

Research Reflection

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The influence of maternal contact on activity, emotionality and social competence in young dairy calves

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Abstract

The study reported in this research communication aimed to assess the influence of maternal contact on calves' activity, fearfulness, and social competence. Calves were either dam-reared for their first 14 d of age (Maternal Contact, $n = 12$) or were separated from their dams within 12 h after birth (Motherless, $n = 12$). Calves of both treatments and the dams of Maternal Contact calves were group-housed and suckling was prevented with udder nets. The general activity (lying, locomotion, swapping between lying and standing) was measured using pedometers in eight Maternal Contact and eight Motherless calves within a 24-d period. Since general activity might be affected by calves' age or the separation of Maternal Contact calves from the dams the 24-d period was additionally divided into two groups (period A: 3rd–13th day of age, period B: 14th–27th day of age). Emotionality and social competence were assessed in the open field, novel object, and confrontation test with an unknown cow at 14, 21, and 28 d of age, respectively. Mann-Whitney- U -tests were performed for statistical analysis. Locomotion was greater in Motherless calves than Maternal Contact calves during the 24-d period (A + B combined, $P < 0.001$) and period B (14th to 27th day of age, $P < 0.001$). There was no treatment difference in duration of lying or in the amount of swapping in any of the periods. After a Bonferroni correction, which we used due to the exploratory character of the study, there were no treatment differences in behaviours indicating emotionality. Compared to Motherless calves, Maternal Contact calves showed increased vigilance ($P < 0.01$) during the confrontation test. The results of this study indicate that mother-reared calves likely searched less for social contact and developed greater social skills than calves that were separated from their mothers soon after birth.

In conventional dairy production, calves are usually separated from their dams within 24 h after birth (EFSA, 2009), which raises public attention and concern (Busch *et al.*, 2017). Alternative systems, which allow cow-calf or specifically dam-calf contact are receiving increased attention (Busch *et al.*, 2017; Beaver *et al.*, 2019; Brombin *et al.*, 2019). However, information about the effect of rearing calves with their dams on calves' behaviour, emotionality, and social competence is still needed.

Rearing with dams shows indications of reduced fearfulness and improved social competence in dairy calves (Krohn *et al.*, 1999; Wagner *et al.*, 2012, 2013, 2015; Buchli *et al.*, 2017), though inconsistencies in behavioural and physiological indicators of emotionality emerged (Le Neindre, 1989; Wagner *et al.*, 2013; Wagner *et al.*, 2015). In cattle, enhanced locomotor activity is an indicator of stress (Pérez *et al.*, 2017) and reduced locomotion can be an indicator of fearfulness (Van Reenen *et al.*, 2005). Calves reared with their dams for a minimum of 30 d are more submissive when confronted with an unfamiliar cow (Buchli *et al.*, 2017) than calves reared without dams. This effect is long lasting, as animals reared with the dams in the first three months of age express more submissive behaviour when integrated into a herd compared to animals separated from the dams immediately after birth (Wagner *et al.*, 2012). Further, compared to separated calves, dam-reared calves show a tendency for increased vigilant behaviour when confronted with an unfamiliar calf (Wagner *et al.*, 2013). The greater social competence in dam-reared calves may be due to the contact to dams or non-related cows, as calves with contact to dams, but not those separated from mothers, could have a social experience with non-related cows. Thus, it is not clear, if contact to either dams or non-related cows leads to the development of social skills, which allow calves to respond more appropriately to social signals from conspecifics (Buchli *et al.*, 2017). Furthermore, while nursing is not the only maternal behaviour, it has calming effects on calves (Hänninen *et al.*, 2008). Therefore, suckled milk was another confounding factor in the previous studies.

The aim of the present study was to investigate the influence of maternal contact in the first 14 d of age on calves' activity, fearfulness, and social competence. We hypothesised that

compared to calves raised with dams (Maternal Contact calves), Motherless calves (calves separated from dams shortly after birth) might be experiencing greater stress and searching more actively for social contact, and thus exhibit greater locomotion in the first weeks of life. Locomotion in Maternal Contact calves was expected to increase after separation from dams. We further hypothesised that Maternal Contact calves would show fewer indicators of fearfulness in a novel situation than Motherless calves as expressed in fewer vocalisations, longer exploration, increased ambulation, and shorter immobility (Wagner *et al.*, 2015). During confrontation with an adult cow, Maternal Contact calves were expected to be more vigilant (Wagner *et al.*, 2013; Buchli *et al.*, 2017) and submissive (Wagner *et al.*, 2012; Buchli *et al.*, 2017) than Motherless calves due to their greater social competence.

Materials and methods

Animals, housing, management

This study was approved by the animal welfare officers of the JLU-Giessen, Germany, and the ARRIVE guidelines were considered when writing this manuscript. The study was conducted at the research farm Oberer Hardthof (Oberer Hardthof 25, 35398 Giessen, Germany) with a total of 24 calves of five breeds: German Holstein ($n = 18$), Jersey ($n = 3$), Simmental ($n = 1$), Brown Swiss ($n = 1$), and Angler ($n = 1$). The calves either had (Maternal Contact, $n = 12$) or did not have access (Motherless, $n = 12$) to their dams in their first 14 d of age. There were four female and eight male calves assigned to the Maternal Contact treatment and five female and seven male calves assigned to the Motherless treatment. The calves were assigned to their respective treatment groups alternating based on the expected date of birth and the mothers' parity with the first calf being arbitrarily assigned to the Motherless group.

