by a 5 min reunion phase. For testing, a group of seven animals was separated from the herd on pasture and was slowly and in a calm manner moved into the waiting pen. Because of limited space availability in the waiting pen, to avoid stress and injuries, a group of seven animals was chosen instead of taking the whole herd. Out of this group, one experimental animal was moved through the alley to the isolation pen. As soon as the animal was in the isolation pen, the door was closed by an experimenter and the isolation phase started. After 5 min had passed, the door was opened again by the same person and the experimental animal were free to walk to the waiting area for the next 5 min (reunion phase). As soon as either the animal reached the end of the alley or the available 5 min were over, the test ended. In the reunion phase the door from the isolation pen to the alley was opened and the experimental animal was free to go through the alley back close to the waiting pen, where the remaining six herd members were located. The waiting pen door remained closed during testing to avoid that waiting animals go through the alley. Thereby, tested animals in the reunion phase could not go into the waiting pen but could walk close to it until only a fence separated the animal from its peers.

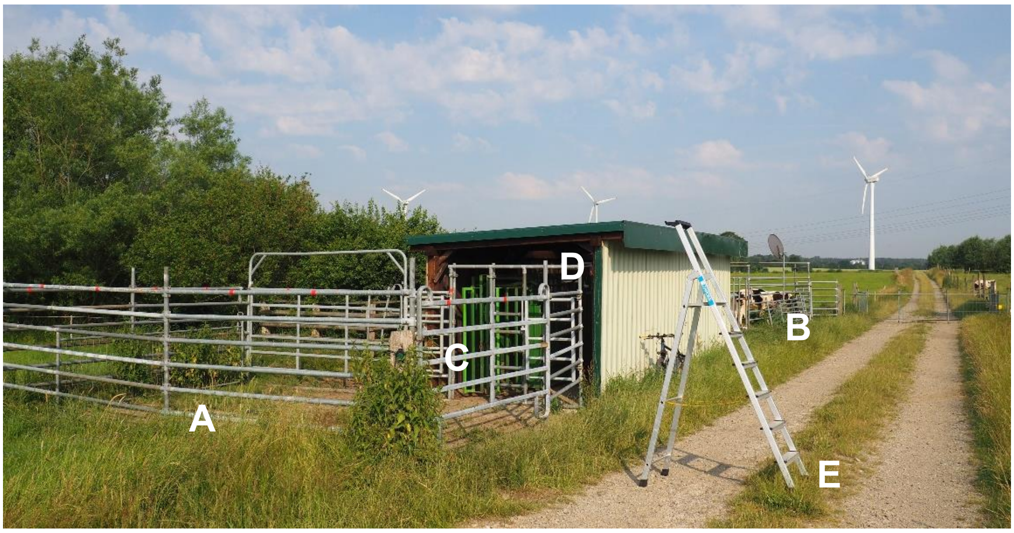

Fig. 2. Test setting of the social isolation and reinstatement test with (A) isolation pen, (B) waiting pen, (C) door to (D) the alley in the shed and (E) leader.

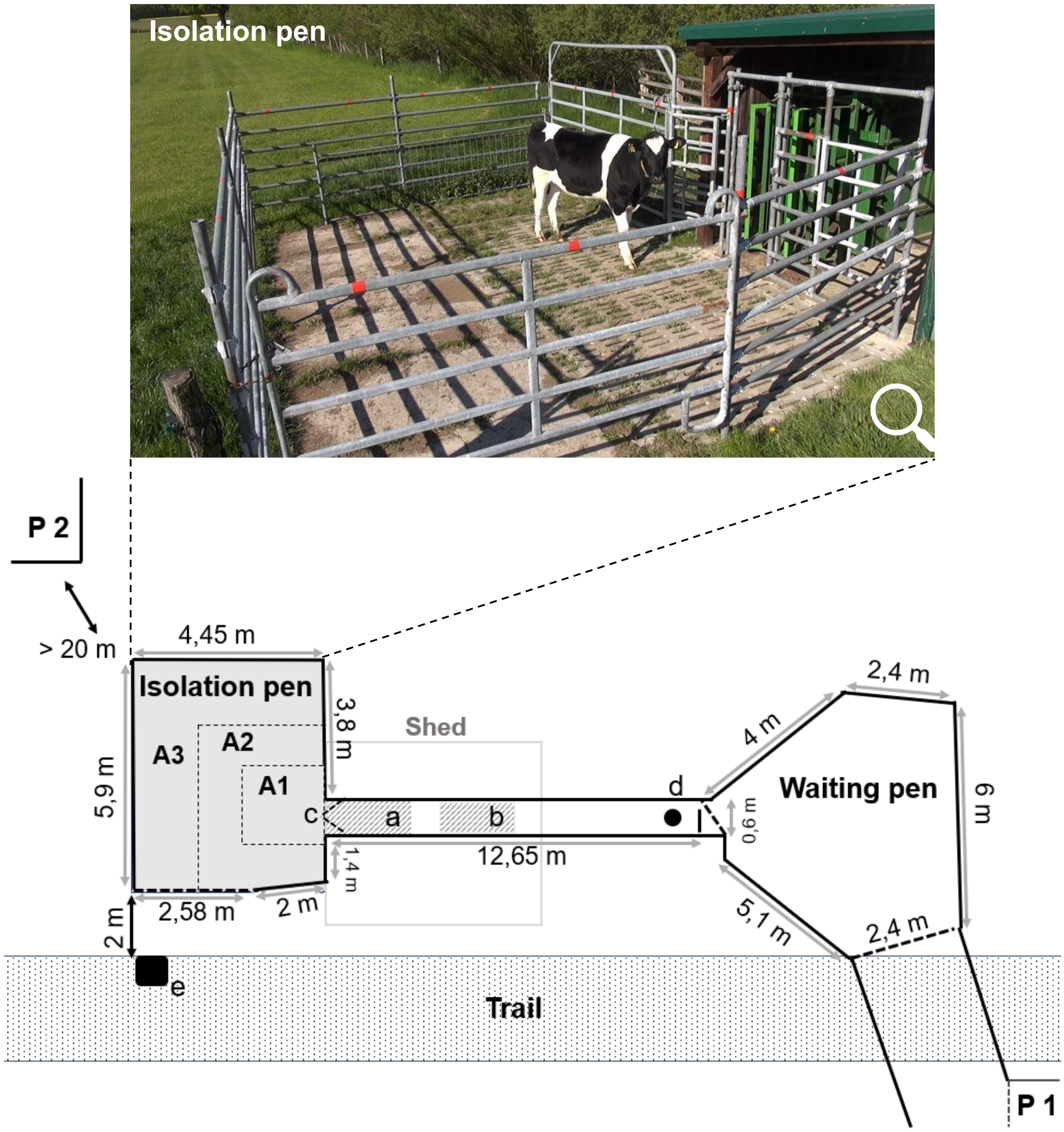

Fig. 3. Sketch of the social isolation test setting with pastures of experimental heifers (P1) and calves (P2). Test setting included a waiting pen and isolation pen connected by an alley, that included one (a) examination sector, (b) scale and a door (c) Cameras were installed (d) at the alley end and (e) held in position by an experimenter, who stood on a leader. For analysis the isolation pen was marked with red tape (see close-up) to subdivide it in three areas (A1, A2, A3).

Always the same three experimenters executed the testing of the animals, one of these was only helping to move the animals, one was additionally closing and opening the doors as well as taking some direct measures (see below), the last one was moving the animals as well as filming the behaviour of the tested animal with a camcorder by standing stationary on a ladder 2 m besides the isolation pen (see below). During testing, the experimenters remained silent, stationary and hidden behind the shed, except the filming person or when operating the door. All tests were conducted between 08.30 h and 10.00 h in the morning. The isolation pen floor and the alley were swept after testing, as soon as an animal eliminated.

Number of vocalisations performed by the tested animal were recorded directly, as well as the latency from opening the door until the test animal reached the alley end. All tests were video recorded. One camera (Sony HD, HDR-CX730) was held in position by one experimenter, who stood constantly during testing on a ladder 2 m away from the isolation pen. A second camera (Sony HD, HDR-CX250) was fixated with a self-constructed adapter on the highest barn centrally located in the alley 2 m away from the waiting pen to record behaviours in the alley. Both cameras were out of animal's range.

Behaviours were recorded with the software BORIS (Friard & Gamba 2016) continuously from videos. Behavioural parameters included e.g. head positions, walking and explorative behaviour (Table 4). Furthermore, latencies that could not be assessed directly during testing were recorded, including latency from opening the door until test animal overstepped the door entrance with its two front legs, with its two hind legs and until it stepped on the scale platform (obstacle) with its two front legs. The scale platform was included as obstacle for analysis, because animals could have hesitated to pass it. The red tapes at the fence functioned as a marker to create a grid laid over the isolation pen, that was used to measure the positions of the animal. The four fields (1 m x 1 m) next to the door were subsumed to area 1, the eight middle fields (1 m x 1 m) to area 2 and the eight outer fields (1 m x 1,8 m) to area 3. Because of the non-rectangular shape of the pen and the side positioned camera, which resulted in fainted squares in distant, fields of area 3 were larger. The fence section (2 m long) near the trail was slightly transverse positioned but ignored due to the minimal deviation.

For inter- and intra-observer reliability two videos including 5 min isolation and 5 min reunion phase of two different animals were coded. The occurrence of some behaviours (startle response, escape attempts, elimination, grazing, locomotion and object play) were coded consistently among observers but could not be tested, because they were not or only rarely observed. For intra-observer reliability calculation of the Cohen's Kappa revealed an 'almost perfect agreement' for head normal (0.93), head high (0.83), exploration (0.88), looking at door (0.89), looking at camera (0.90) and a 'substantial agreement' for walking (0.80) and vigilance (0.78) (Landis & Koch 1977). For inter-observer reliability the outcome was an 'almost perfect agreement' for looking at door (0.82), head high (0.80), a 'substantial agreement' for head normal (0.77), exploration (0.77), walking (0.75), vigilance (0.77) and a 'moderate agreement' for looking at camera (0.66) (Landis & Koch 1977).

Table 4 Recorded behaviours and definitions of the social isolation test. D, duration; F, frequency

Individual behaviour	
Looking at door (D, F)	Looking/orienting directly at door when farther away or, when standing below 50 cm in front of the door, orienting at the door within angle of 45° of each side of the medial axis of the head
Looking at camera (D, F)	Looking/orienting directly at camera
Vigilance (D, F)	Head position above horizontal to the withers, ears erected
Head high (D, F)	Head position above horizontal to the withers but without ears erected
Head normal (D, F)	Head position horizontal to or below horizontal to the withers
Exploration (D, F)	Sniffing or licking on floor, door or fence
Walking (D, F)	More than two moving steps with forelegs resulting in a change of position
Vocalisation (F)	Any vocalisation of the animal (measured directly during testing)
Self-grooming (D, F)	Licking, rubbing and scratching the own body
Startle response (F)	Jumping or running away in response to door opening
Startle response undefined (F)	Short wince, jumping or running away in response to an undefined stimulus
Elimination (F)	Defecation and urination
Grazing (D, F)	Eating grass from outside the pen
Escape attempts (F)	jumping against and trying to get over the barrier
Solitary play (D, F)	
Locomotion play	Comprising locomotor play (i.e. gallop, leap, jump, buck, turn; according to Jensen et al. 2000)
Object play	Comprising object play (i.e. butting bars in a playful manner, according to Jensen et al. 2000)

2.3.3 Maternal Behaviour Observations

Permanently installed video cameras in two out of six calving pens were used to record cow's maternal behaviour and behaviour performed by calves of the mother-bonded reared experimental animals. Video data from year 2017 of the three oldest experimental animals were not detectable, thus, videos of only six experimental animals of the contact treatment group and their mothers were available. A camera system was used with time-lapse recording and captured the indoor and outdoor part of the calving pens. For analysis, two pictures per second were used to enable a continuous playback. To investigate behaviours early in calf's live, the first 12 h after birth were analysed. Additionally, 4 h on the third day of calf's' life, when the calf was more active, were examined. For this purpose, a time span was chosen where no or very little disturbances could be found and were activity with a higher likelihood appeared. It turned out that time spans between 11:00 to 13:00 am and 2 h directly after evening milking met the criteria.

Behaviour parameters were focused on interactions between mother and calf such as contact, suckling, proximity and mother cow's orientation towards her calf (Table 5). Sniffing and licking on the video recordings were difficult to distinguish, these two elements were combined. All behaviours were recorded using focal animal sampling and continuous recording of behaviour (Martin and Bateson 2011) with the open-source software BORIS (Friard & Gamba 2016). Coding was performed by one person who was blind by changing video names. Videos were cut down to 24 h segments to simplify the playback, to avoid transmission errors due to high data rate and, so far, it was not possible in BORIS to jump to specific time points that were above 24 h.

It was noticed during coding, that the video time and the timeline from BORIS was not always identical. Either less time on the video timeline has passed (max. 07.31 min in 12 h coding) or more time (max. 04.29 min in 12 h coding) compared to the timeline of BORIS. For example, the timeline of BORIS reached 12 h, while the timeline on the actual video ended by 11.52 h. Due to the low degree of deviation (<10 min), this anomaly was ignored during analysis but discussed.

For intra-observer reliability 10 different video samplings each 30 minutes long from 5 different other animals were observed twice from the same observer. Some behaviours and conditions (disturbance, out of sight, outdoors alone, solitary play, head play, covering up) were not or only rarely observed and could not be included in the calculation. Calculation of the Cohen's Kappa revealed an 'almost perfect agreement' for within body length (0.91), above body length (0.85), head directly directed to calf (0.82), head directed to calf (0.87), head away from calf (0.88), head lock (0.99), contact (0.91) (Landis & Koch 1977).

Table 5 Recorded behaviours and definitions for maternal contact observations. D, duration; F, frequency

Individual Behaviour	
Standing (D)	Animal is in upright position, elevated on all four legs
Lying (D)	Animal's body is on the ground, legs bearing no weight
Solitary Play (D)	Animal is performing locomotor play (i.e. gallop, leap, jump, buck, turn) or object play (e. g. butting bars in a playful manner) according to Jensen et al. (2000)
Disturbance (D)	Keeper intervene e.g. perform medical inspection, bottle-feed calf and/or mother cow's head is fixated in the head lock so that she cannot walk away/change her position
Out of sight (D)	Animal is not visible e.g. in outside area or behind other animal
Head lock (D)	Mother cow's head is in the head lock while head lock is not closed, she is eating and can move freely
Outdoors alone (D)	Cow (or calf) is outside (with hindlegs over border) while calf (or cow) is indoors with all body parts
No outdoor access (D)	The door to the outdoor run is closed
Contact behaviour	
Contact (D)	Muzzle of the cow (or calf) in close proximity (< 10cm) to (any part of) the calf's (or mother's) body -including licking, sniffing, nudging
Suckling (D)	Calf standing antiparallel to mother cow with head under mother cow's body (>10 sec)
Potentially suckling (D)	Calf standing behind mother cow's body in a potential suckling position but is covered by its mother, suckling not visible
Head play (D)	Animals leaning with foreheads on each other both rubbing forehead against each other, sometimes lateral rubbing and pressing head against the head or neck of the other animal.
Covering up calf (F)	Mother cow is covering up her calf with straw
Orientation	Only when mother cow is lying, neglecting short side movements (e.g. head throw to flick flies)
Head directly directed to calf (D)	Mother cow's head is directed directly towards her calf, calf is positioned within an angle of 45° to each side of the medial axis of the head.
Head directed to calf (D)	Mother cow's head is directed towards her calf, calf is positioned in an area more than 45° up to 90° angle from the medial axis of the cow's head
Head directed away (D)	Mother cow's head is directed away from her calf, calf is positioned 180°-260° from midpoint of mother cow's head, between her ears
Proximity	
Within body length (D)	Head of the mother cow is positioned to calf within her own body length
Above body length (D)	Head of the mother cow is positioned to calf above her own body length

2.4 Statistical analysis

Statistical analyses were carried out in SPSS (version 25, IBM SPSS. year 2017). All data were analysed descriptively with the help of graphic representations (box plots/scatter plots). The initiated and received behaviours in the social behaviour observations were analysed separately and the main variables were the frequency and duration of socio-positive and the frequency of aggressive as well as subordinate behaviours. Further, the relative weight of experimental animals in the group was determined for analysis by subtracting the individual weight from the average group weight, because weight might have an influence on social behaviour. For the social isolation and reinstatement test, the isolation phase and reunion phase were analysed separately. Duration of looking at door was the main variable. Further the duration of positioned in area 1, vigilance, exploration, walking and the frequency of vigilance and vocalisation were tested for the isolation phase. In the reunion phase main variables were latencies, as well as the duration of exploration, vigilance, walking and the frequency of vigilance were evaluated. No hypothesis was formulated for self-grooming, the data was therefore in the first instance analysed only descriptively. A conspicuous difference between treatments was then investigated statistically.

ANOVA calculation was performed for all behaviours that were of interest for our prediction and that occurred in sufficient frequency to allow for analysis. For social behaviour observations the ANOVA model were conducted with treatment (Contact/Automat), group (Heifer 1/Heifer 2/Calves 1/Calves 2) and their interaction as fixed effects, as well as age and weight as covariate. For the social isolation and reinstatement test ANOVA model were conducted with treatment (Contact/Automat), group (Pasture 1/Pasture 2) and their interaction as fixed effects, as well as absolute age and weight as covariate. For the reunion phase the total test time was added as covariate for variables except latencies, because it varied between animals. To test model assumptions, residuals were checked for normality using a Shapiro-Wilk test and visually for homogeneity of variance. The models were not reduced but full models tested. In the results section all ANOVA results regarding the treatment and all other significant factors are reported, non-significant factors are not reported.

Maternal contact observations were analysed primarily descriptively. The distribution of lying orientation directly directed to calf (calf is positioned within an angle of 90° around the midpoint of cow's head) and directed to calf (calf is positioned in an area more than 45° up to 90° angle from the medial axis of the cow's head) were consistent within individuals, e.g. Cow 1 who often lied directly directed to calf, also lied often directed to calf. Due to this result, the data from lying directly directed to calf and directed to calf was summarized, so that results are presented in only one variable (Cow lying towards calf). To test for associations between maternal behaviour after calving and socio-positive interactions of experimental animals in the herd preliminarily, Spearman rank correlation coefficients were calculated despite the small sample size (n = 6) with the total duration of maternal contact, i.e. the sum of duration of maternal contact within the first 12 h after calving and during 4 h of the 3rd day.

3 Results

3.1 Social behaviour observations

Regarding initiated social behaviours, 332 events of socio-positive behaviours per 90 h, 242 events of aggressive behaviours and 138 events of subordinate behaviours were observed. For the received social behaviours, 254 events of socio-positive interaction per 90 h, 258 events of aggressive behaviours and 105 events of subordinate behaviours were observed. One animal of the *Contact* group performed tongue rolling (14 events/5 h) and one *Automat* animal performed bar biting (5 events/5 h). Cross-suckling was not observed.

Contact animals (n = 9) showed more subordinate behaviours than artificially reared animals (Fig. 4, Table 6). The two treatments did not differ in the frequency and duration of initiated socio-positive behaviour or initiated aggressive social interactions; yet a significant interaction between treatment and group was found for frequency of socio-positive behaviour. Subordinate behaviour tended to differ between groups with individuals of group C1 displaying it most rarely (Table 6, Annex 1, Fig. A1.1). The relative weight affected initiated subordinate behaviour (regression coefficient: -0.259) and initiated aggressive behaviour (regression coefficient: 0.333) (Table 6).

Table 6 ANOVA results for social interactions (F, frequency; D, duration) of animals reared with cow-calf contact (*Contact* n = 9) and animals reared artificially (*Automat* n = 9) during 5 h observation. Only treatment and significant (p < 0.05) other factors are reported (treatment x group (treat. x gr.), relative weight (rel. weight), group (C1 = Calves 1, C2 = Calves 2, H1 = Heifers 1, H2 = Heifers 2)). SE, standard error; df, degrees of freedom for group, for cases per animal; n², eta-squared effect size.

		Estimated mean ± SE		ANOVA			
Initiated behaviour		Contact	Automat	F	df	P	η²
Socio-positive behaviour, F	treatment	18 ± 3.29	17.39 ± 3.48	0.00	1,33	0.951	0.001
	treat. x gr.			4.27	3,90	**0.039**	0.587
	C1	25.08 ± 6.25	4.45 ± 4.45				
	C2	11.36 ± 8.50	21.54 ± 9.11				
	H1	21.58 ± 4.25	35.93 ± 4.91				
	H2	13.99 ± 6.26	7.16 ± 8.71				
Socio-positive behaviour, D	treatment	111.15 ± 74.86	113.27 ± 79.19	0.00	1,10	0.974	0.000
Aggressive behaviour, F	treatment	10.77 ± 3.92	13.49 ± 4.15	0.50	1,77	0.500	0.061
	rel. weight			11.18	1,90	**0.009**	0.554
Subordinate behaviour, F	treatment	11.52 ± 1.50	4.40 ± 1.59	10.31	1,45	**0.028**	0.696
	rel. weight			46.19	1,90	**0.000**	0.837
	group			9.01	3,28	**0.056**	0.903

Continuing Table 6.

		Estimated mean ± SE		ANOVA			
Received behaviour		Contact	Automat	F	df	P	η²
Socio-positive behaviour, F	treatment	14.931 ± 2.32	14.79 ± 2.46	0.00	1,25	0.980	0.000
Socio-positive behaviour, D	treatment	65.03 ± 30.36	123.50 ± 32.12	1.58	1,43	0.221	0.161
Aggressive behaviour, F	treatment	12.25 ± 3.17	14.76 ± 3.35	0.10	1,34	0.768	0.029

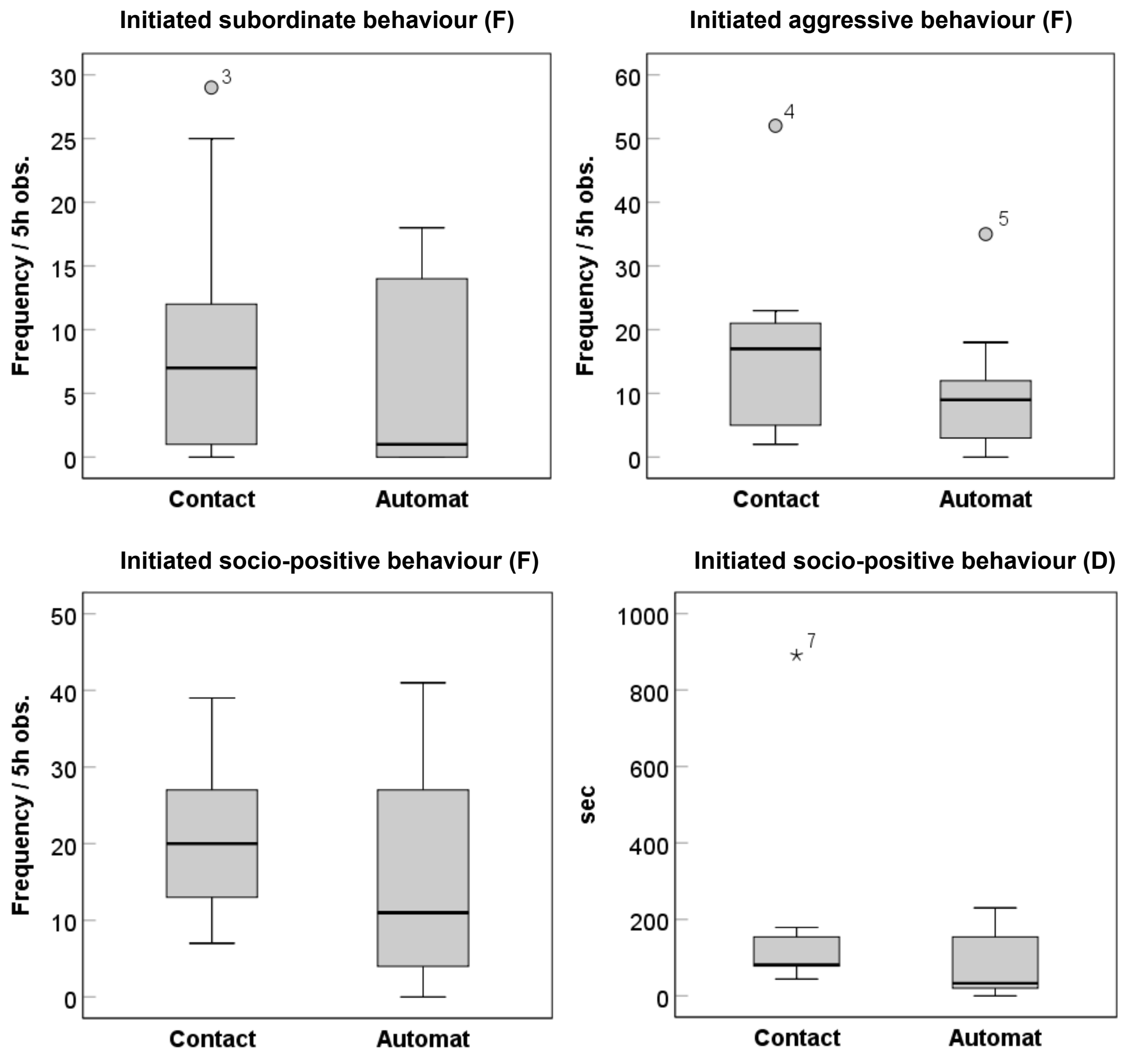

Fig. 4. Initiated social behaviours of experimental animals during 5 h observations. Boxplots show F, frequencies of initiated subordinate behaviour, aggressive behaviour, socio-positive behaviour and D, duration of initiated socio-positive behaviour of the different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

Regarding behaviours experimental animals received during observation, no treatment effect was observed for the frequency of socio-positive behaviours, duration of socio-positive behaviours and aggressive behaviours (Fig. 5, Table 6). The relative weight and group had no influence on received social behaviours (Table 6).

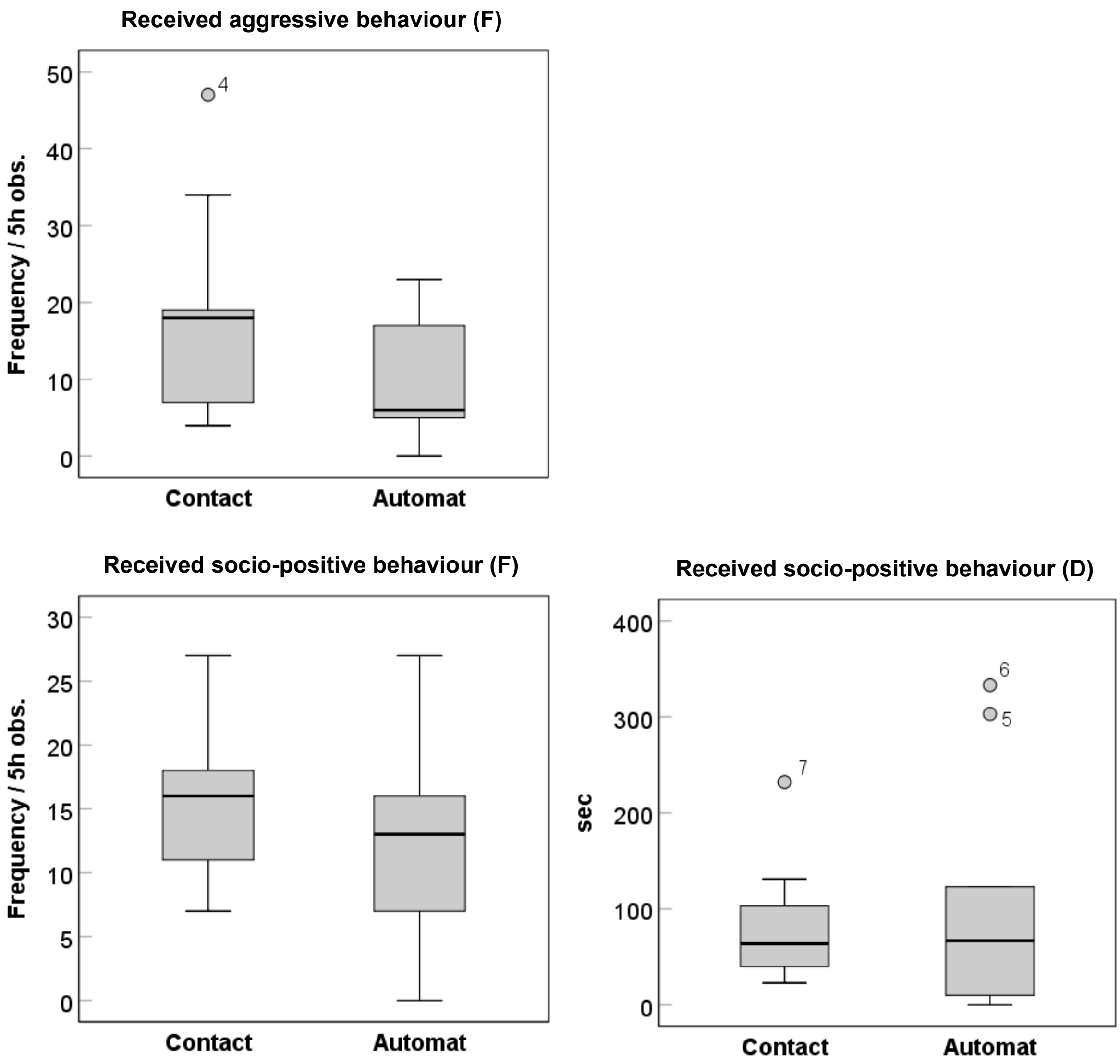

Fig. 5. Received social behaviours of experimental animals during 5 h observations. Boxplots show F, frequencies of received aggressive behaviour (top) and socio-positive behaviours (left) and D, duration of received socio-positive behaviours (right) of the different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

3.2 Social isolation and reinstatement test

Flight attempts and any play behaviour were not observed in both treatment groups during the social isolation and reinstatement test. Only one event of startle response in response to the door opening was observed in one artificially reared animal. During the 5 min social isolation, mother-bonded reared animals looked longer to the closed door that leads to the peers (Fig. 6, Table 7), tended to spend more time closer (Area 1) (Fig. 6, Table 7) to the door and showed more events of vigilant behaviour than artificial reared animals (Fig. 7, Table 7). No treatment effects were found for the duration of vigilance, exploration and walking, as well as for vocalisation events (Fig. 7, Table 7). Regarding age, weight and group affiliation (Pasture 1/Pasture 2) no significant differences were found. A treatment difference for self-grooming was visible while comparing the boxplots descriptively. After testing, results represented that mother-bonded reared animals performed less self-grooming compared to *Automat* animals (Fig. 7, Table 7).

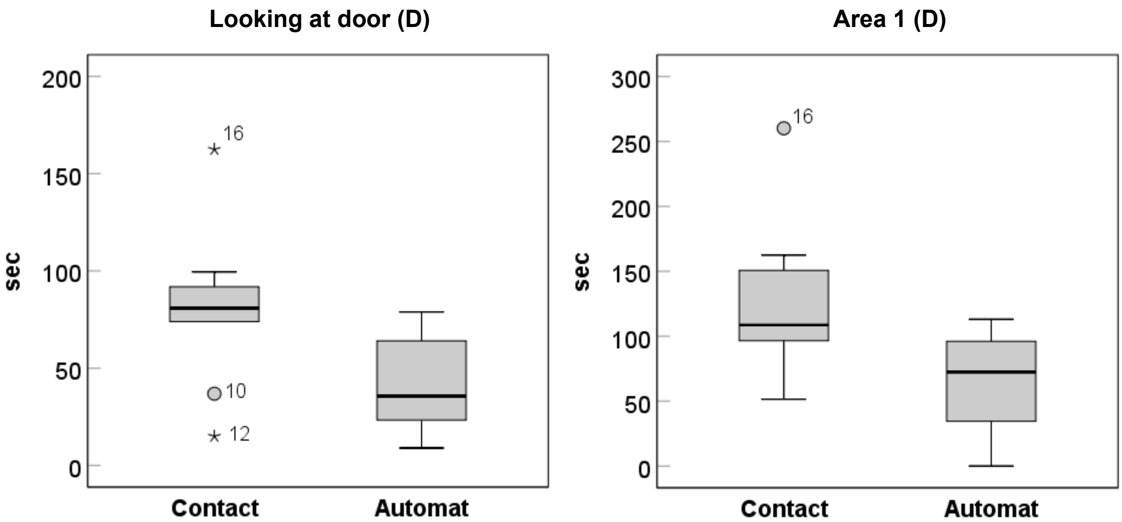

Fig. 6. Individual behaviour observed in 5 min Isolation phase. Boxplots show durations (D) of looking at door and staying in area 1 of the different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

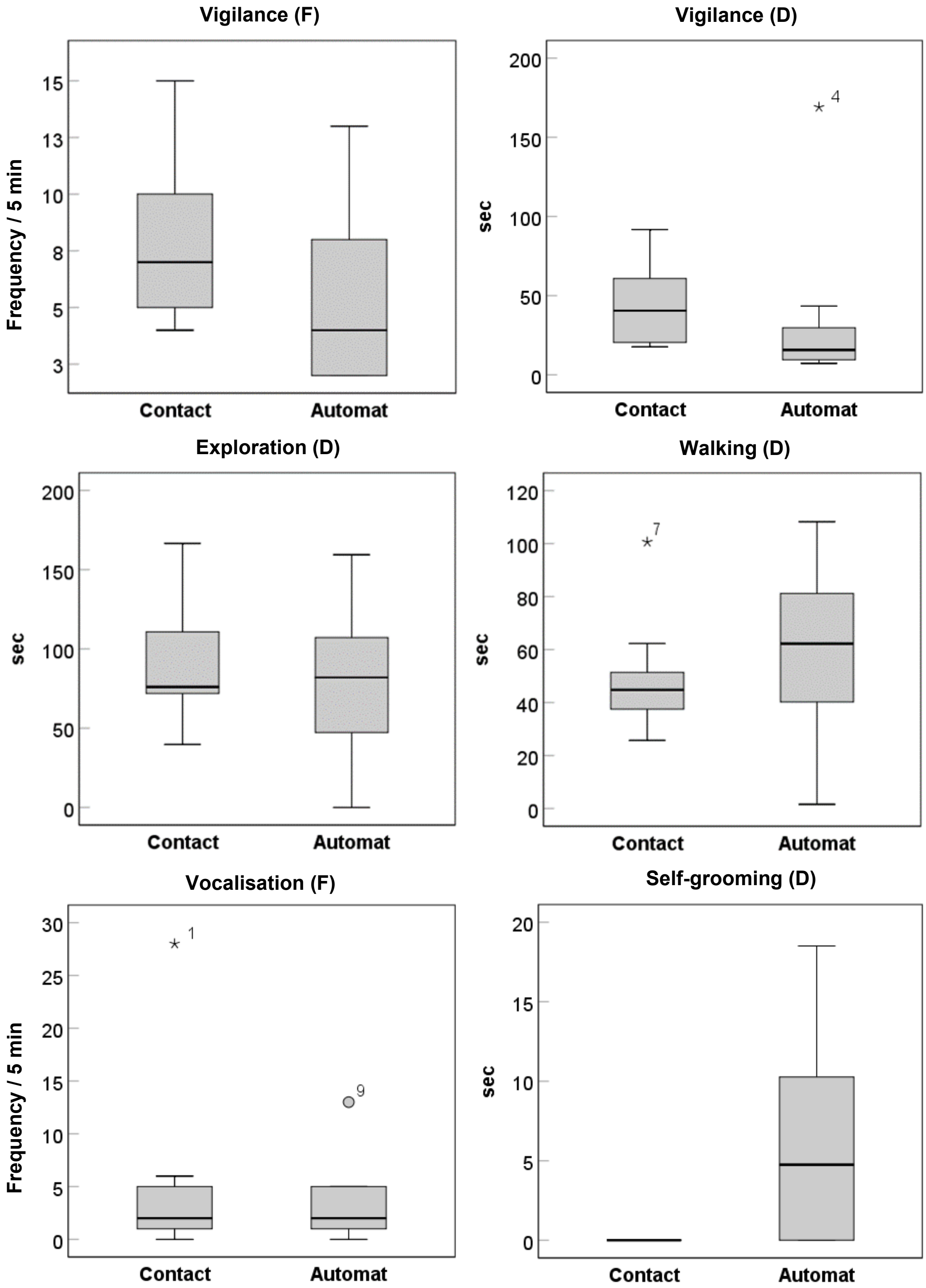

Fig. 7. Individual behaviour observed in 5 min Isolation phase. Boxplots show the frequency (F) of vigilant behaviour, vocalisation and duration (D) of vigilance behaviour, exploration and walking of the different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

Table 7 ANOVA results for behaviour (F, frequency; D, duration) of experimental animals (*Contact* n = 9, *Automat* n = 9) during social isolation test with estimated mean ± standard error. All treatment effects and only significant ($P < 0.05$) results for other factors (treatment x group, age, group, weight, latency alley end) are reported. SE, standard error; df, degrees of freedom for group, for cases per animal; n^2, eta-squared effect size.

		Estimated mean ± SE		ANOVA			
Isolations Phase		Contact	Automat	F	df	P	η²
Looking at door (D)	treatment	72.20 ± 12.31	52.55 ± 11.92	8.04	1,12	**0.014**	0.388
	weight			4.00	1,12	0.069	0.250
Area1 (D)	treatment	111.91 ± 19.50	78.07 ± 18.89	5.47	1,12	0.072	0.550
Vigilance (F)	treatment	9.42 ± 1.51	5.06 ± 1.46	25.72	1,12	**0.000**	0.666
Vigilance (D)	treatment	44.7 ± 16.52	33.92 ± 16.00	0.30	1,10	0.651	0.156
Exploration (D)	treatment	86.07 ± 15.00	82.08 ± 14.53	0.03	1,12	0.891	0.021
Walking (D)	treatment	53.96 ± 9.37	65.49 ± 9.07	0.13	1,12	0.781	0.107
	treatment x group			6.57	1,12	**0.025**	0.354
	Pasture 1	32.25 ± 16.51	75.91 ± 18.68				
	Pasture 2	75.67 ± 29.37	55.07 ± 21.43				
Vocalisation (F)	treatment	7.29 ± 2.48	2.55 ± 2.40	0.48	1,12	0.608	0.305
	treatment x group			4.13	1,12	0.065	0.256
Self-grooming (D)	treatment	1.00 ± 1.80	5.66 ± 1.75	10.62	1,12	**0.037**	0.751
Reunion Phase							
Exploration (D)	treatment	49.08 ± 9.60	31.86 ± 9.28	2.82	1,11	0.261	0.632
	group			8.92	1,11	**0.011**	0.426
	latency alley end			8.50	1,11	**0.014**	0.436
Vigilance (F)	treatment	3.91 ± 1.84	1.23 ± 2.18	0.04	1,16	0.873	0.021
Vigilance (D)	treatment	8.32 ± 21.831	29.18 ± 21.11	0.78	1,11	0.489	0.325
Latency front legs	treatment	57.81 ± 32.46	140.54 ± 31.44	0.71	1,12	0.548	0.397
	treatment x group			4.98	1,12	**0.046**	0.293
	Pasture 1	199.80 ± 57.20	185.62 ± 64.73				
	Pasture 2	-84.17 ± 70.60	95.46 ± 74.25				
Latency hind legs	treatment	140.64 ± 40.86	171.83 ± 39.58	0.89	1,12	0.410	0.212
	age			4.86	1,12	**0.048**	0.288
	weight			4.23	1,12	0.062	0.261
Latency obstacle	treatment	159.33 ± 40.48	180.22 ± 39.21	0.31	1,12	0.626	0.117
	age			4.67	1,12	**0.052**	0.280
	weight			0.31	1,12	**0.048**	0.287
Latency alley end	treatment	173.43 ± 39.18	187.87 ± 38.05	0.13	1,12	0.755	0.062
	age			4.00	1,12	0.069	0.250
	weight			4.52	1,12	**0.055**	0.274

In reunion phase, 11 out of 18 (*Contact* = 5, *Automat* = 6) experimental animals were able to reach the end of the alley to meet their peers. Only one artificially reared animal performed locomotion play (1 event/5 min). The rearing treatment did not affect animals' latency of reaching the alley end, passing the obstacle and passing the front door with front legs and hind legs (Table 7, Annex 2, Fig. A2.1). Further, no treatment effect could be found for the frequency of exploration and frequency and duration of vigilance behaviour (Table 7). A group effect was found for calves from pasture 2 explored longer than heifers from pasture 1 (Table 7). The latency until animals reached the alley end influenced the duration of exploration (Table 7). Age influenced animals' latency to pass the door with their hind legs with younger animals being quicker (regression coefficient: -2.433), as well as weight influenced the latency to pass the obstacle with heavier being quicker (regression coefficient: 3.020) (Table 7).

3.3 Maternal behaviour observations

In the first 12 h after calving, experimental animals of the *Contact* group (n = 6) received close maternal contact (e.g. licking, sniffing) for 1.25 h up to 2.21 h (Fig. 8). Therefore, cows spent 10.4 % - 18.4 % of the first 12 h after calving with licking or sniffing the calf. The highest amount of maternal contact was initiated by *cow 15*, the shortest by *cow 11.* The first sniffing or licking contact between cows and calves occurred within 0.08 min up to 8.02 min after birth. Three out of six cows ate the placenta and three cows were observed to cover up her lying calf with straw (*cow 10* = 9 events/12 h, *cow 11* and *15* = 1 event/12 h).

On the third day of calf's life, the observed maternal contact during 4 h initiated by *cow 1, 7* and *11* with a maximum of 5 min was relatively low, while *cows 2, 10* and *15* spent 13 min up to 19 min sniffing or licking their calves (Fig. 8). During the third day cows spend 1.3 % - 4.8 % of their time during the 4 h observation period with sniffing and licking their calf. Again, the calf of *cow 15* received the longest duration of maternal contact.

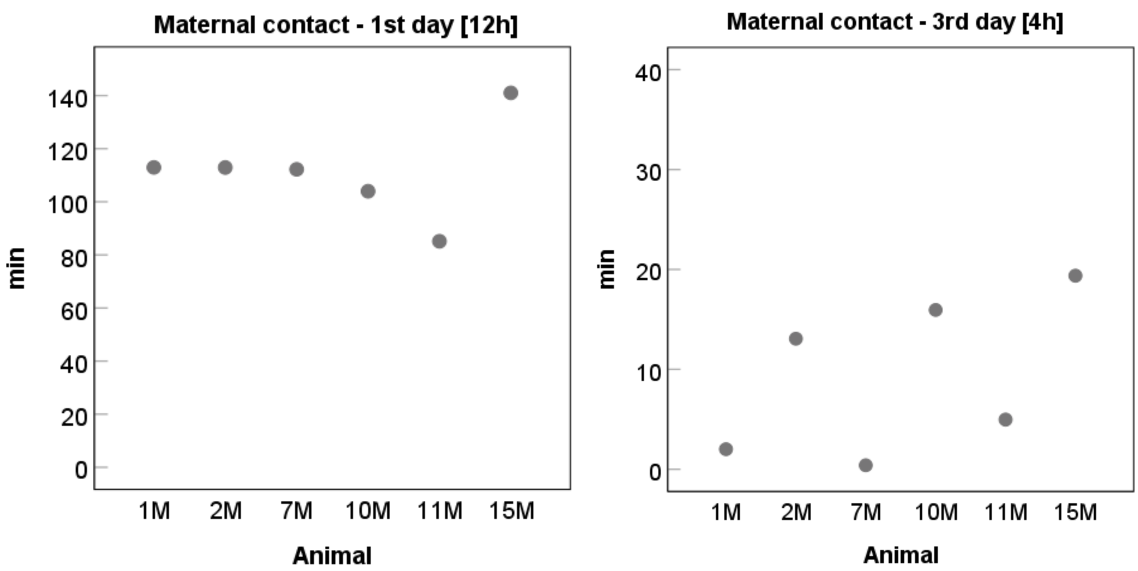

Fig. 8. Duration of sniffing and licking (maternal contact) mothers of calves from the contact treatment group (n = 6) performed in the first 12 h after calving (left side) and during 4 h on the third day of calf's life (2 h at midday and 2 h after evening milking) (right side). Note different scales.

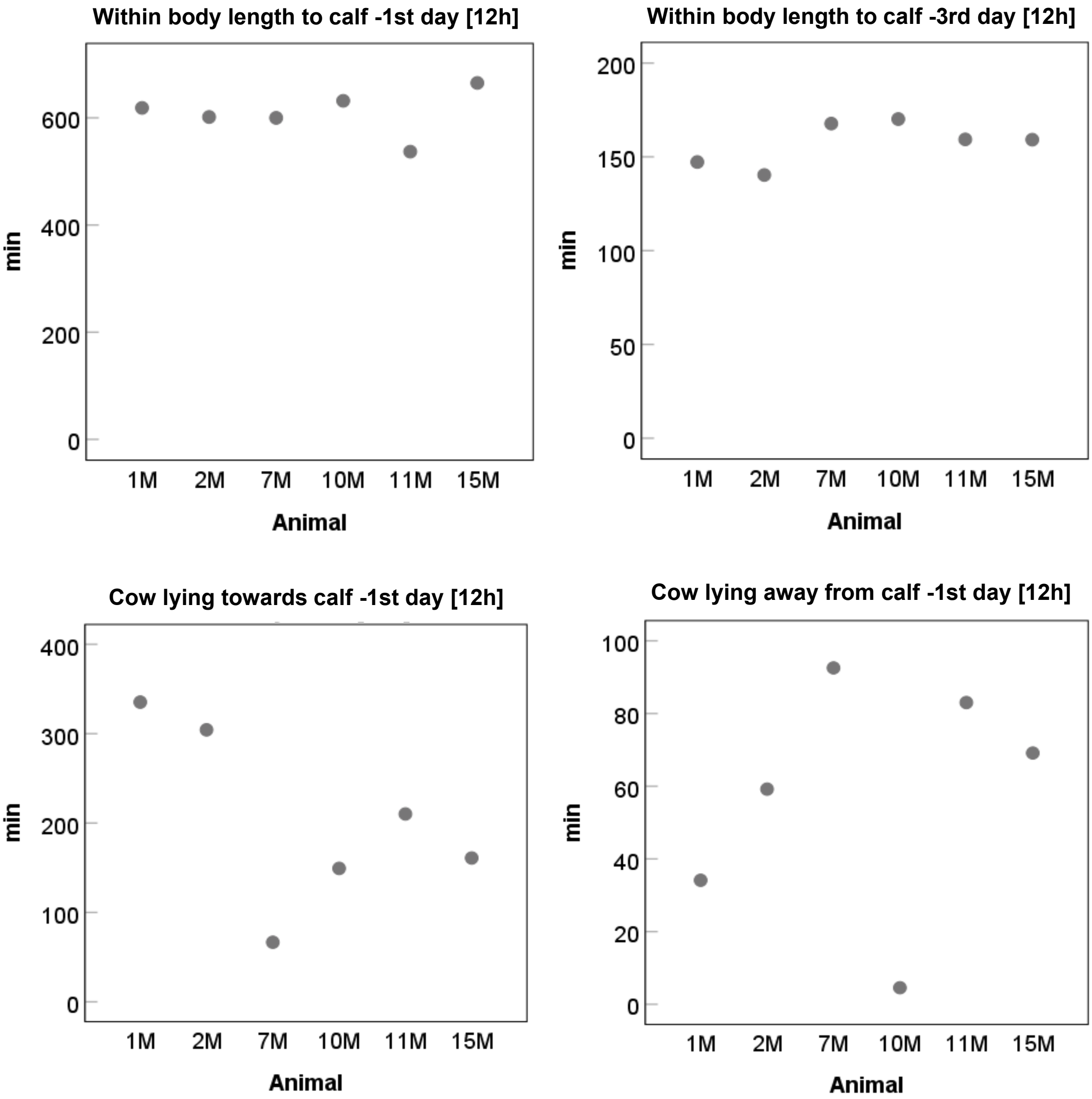

Fig. 9. On the top, duration of mother cow's proximity to calves of the contact treatment group (n = 6) in the first 12 h after calving (left) and during 4 h on the third day of calf's life (right). Below, duration of cow lying directly towards and towards the calf (left) and away from calf (right) in the first 12 h after calving.

Most of the time (74.6 % - 92.3 %) the calf was within its mother's body length on the first day after calving; the longest duration was observed for *cow 15* (11.08 h). On the third day cows spent 58.5 % up to 70.9 % of the 4 h observation in such close proximity to the calf (within body length) (Fig. 9).

In the first 12 h after calving, total lying duration of the cow ranged from 2.34 h to 6.09 h. Cows were orientated 1.07 h up to 5.58 h of their lying time directed towards the calf, therefore cows spend 41.9 % up to 97 % of their total lying time in orientation towards its calf. *Cow 7* was the only cow, who spent more time lying away than towards the calf. The shortest measured duration of lying away from the calf was measured for *cow 10* (0.05 h), who also had the shortest duration of total lying (2.34 h) on the first day (Fig. 9).

Regarding the development of the calves in the first days of life, observation revealed that four calves suckled within 12 h, they needed 59 min up to 185 min for the first successful suckling. *Calf 10* did not suckle within 12 h and *calf 11* only with help (guiding to teat several times) after 6.59 h (Fig. 10). All calves born during the day got colostrum by a caretaker within 3 h, the other calves (*calf 1, 7*), got it on the next day, 4.56 h and 6.20 h after birth. The same three animals, who suckled for a reasonable amount of time in the first 12 h after calving (*calf 1, 7, 15*), also performed playing behaviour within the first day. Additionally, these calves spent a higher amount of time playing on the third day, compared to the other three calves. *Calf 15* who received the most maternal contact by its mother, *cow 15*, engaged the most time in solitary play on the first day. *Calf 11,* who received less maternal contact compared to the other calves, did not show any play behaviour and suckling duration was relatively short, even on the third day of life (Fig. 10).

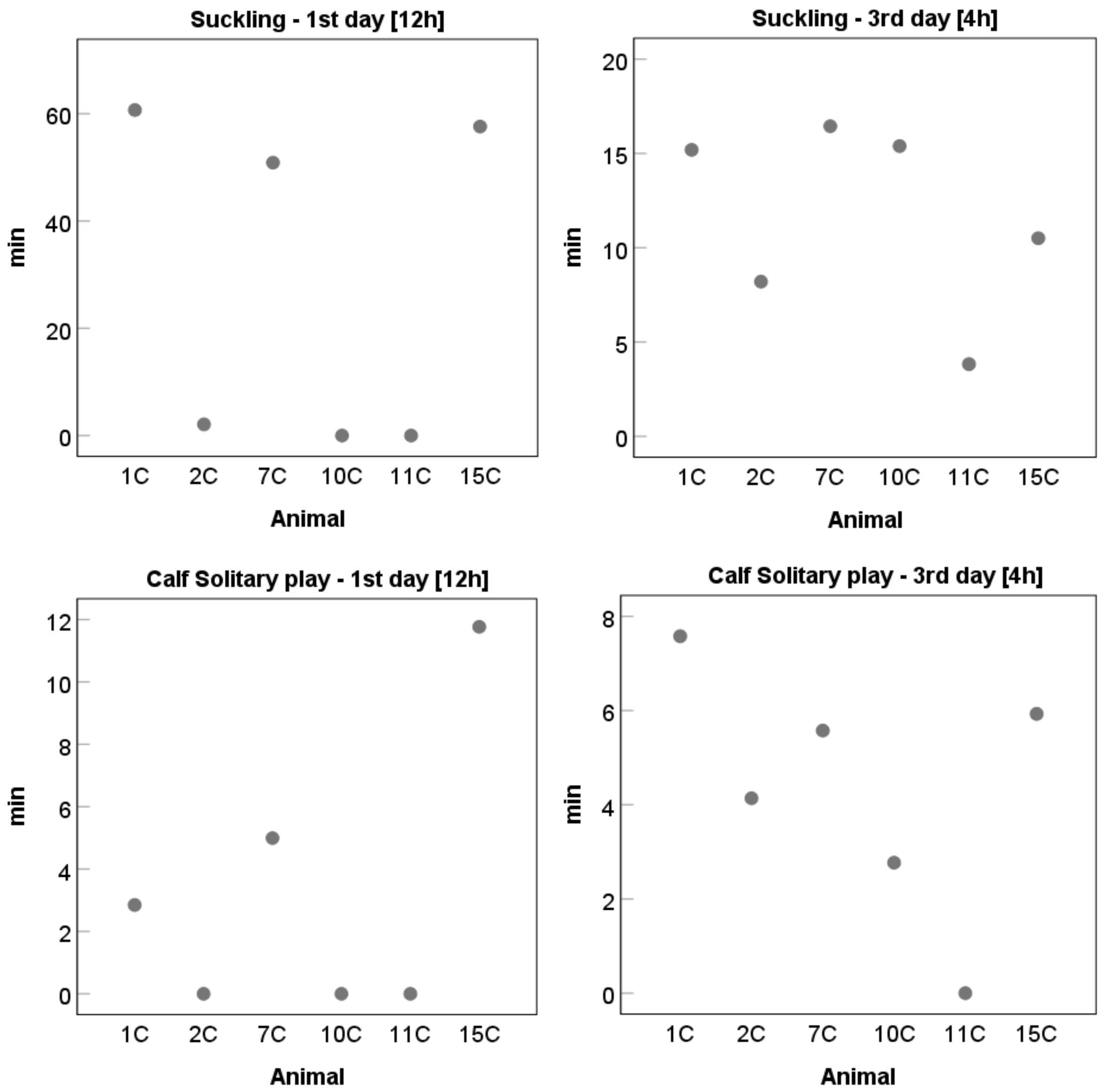

Fig. 10. Observed behaviour of calves from the mother reared treatment group. Scatter plots show duration of calf suckling and solitary play in the first 12 h after calving (left side) and within 4 h on the third day of calf's life (right side).

Correlations were found between experimental animals' social behaviour later in life and received duration of maternal social contact after calving. The duration of initiated socio-positive behaviours (*p* = 0.043) and the frequency of received positive social behaviours (*p* = 0.016) correlated positively with the received total duration of maternal contact on the first and third day after birth. Summing up all (initiated + received) socio-positive behaviours revealed a correlation for the frequencies (*p* = 0.026) and a tendency for duration (*p* = 0.052) (Fig. 11).

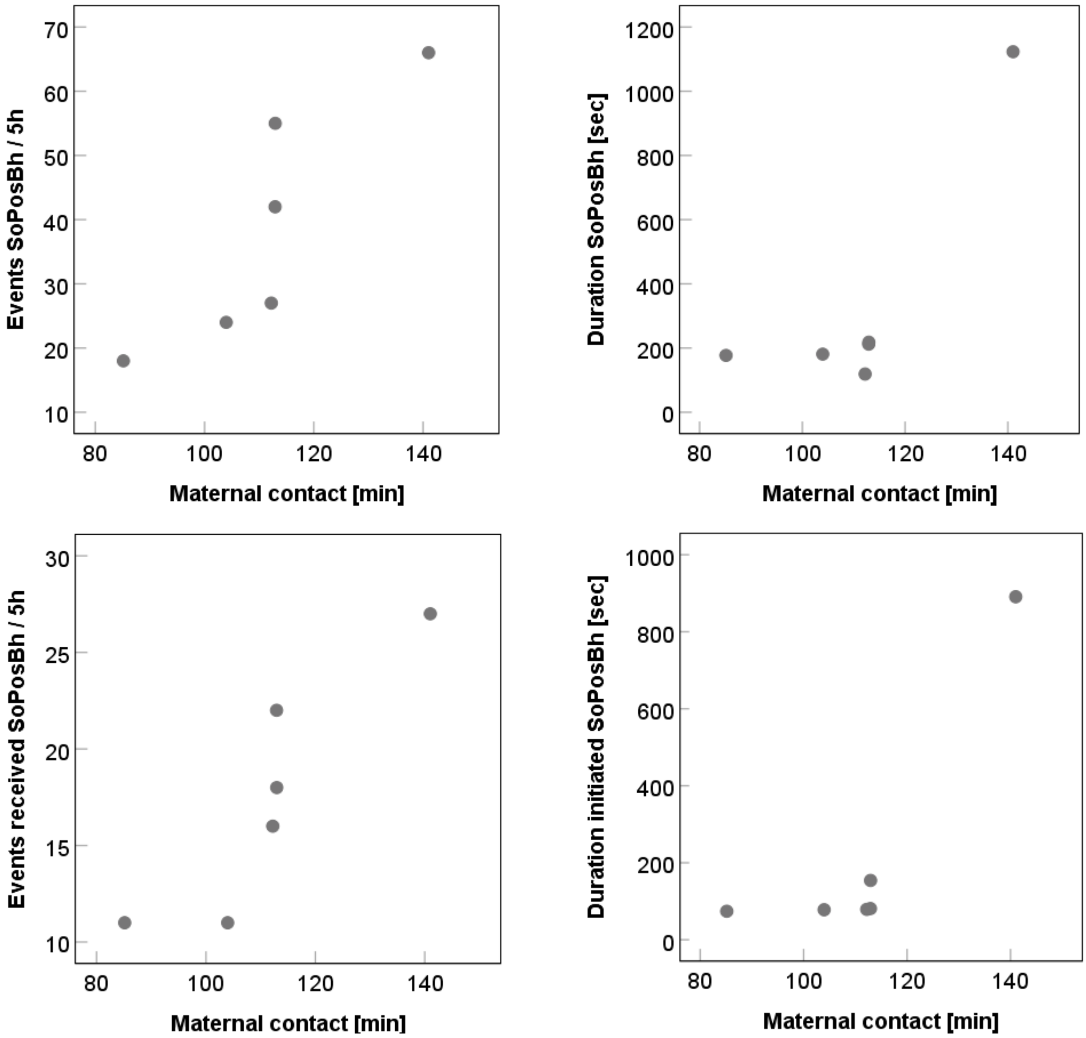

Fig. 11. Relation of maternal contact mother-bonded reared animals (n = 6) received on the first and third day of life and socio-positive behaviour displayed later in life during 5 h of observations. Scatter plots show on the top the frequency (F) and duration (D) of all socio-positive behaviours and below the frequency of received and duration of initiated socio-positive behaviours in relation to duration of received maternal contact.

4 Discussion

4.1 Social behaviour observations

Mother-bonded reared animals performed more subordinate behaviour, i.e. submissive gestures and avoidance behaviour, compared to artificially reared animals. This is in line with our hypothesis and confirms previous results in heifers confronted with a social challenging situation, i.e. integration into a new herd (Wagner et al. 2012), and calves that were confronted with an unfamiliar cow (Buchli et al. 2017). Our study gives reason to assume that animals reared with cow contact also display more submissive behaviours in everyday situations. Subordinate behaviours play a major role in dominance relationships by signalling animals' inferior status towards a conspecific thus avoiding further aggressions (Phillips 2002, pp.89). From the latter, one can assume that displaying the subordinate status is an important social competence. Cows reared with mother contact, who performed more submissive gestures, might have social benefits by avoiding aggressions (Wagner et al. 2012).

No consistent differences between treatments in initiating and receiving socio-positive behaviours, as well as aggressive behaviours were found. Similar results were found in challenging situations, such as integration into a herd (Wagner et al. 2012) or confrontation test with conspecifics (Wagner et al. 2013, Buchli et al. 2017). All experimental animals were housed most of the time in groups (*Automat* animals: maximum of six days single housed after birth) and had therefore social contact. General social contact is one important condition to establish social competence (Cantor et al. 2019). Maternal contact might not be crucial for the development of positive and agonistic social-specific behaviour alone, which could be the reason why no differences were found between treatments. Furthermore, animals were only observed for a limited time interval, which could be not sufficient, since social interactions did not occur regularly. It is plausible, that we found a weight effect for withdrawal behaviour, because weight is linked to social status (Arave et al. 1975).

We observed one animal from the *Contact* treatment, who repeatedly performed tongue rolling for a noticeable number of events. Oral stereotypies i.e. tongue rolling and cross-suckling were observed in artificially reared calves in weeks 2,4 and 8 after birth, but not in calves who could suckle their mothers during that time (Fröberg & Lidfors 2009). However, the occurrence of tongue rolling is mainly associated with feeding management (Sato et al. 1994). Cross-suckling seems to be more affected by rearing conditions in calves (Margerison et al. 2003) but was not observed in our study. This is an indicator for multifactorial reasons for the exhibition of this abnormal behaviour e.g. based on poor management systems. In our study, calves were weaned later and got up to 12 kg/day milk portions per day, while in Fröberg and Lidfors (2009) study they got only 9 l per day. These differences were possibly already enough to cause the difference in the occurrence of cross-suckling. Further, cross-suckling is more present at a younger age, in un-weaned calves since in our study half of the experimental animals were above 10 months old and weaned, it is less likely that animals perform it. But there is also evidence for the occurrence of cross suckling as a behaviour problem later in life especially in animals that performed cross-sucking before weaning (Keil et al 2001).

4.2 Social isolation test and reinstatement test

Mother-bonded reared heifers' and calves' propensity to look longer towards and, by trend, to stay longer close to the door can be interpreted as being more attracted to the peers than artificially reared animals. Therefore, we found evidence that the sociality of individuals reared with mother contact is enhanced compared to artificially reared ones. The higher motivation of animals reared with mother contact to join their peers might be evidence for the stronger desire to be in contact with the peers, that arise because of the maternal contact these animals received during rearing.

In other studies, animals reared with mother contact spent more time near the walls (Neindre et al. 1989) and show more active flight attempts (Wagner et al. 2013) in isolation, which was as well interpreted as higher motivation to re-join the group. However, no escape attempts during isolation were observed in our study and almost no escape attempts were found in Buchli et al. (2017) study. Animals of our study had visual contact to their peers all the time, while in other studies barriers where opaque, which might be a reason why animals did not show any strong behaviour responses such as trying to escape in our study.

For cattle, the dam is an important bonding partner (von Keyserlingk & Weary 2007) and in a challenging situation they tend to stay close to their bonding partner (Waiblinger et al 2006). The desire of cattle to keep the contact to conspecifics, thus, a high sociality seems advantageous for them, since it was shown that the presence of a conspecific leads to a stress deduction when being separated (Færevik et al. 2006). Rearing animals with mother contact, who showed a higher sociality, could lead to more stress resistant animals. But as a consequence, also the housing and management conditions have to be adapted to the needs of highly social animals to respect this desire e.g. allowing the contact to others in challenging situations and ensure stable groups to allow the maintenance of social bonds between animals.

Similarly to Wagner et al. (2013) study, more events of vigilant behaviour were observed in mothered compared to artificially reared animals during isolation. They already discussed the ambivalent notion of vigilance, in which it can be interpreted as fear or as environmental scanning. Furthermore, the behaviour seems to be dependent from environment factors, which are difficult to control (Buchli et al. 2017). Animals reared with mother contact in our study, who looked longer to the door that leads to its conspecifics, did also show more events of vigilant behaviour, which could lead to the interpretation of vigilance in this test setting as environmental scanning to search for contact to conspecifics. However, this result should be considered with caution, since a difference was only measured for the frequency but not duration of vigilant behaviour and the isolation pen was not isolated from other environmental factors. Also, behaviour definitions in the ethogram could have led to a misinterpretation, since a higher number of vigilance events can be a result of ear movements. “Vigilance” was coded when the head is high and both ears were erected, but as soon as one or both ears were non-erected and the head was still high, the animal showed the behaviour “head high”. As a result, a very small movement could have led to a high number of vigilance events that may not represent the situation appropriately. The rate of transition between head high and vigilance reflect this assumption, in mean 56.1 % of cases coded as head high was followed by vigilance and in mean 81.7 % of cases coded as vigilance was followed by head high. Besides, the behaviour “head high” needs even more clarifications, because it was also coded during exploration of high located objects even though in this situation the head is hold always automatically in a higher position. Further studies should reconsider the differentiation between head high and vigilance and should exclude exploration in combination with head high. Additionally, it would be interesting to investigate how often vigilance is performed together with looking to the door that leads to conspecifics to clarify if these behaviours are connected. Results could help to get a more accurate picture of the notion of vigilance.

The treatment had no effect on the frequency and duration of exploration in isolation and reunion phase, which is in line with the study of Wagner et al. (2013). Probably the motivation to explore was not the primary ambition for tested animals, because all animals walked through the experimental setting before. On the other hand, heifers from pasture 1, who saw the test setting for the second year, explored less compared to calves from pasture 1 in the reunion phase of the test. This might be an indication that exploration is dependent on familiarity of the environment and that rearing did not affect this behaviour. It was observed before that exploring increased with the appearance of something that is novel for the animal (Herskin et al. 2004) and that a new environment can initiate exploration (Phillips 2002, pp. 86).

The function of vocalisation in cattle is to remain in contact with bonding partners (Phillips 2001, pp. 96). When mother-bonded reared animal's sociality is more pronounced compared to artificial reared animals, it would be plausible that mothered animals vocalise more to maintain the contact. However, in this study, rearing did not affect vocalisation. One explanation might be, that the animals had constantly visual contact to its peers and that a difference in vocalisation is only identifiable when conspecifics are out of sight and the stress level is higher. This suggestion would be in line with the idea that more vocalisation in cows are a result of a strong stress response (van Reenen et al. 2004) and would explain why in a test arena with solid wooden walls mothered female calves vocalised more compared to *Automat* calves (Wagner et al. 2013). Another explanation why in this study no difference was found, might be that the presence of a person during the test may have affected the vocalisation behaviour as it has been observed in sheep (Le Neindre et al. 1993).

More walking in cattle is associated with fear response in social separation but is less distinctive in a familiar environment (de Passillé 1995). No significant differences in the duration and frequency of walking between treatments were found in this study, which is an indication for similar fear levels between the two treatment groups. We deliberately chose a familiar test pen to minimize the potential confounding effect of fearfulness on behaviour of our experimental animals. The lack of a difference in walking behaviour indicates that we choose the right setting for our research question.

Animals of both treatments performed self-grooming and *Automat* animals showed more self-grooming than *Contact* animals. This stands in contrast to other studies, which observed no difference between treatments (Wagner et al. 2013). But in a different situation, during the integration into a new group, animals reared with mother contact showed more self-grooming behaviour compared to artificial reared animals (Wagner et al. 2012). It was assumed that mother-bonded reared animals performed more self-grooming because they were more relaxed. In our study it seems that *Contact* animals had a higher motivation to join their peers and potentially were more stressed than artificially reared animals, which could be a reason why they performed less self-grooming. However, this is only one possible explanation and the varying findings in different studies support Wagner et al. (2012) notion that self-grooming in stressful situations might have different explanations and that further research is needed to distinguish between them.

4.3 Maternal contact observations

As expected, in the first hours, mother cows spent a lot of their time licking the calf. Kiley-Worthington et al. (1983, pp. 73) observed in 12 cows an average licking of 22.2 minutes in the first three hours after birth, which result in 1.29 h when being projected to 12 h and is similar to what we described. The same authors also declined a high individual variation, which is in line with our findings. The licking is known to be important for the bonding between cow and calf (von Keyserlingk & Weary 2007). Using the percentage of received maternal contact on the first day 10.4 % - 18.4 % and third day 1.3 % - 4.8 % of calves' life, revealed a relatively decrease of maternal contact over the days. But percentages should be compared with caution due to the different observation lengths and times on each day. However, also Jensen (2011) found less sniffing and licking initiated by mother cows over increasing days *post-partum* as well as Kiley-Worthington et al. (1983, pp. 97), who observed cow-calf pairs over a period of six month. The observed placentophagia (eating placenta) is a natural behaviour in cattle and was described before (Hudson & Mullord 1977, Edwards 1983, Kiley-Worthington et al. 1983, pp. 77, von Keyserlingk & Weary 2007).

Interestingly, half of the observed mother cows (n = 3) covered up her calf with straw, a behaviour that could be interpreted as nestbuilding-like behaviour. Nestbuilding behaviour is rarely observed in cattle (von Keyserlingk & Weary 2007). Wehrend et al. (2006) described it as “the animals pushed the straw to form a resting place using their heads and their front limbs” (pp. 166) and saw the behaviour in 33 out of 87 animals but it was only recorded before parturition. In our study, the behaviour looked similar as the one described by Wehrend et al. with the exception, that the cow put the straw over the calf in the first hours after calving and the behaviour emerged often in combination with licking the calf before or/and afterwards. On pasture calves prefer to lay covered in high vegetation (Langbein & Raasch 2000), a behaviour which is difficult to perform in the calving pen and modern farming. One explanation could be that the mother tried to cover the calf to compensate the missing hiding possibility. The calf from the cow *10*, who was observed to cover up her calf with straw 9 times, did not suckle within 12 h. The median latency for the first suckling after calving is 4 h in a study with 21 cow-calf pairs (Ventorp & Michanek 1991) and in a different study by Kiley-Worthington et al. (1983, pp. 71) the first teat searching and also short bouts of suckling were observed already within 1.5 h - 2 h after birth. The calf from *cow 10* needed a lot of time to suckle its mother for the first time. Further, the cow of this calf had the shortest lying duration and when lied down, she was nearly never orientated away from her calf. Potentially, with this behaviour the restless cow tried to protect her calf, who apparently had some deficits at the beginning of its life. An observation that would strengthen the assumption from Stěhulová et al. (2013), who suggested that cows can adapt their maternal care to the needs of their calf.

Edwards (1983) detected an increase of the average distance between cow and calf over the first hours after calving. The same results were found in our study, in which the cow spent 74.6 % - 92.3 % of 12 h observation on the first day in direct proximity to its calf but only a percentage of 58.5 % - 70.9 % in 4 h observation on the third day of calves' life. Also, Kiley-Worthington et al. (1983, pp. 78) found that cows spent a higher percentage of time near the calf in the first three hours *post-partum* than later in time. But it has to be considered that at least the space allowance of the indoor area of the calving pen (= lying area) was only 3 m x 4 m, which could have contributed to the high percentage of cows positioned in direct proximity to the calf.

Calves who suckle within 12 h after birth also performed locomotion play on the first day and they also performed more playing behaviour on the third day compared to animals who did not suckle within 12 h. This result is only descriptive and could not be tested according to the small sample size. In Krachun et al. (2010) study, running appeared less in calves with lower energy intake. Although, in this study the calves were separated from its mother within 12 h after birth and the data collection begun at 3 weeks of age it would be plausible that calves who explore their environment and mother, thus, start to suckle and be in general active, also engage more in locomotion play. A positive correlation between calf's activity and first-time suckling was already described (Ventorp & Michanek 1991). Furthermore, licking stimulates calves' activity (von Keyserlingk & Weary 2007). It would explain why the calf who received the most maternal contact performed the most observed solitary play while the calf who received the lowest amount of maternal care did not play at all and that suckling was only observed rarely on the third day.

That the socio-positive behaviour later in life of experimental animals correlated with the maternal contact these animals received after birth means that there is a connection between the allowance of maternal contact and the development of social traits. This finding may support the assumption that received maternal contact in the first hours after birth have already a positive effect on the development of socio-positive behaviour later in life. This then might be also the reason why no differences between treatments regarding the frequency and duration of socio-positive interactions later in life were found, because all animals stayed at least 12 h together with their mothers.

The time deviations that were observed during coding with BORIS, as described in the methods, give reason to assume, that there was a transmission error between the embedded video and the timeline of BORIS. Furthermore, a small number of pictures from the camera recordings were missing due to unknown technical interruptions in the system, which may have caused the different video lengths. Below 1.4 % of 12 h total observation time per observed animal was affected from the deviations. Due to the minor divergences, we did not expect that the deviations influenced the results.

4.4 Conclusion

The results of the study give evidence that in dairy cows rearing with contact to the mother early in life have long term effects on social traits. The social competence and sociality are enhanced in animals from mother-bonded rearing. Furthermore, maternal care these animals received after birth are connected to the socio-positive behaviour displayed later in life. Some observations from the first hours after calving e.g. that cows cover up their calves with straw are indicators for the profound care, they are able to provide for their calves, and were not described before. It becomes apparent with our findings and findings from other researchers, that the social environment has an important impact on the development of cattle's' social life. Future research should gain more knowledge regarding the effect mother rearing has on personality and how changes in personality due to artificially rearing affects later behaviour and welfare. The results could be used to induce evidence-based decisions and political changes in how we treat these animals, who show clear signs of a complex social life that is negatively affected by human made housing management.

5 Summary

Calves in dairy farming are separated from their mothers within 24 h after birth and reared artificially. Growing up without mother contact is known to affect animals' social behaviour, especially the social competence negatively. In this study, eighteen female Black-and-White-German-Holstein heifers and calves reared with (*Contact*, n = 9) or without maternal contact (*Automat*, n = 9) in the first 12 weeks of life were included, to investigate the effect rearing has on different social traits. For each animal the spontaneous social behaviour was assessed in 5 h social observations per animal in the home pen, while using focal animal sampling and continuous recording. To test for sociality, all animals participated in a 5 min isolation test followed by a 5 min reinstatement phase in a familiar environment. Behaviour during the test was analysed from video recordings, only vocalisations were noted directly. Video material from the calving pens provided insights into the quality and quantity of maternal care *Contact* animals (n = 6) received on the 1st (for 12 h) day and on the 3rd day (for 4 h) of their life. Further it was analysed, whether maternal contact behaviour (sniffing, licking) was positive associated with *Contact* animals' involvement in socio-positive interactions later in life.

Data were analysed using ANOVA with treatment, group and their interactions as fixed factors, as well as age and weight as covariates. *Contact* animals showed more subordinate behaviours in the herd (*P* = 0.028) and while in isolation they looked longer (*P* = 0.014), tended to spent more time closer to the closed door that lead to the peers (*P* = 0.072), as well as showed more events of vigilance behaviour (*P* < 0.001) than artificially reared animals. However, the treatment did not affect the frequency and duration of socio-positive behaviour, nor animals' latency to re-join their peers during the reinstatement-phase. Correlations between the amount of maternal contact mother-bonded reared animals received and their initiated socio-positive interactions (*P* = 0.043) and received socio-positive interactions (*P* = 0.016) later in life were found. Interestingly, three cows show a nestbuilding-like behaviour that was rarely descripted before. The results give reasons to assume that cows maternal care is from high quality and that the allowance of maternal contact affects animals' sociality and their social competence later in life.

6 Zusammenfassung

Kälber von Milchkühen werden normalerweise innerhalb von 24 Stunden von ihren Müttern getrennt und künstlich aufgezogen. Das Aufwachsen ohne Mutter hat einen negativen Einfluss auf das Sozialverhalten der Tiere, insbesondere deren Sozialkompetenz ist betroffen. Für diese Studie wurden achtzehn weibliche Färsen und Kälber der Rasse schwarzbuntes Holstein-Rind ausgewählt, die entweder mit Kontakt zur Mutter in den ersten 12. Lebenswochen aufgezogen wurden (*Kontakt*, n = 9) oder ohne Mutterkontakt (*Automat*, n = 9). Das spontane Sozialverhalten jedes Tieres wurde in 5 h Sozialbeobachtungen pro Tier durch Fokusstierbeobachtung mit kontinuierlicher Aufnahme erfasst. Um die Sozialität zu untersuchen, nahmen alle Tiere an einem fünf-minütigen Isolationstest, gefolgt von einer fünf-minütigen Phase, in der die Tiere zur Gruppe zurückkehren und den Sozialkontakt somit wiederherstellen konnten. Das Verhalten der Tiere wurde anhand von Videoaufnahmen, die während des Tests erfolgten, analysiert. Lediglich die Lautäußerungen der Tiere wurden direkt erfasst. Weiterhin gab Videomaterial aus den Abkalbebuchten Aufschluss über die Qualität und Quantität der mütterlichen Fürsorge, die Tiere aus der muttergebunden Aufzucht (n = 6) am ersten Tag (12 Stunden) und am dritten Tag (4 Stunden) nach der Geburt erhielten. Es wurde untersucht, ob es eine positive Assoziation zwischen der erhaltenen mütterlichen Fürsorge und der Beteiligung an sozio-positiven Interaktionen später im Leben der Tiere gibt.

Die Daten wurden statistisch mit einem ANOVA Modell ausgewertet; mit Aufzucht, Gruppenzugehörigkeit und deren Interaktion als festen Faktor und Alter sowie Gewicht als Kovariaten. *Kontakt* Tiere zeigten mehr Unterlegenheitsgesten in der Herde ($P = 0.028$), verbrachten in Isolation tendenziell mehr Zeit in der Nähe ($P = 0.072$) und sahen länger zum Ausgang, der zu den anderen Gruppenmitgliedern führte ($P = 0.014$). Auch zeigten sie eine höhere Frequenz an Wachsamkeit ($P < 0.001$). Jedoch beeinflusste die Aufzucht weder die Anzahl und Dauer des positiven Sozialverhaltens noch die Latenz bis die Tiere zur Gruppe zurückkehrten. Die Dauer des mütterlichen Kontakts, den muttergebunden aufgezogene Tiere nach der Geburt erhalten haben, korrelierte positiv mit ihren gezeigten ($P = 0.043$) und von anderen Tieren erhaltenen ($P = 0.016$) sozio-positiven-Verhalten später im Leben. Interessanterweise zeigten drei Muttertiere Nestpflege-Verhalten, das bisher kaum beschrieben wurde. Die Ergebnisse zeigen, dass Muttertiere ihre Kälber mit ausgeprägter Fürsorge behandeln und dass der Kontakt zur Mutter in der Aufzucht die Entwicklung der Sozialkompetenz und Sozialität der Tiere beeinflusst.

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8 List of figures

9 List of tables

10 Acknowledgement

Writing a master thesis needs more than just being a master student. I learned that rather knowledge and expertise is the main issue during the progress, but dealing with self-motivation, own perfectionism, frustration and a lot of different personalities.

Though my way of completing this thesis my supervisor Susanne Waiblinger have taught me a lot. I am very thankful for her helpful and wise comments. From all my heart, I also thank my companion and king, Skalde Runge. He is my biggest fan, my best discussion partner and the most beautiful person, who never stop believing in me. Who knows if we would have ever met without this project? Further, I would thank my mother for her unconditional support during all steps in my life, independently from how crazy they are. A huge gratitude also goes to my fellow students for all the times they cheered me up when I needed it and all the funny moments during the master program. Finally, I want to mention, that I fell in love with my eighteen study participants, who showed me how incredible sensible, playful, curious and lovely cattle are. I am sure, if everyone would spend some time with them, more people would reconsider their milk and meat consumption.

Annex 1 - Behaviour during social behaviour observations

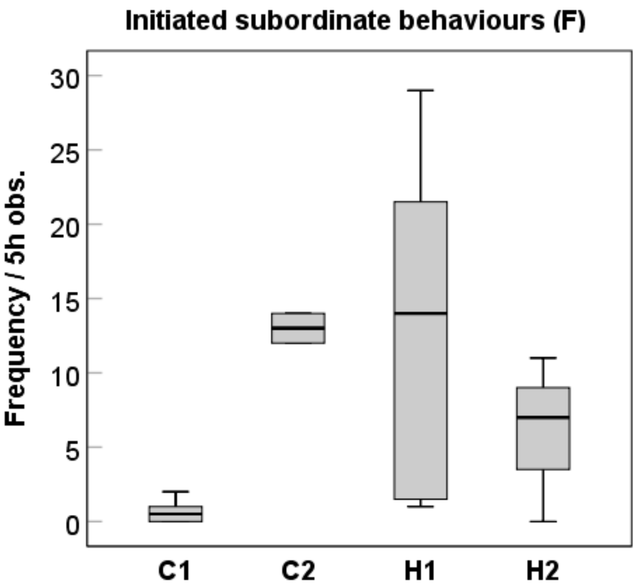

Fig. A1.1 Initiated subordinate behaviour frequency (F) among different groups (C1: Calves 1, C2: Calves 2, H1: Heifers 1, H: Heifers 2). Number of experimental animals varied between groups (C1 n = 6, C2 n = 2, H1 n = 7, H2 n = 3)

Annex 2 - Behaviour during social reinstatement test

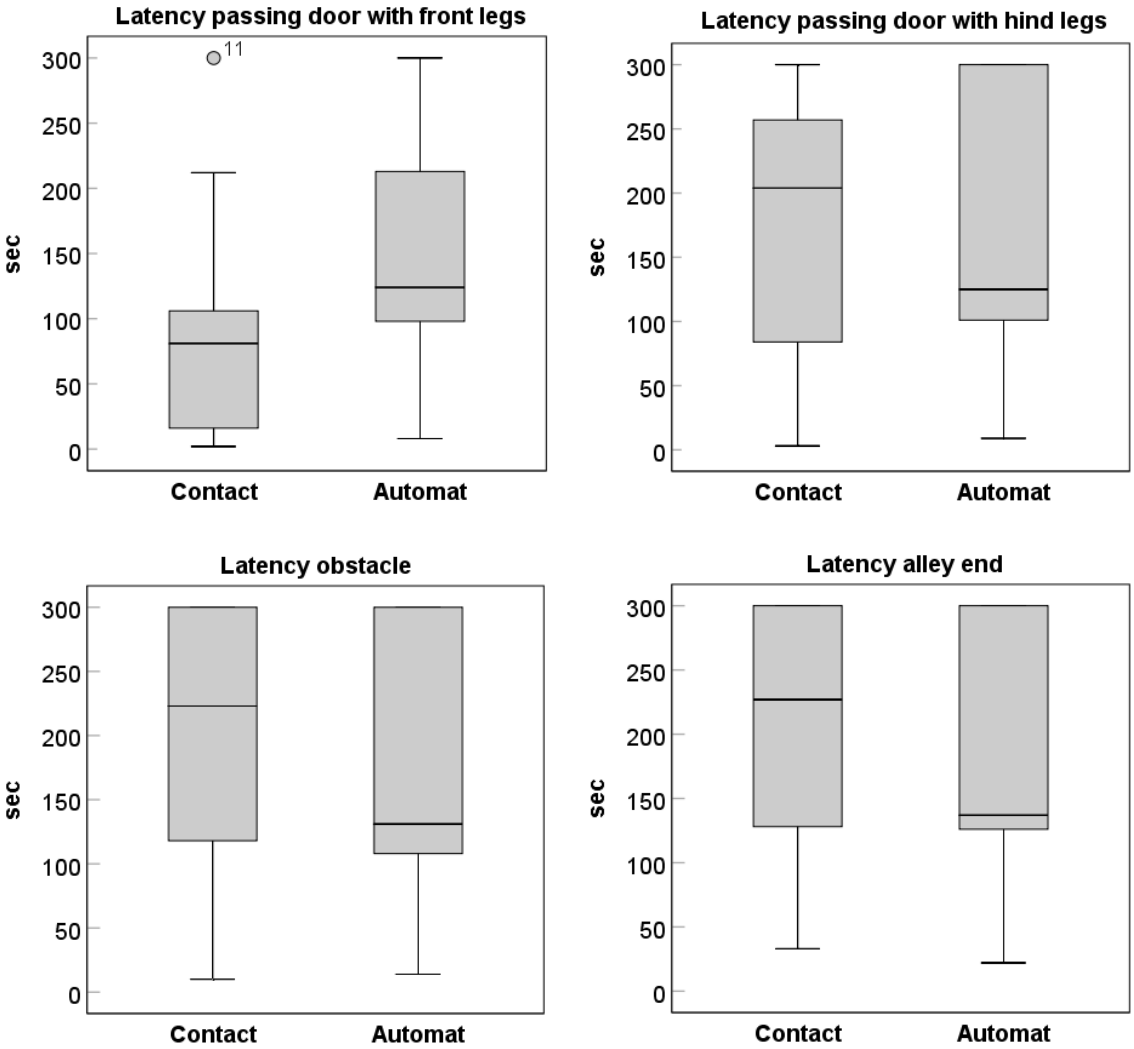

Fig. A2.1. Measured latencies of experimental animals reaching different setpoints in the alley in 5 min Reunion phase after opening the door. Boxplots show animal's (a) latency of passing the door with front legs, (b) latency of passing the door with hind legs, (c) latency of stepping on obstacle (scale) with front legs, (d) latency until reaching the alley end (group members) of treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

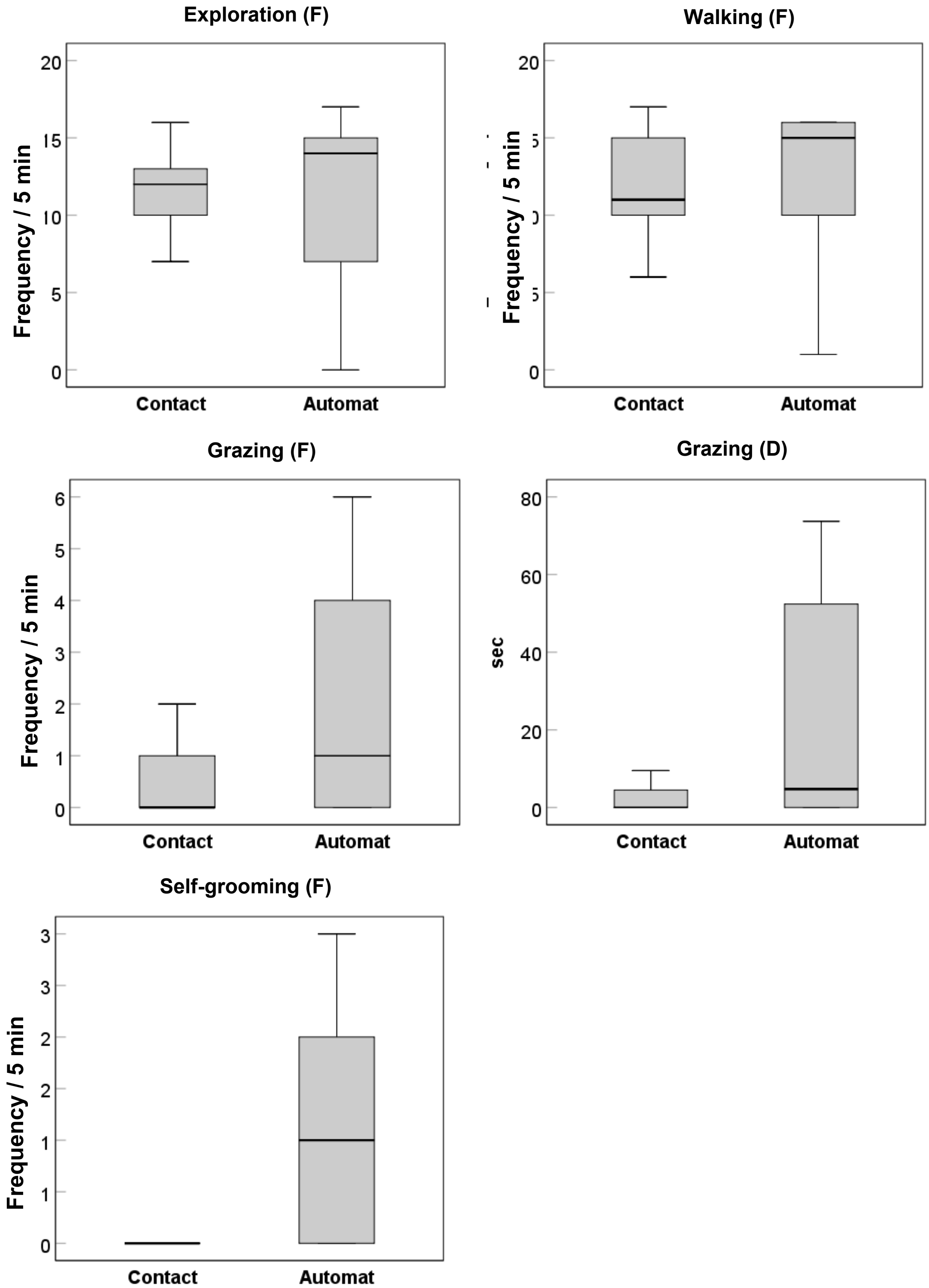

Fig. A2.2. Individual behaviour observed in 5 min Isolation phase. Boxplots on the left- side show frequencies (F) of exploration, grazing, self-grooming and on the right-side the frequency of walking and the durations (D) of grazing behaviour of the different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

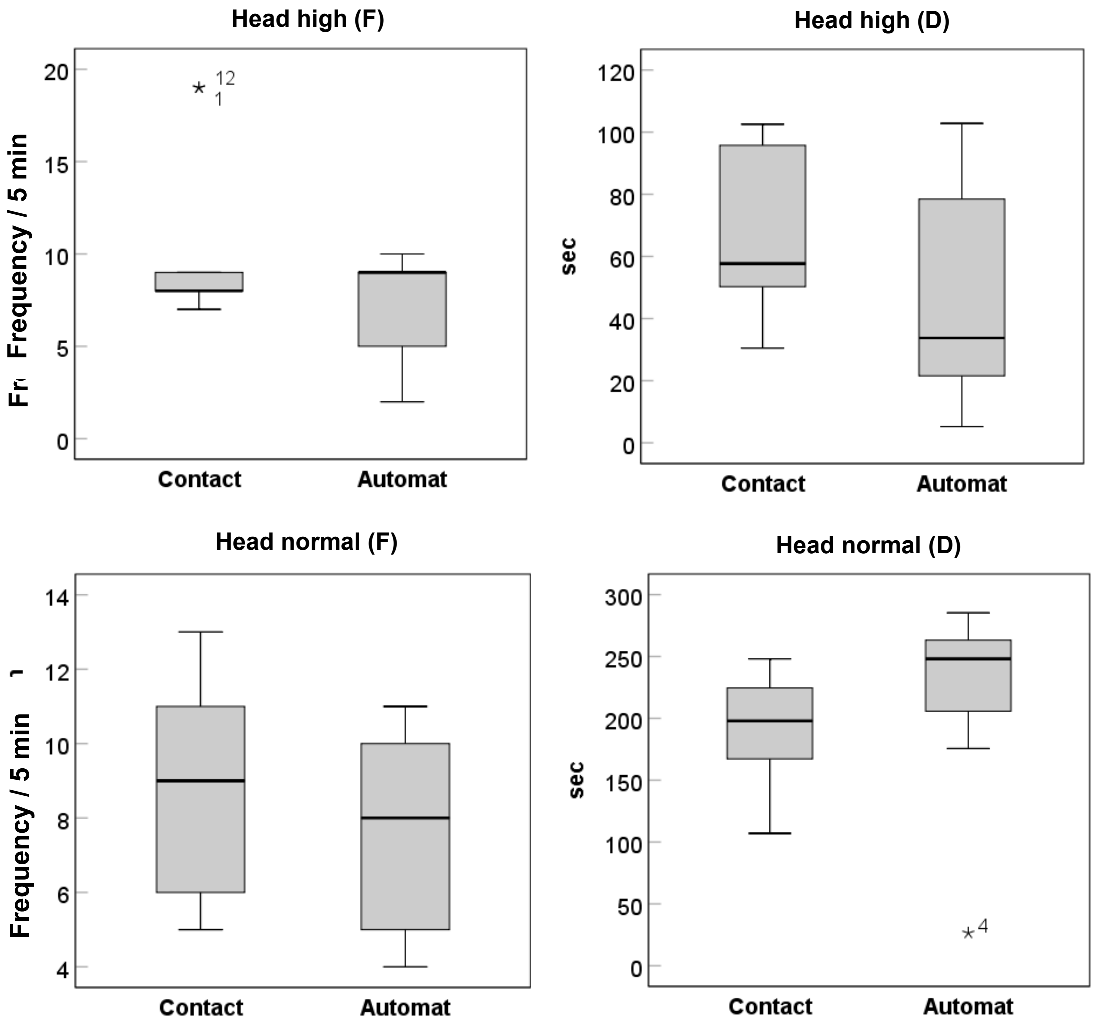

Fig. A2.3. Individual head and ear position observed in 5 min Isolation phase. Boxplots on the left side show frequencies (F) and on the right-side durations (D) of head high and head normal position of different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

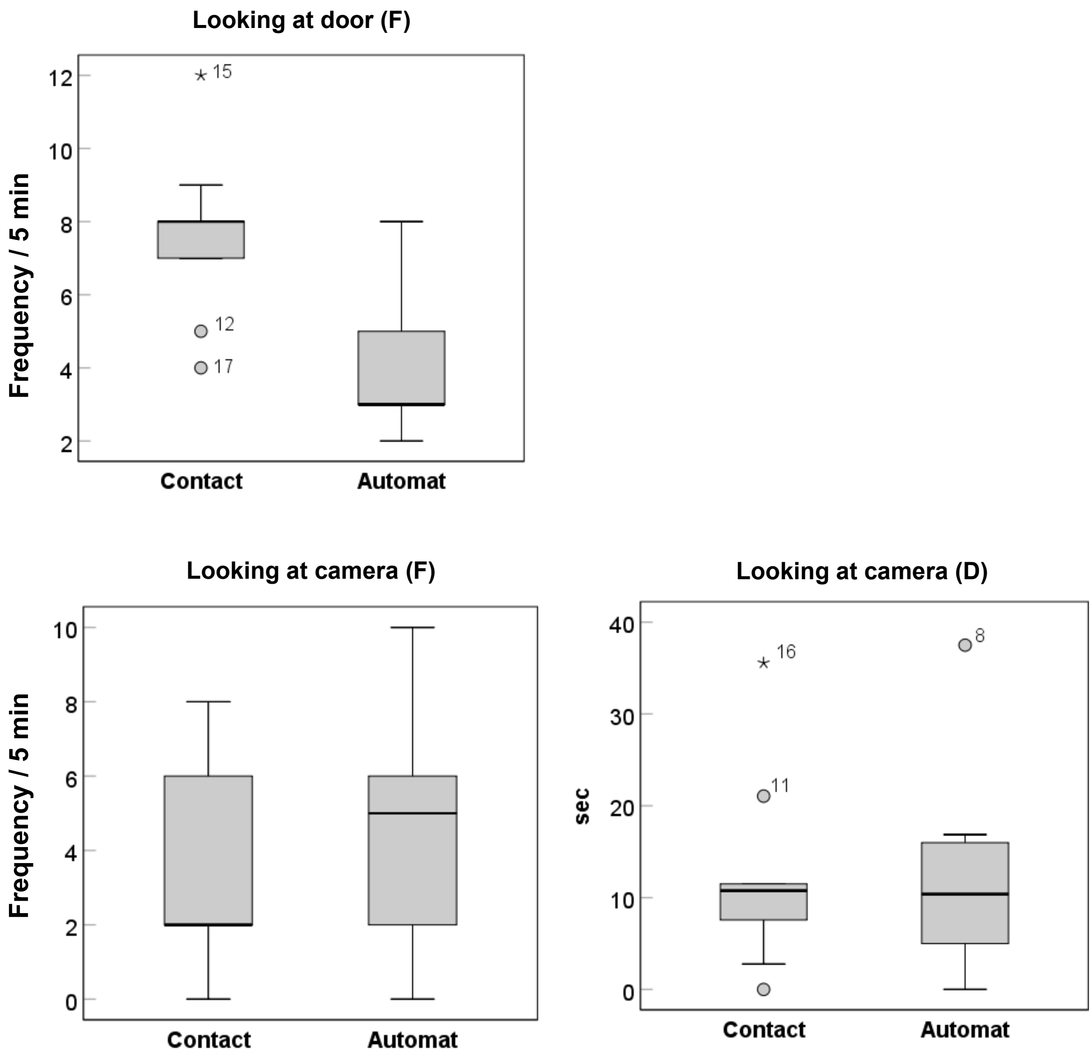

Fig. A2.4. Orientation behaviour observed in 5 min Isolation phase. Boxplots show on the left side frequencies (F) and on the right-side durations (D) of experimental animal's looking to door, looking to camera of different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

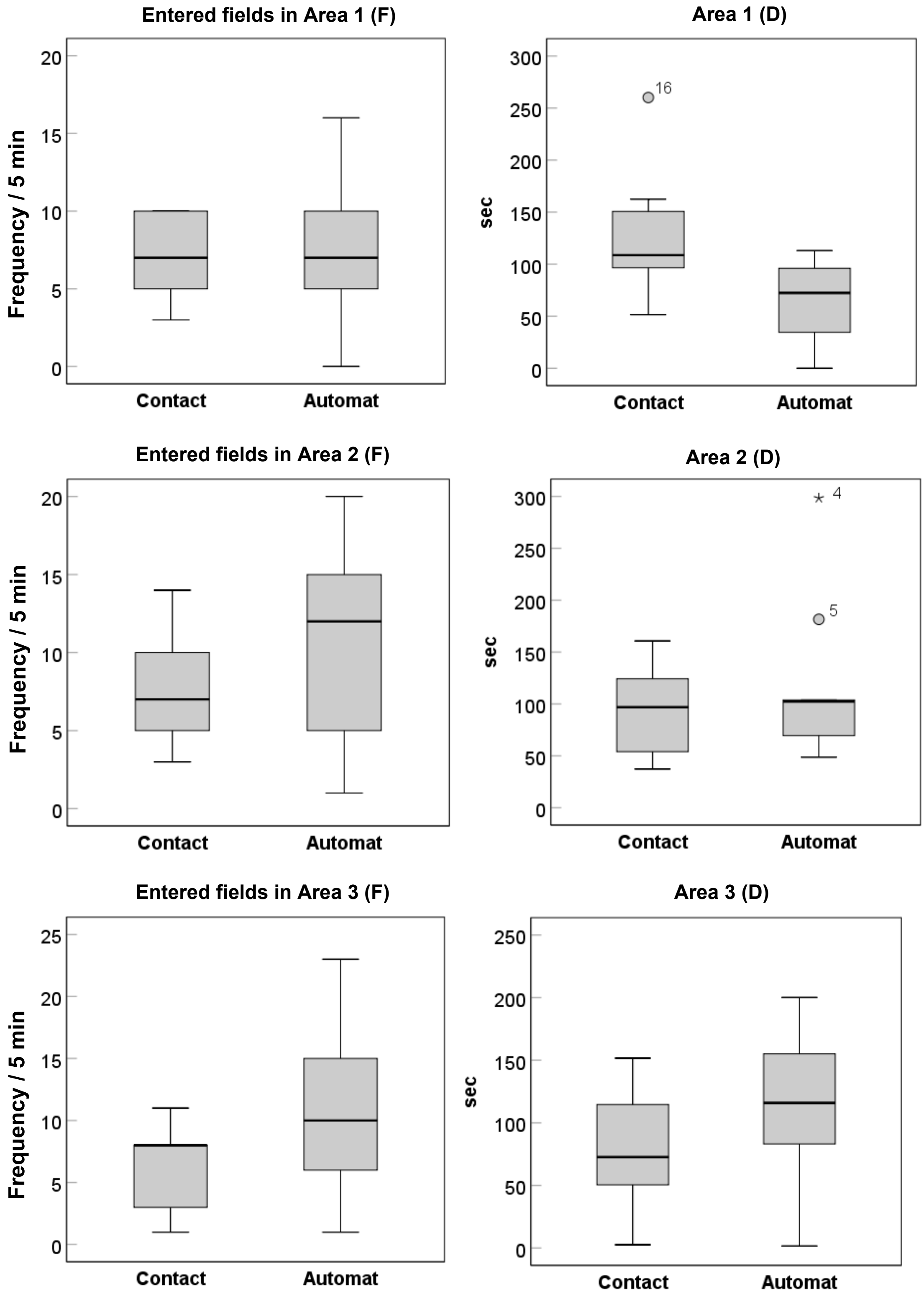

Fig. A2.5. Position of experimental animals in the Isolation pen in 5 min Isolation phase. Boxplots on the left side show frequencies (F) of entered fields and boxplots on the right-side show durations (D) of staying in Area 1, Arena 2, Area 3 of different treatment groups, *Contact* (n = 9) and *Automat* (n = 9).

Annex 3 - Behaviour during maternal contact observations

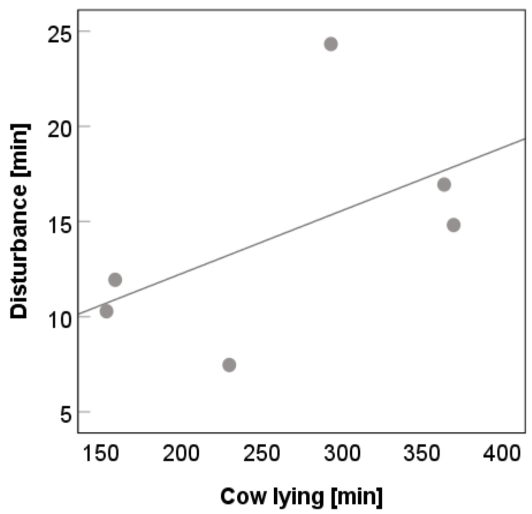

Fig. A3.1 Relation of cows' total lying duration in the first 12h after calving and duration of disturbance (caretaker intervened).

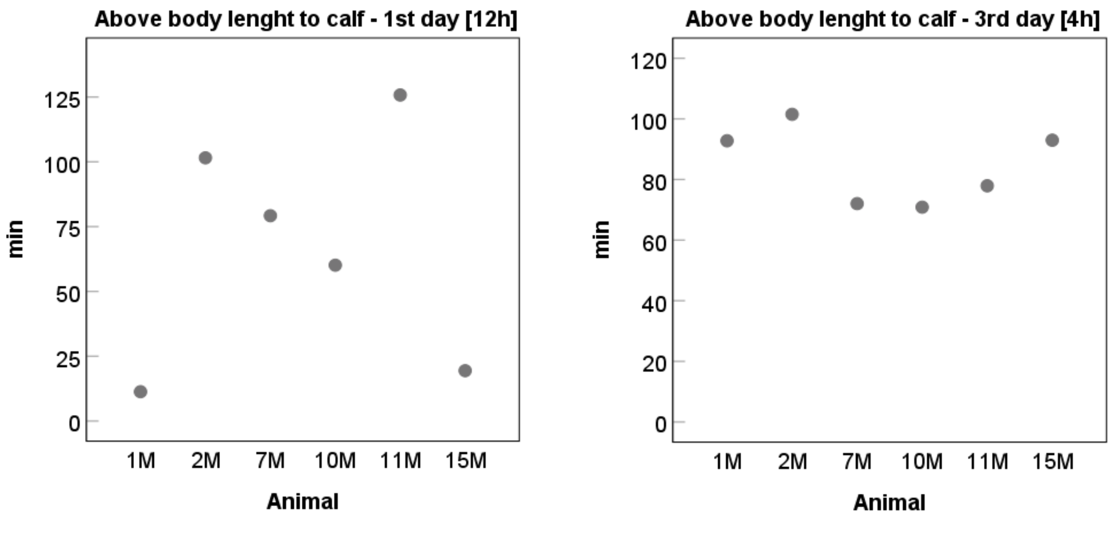

Fig. A3.2. Cow's proximity to calves of the mother-bonded reared treatment group. Scatter plots show the duration cow's lying above body length to calf in the first 12h after calving (left side) and during 4h on the third day of calf's life (right side).

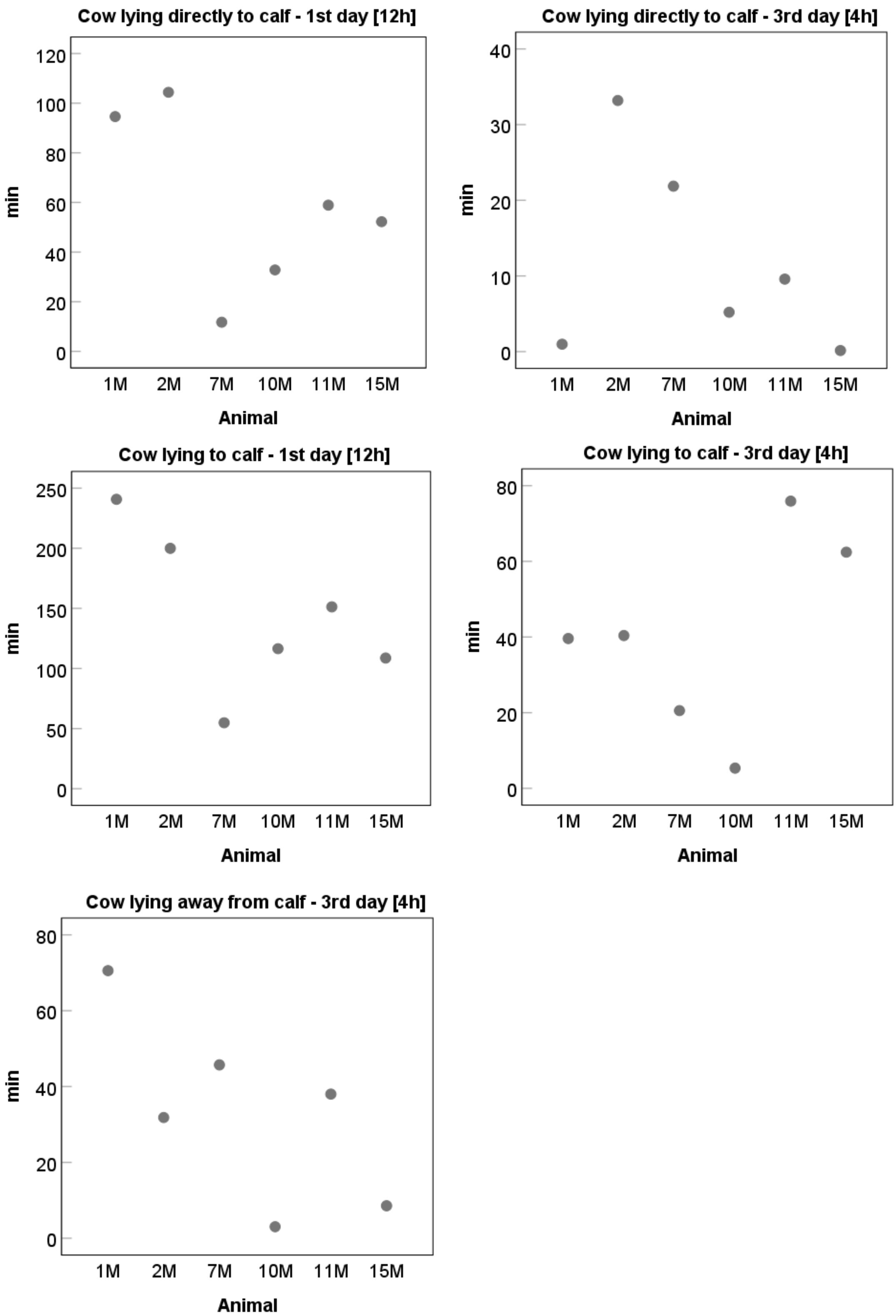

Fig. A3.3. Cow's orientation towards calves of the *contact* treatment group. Scatter plots show the mother cow's duration of lying directly, lying to calf and lying away from calf in the first 12h after calving (left side) and 4h on the third day of calf's life (right-side).

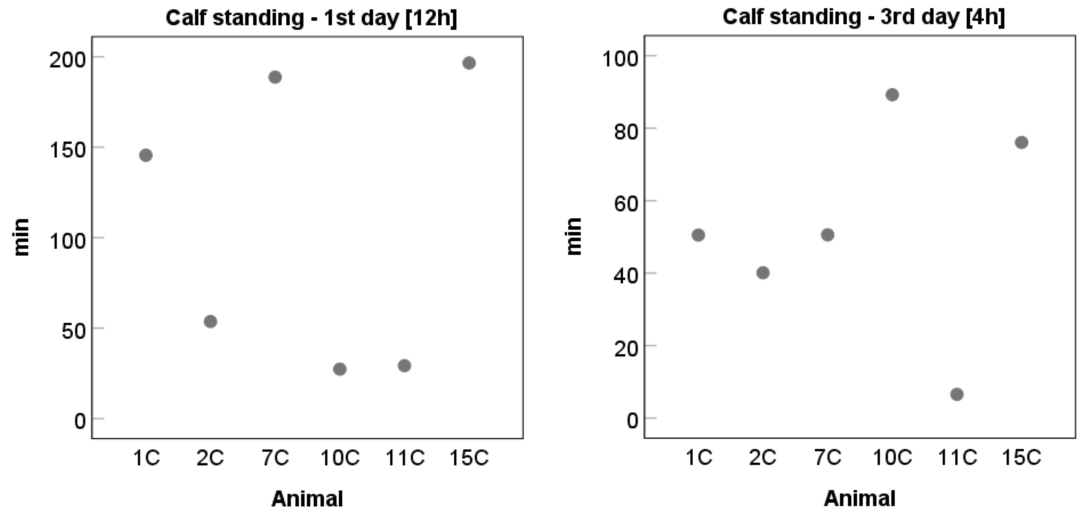

Fig. A3.4. Observed calf behaviour of the mother-bonded reared treatment group. Scatter plots show duration of calf standing in the first 12h after calving (left side) and within 4h on the third day of calf's life (right side).

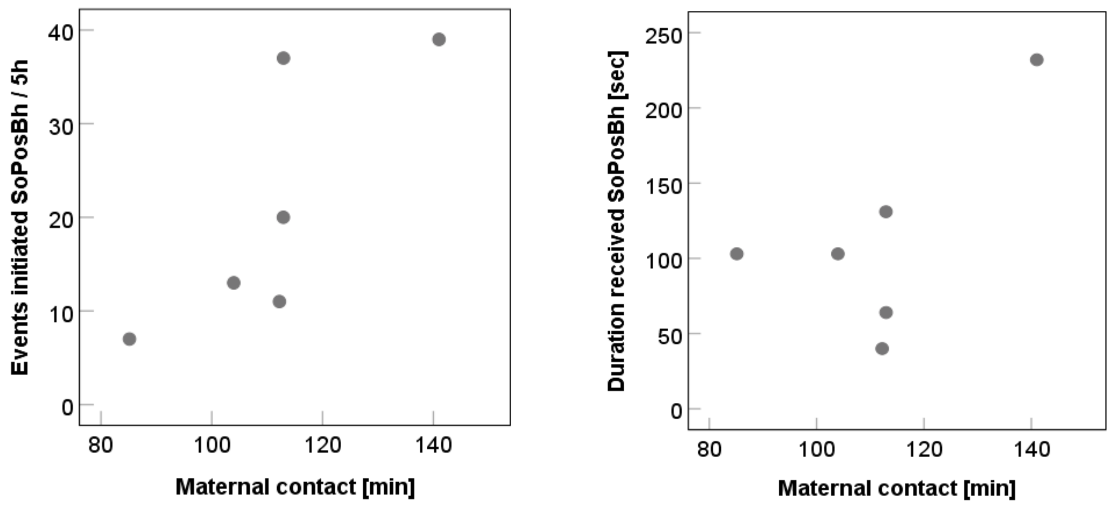

Fig. A3.5. Relation of maternal contact animals of the mother-bonded reared treatment group received on the first and third day of life and socio-positive behaviours displayed later in life during 5h of social observations. Scatter plots show on the left side the frequency (F) of initiated and on the right-side duration (D) received socio-positive behaviours in relation to duration of received maternal contact