Six to eight weeks prior to calving, eleven primiparous and twelve multiparous cows between the age of two and five years were dried off (multiparous only) and housed in a pen with deep-bedded loose housing where they were kept until calving. The design of the dairy unit is shown in online Supplementary Fig. S1 together with a full explanation of accommodation at all stages of the experiment. After birth, calves of both treatments and the dams of Maternal Contact calves were housed within the same group in a single pen ($5 \text{ m}^2/\text{animal}$) adjacent to the calving pen until the calves were 14 d old. This was done to allow calves of both treatments social contact with other calves and the dams of Maternal Contact calves. In the first 14 d of age, the group consisted of up to eight animals (four calves and four mothers) and calves differed in age (one to 14 d). Within the first 12 h after birth, all calves received 2–3 l colostrum from a bucket. From then on, the calves were bucket-fed with whole milk (4–8 l/d in total) twice a day at $\sim 6:00$ and $\sim 16:00$. The amount of whole milk was adjusted to the calves' milk intake and increased with increasing age. Water, hay, silage, and concentrate were supplied *ad libitum* from the first day on.

Experimental design and procedure

Treatment

Motherless calves were separated from their mothers no later than 12 h after birth and the dams spent the first 24 h after separation in another part of the barn where audible but no tactile or visual

contact with the calves was possible. After this initial, 24-h separation, the mothers of Motherless calves were brought to the production herd which was positioned in the same barn as the calves, adjacent to them but separated by a feeding alley (online Supplementary Fig. S1). Maternal Contact calves had almost uninterrupted contact with their mothers for the first 5 d of age and were separated from the mothers only during milking in the morning and in the afternoon for approximately 15 min. To prevent the calves from suckling, the dams were equipped with udder nets (Siepmann GmbH, Wittener Landstraße 19, 58313 Herdecke, Germany, HK-Zitzenschutz, size L) no later than 12 h after calving. During 6–14 d of calves' age, Maternal Contact calves had contact to their dams in the night-time between the milking sessions, while in the daytime, dams were in the separation pen. At 14 d of age, all calves were moved outside the barn and kept in groups of three to five animals in commercial pens with igloos. The pens had concrete floors and the igloos were straw bedded. The dams were moved to the production herd at this point.

Behavioural tests for assessment of emotionality and social skills

We assessed calves' behaviour patterns and social interactions (descriptions of the behaviours in online Supplementary Table S1) in novel environments and challenging situations. All calves were tested for 15 min in the open field (novel environment and social isolation), novel object (novel experience and social isolation), and confrontation test with an unfamiliar cow (novel social experience) at 14, 21, and 28 d of age, respectively.

The test arena ($5 \text{ m} \times 3.5 \text{ m}$: see online Supplementary Fig. S1) was positioned outside of the barn and enclosed with a fence (Texas Trading Zaunelement P10, height: 1.75 m) covered with a black plastic tarp on two sides. After eleven calves were tested, the tarp was replaced with black wooden boards. The two remaining sides of the arena were the outer walls of the barn. The floor was visually divided into $0.5 \text{ m} \times 0.5 \text{ m}$ squares with a silver-coloured spray. Calves in the test arena had audible, but no physical or visual contact to animals outside the arena. After each test, the arena was brush cleaned with water.

Open field test

All except five tests (four by JS and one by JS & AV) were performed by NS. The majority ($n = 21$) of calves were tested between 14:00 and 14:30. For testing, each calf was guided from the calving pen into the test arena on a halter. The halter was removed at the entrance of the arena and the calf was left alone inside the arena. After the completion of the test, the calf was led from the test arena to an igloo pen positioned outside of the barn.

Novel object test

The novel object test was performed in the same test arena as the open field test. A concrete-filled, red traffic cone with an attached metal handle at its tip (total height: 0.56 m) was used as a novel object and placed in the centre of the test arena on the ground before the test began. All except eight tests (six by JS, one by JS & AV, one by AV & AS), were performed by NS. The majority of calves ($n = 21$) were tested between 14:00 and 14:30. The same procedure as in the open field test was used to bring calves into the test arena and back to the igloo pen.

Confrontation test

Six tests were conducted by JS, one by AV & AS, and 17 tests were conducted by NS. The majority of calves ($n = 21$) were tested between 10:00 and 11:00. Before the test, always the same unfamiliar cow from the production herd was brought to the test arena either by JS, NS, or farm staff. The cow was tied to the fence surrounding the arena opposite to the entrance. She was released immediately after the calf entered the arena. The calf was brought to the arena following the same procedure as in the previous two tests. After completion of the test, the cow was brought back to the production herd by the experimenter, while the calf remained in the test arena. As soon as the cow was returned to the herd, the calf was brought back to the igloo pen.

Data recording

Social interactions

All social interactions on day 1 of calves' life were recorded for 30 min immediately after birth and after 12 h. Since this was not the focus of the study, the data is presented descriptively in the supplementary material (online Supplementary Table S2).

General activity

To measure calves' general activity, eight Maternal Contact and eight Motherless calves were equipped with pedometers (ENGS Systems, POB 77 Rosh Pina 1200, Israel) on the right front leg no later than 48 h after birth. Due to late delivery of the pedometers, activity data was unavailable for the first eight calves of the study. The pedometers were removed after completion of the confrontation test. Data from the third to the 27th day of age were used to evaluate the calves' locomotion (steps/hour), lying behaviour (abdomen touching the ground; minutes/hour), and swapping between standing and lying (changes/hour) (Alsaad *et al.*, 2012).

Assessment of behavioural patterns indicative of emotionality and social competence

Calves' behaviour in each test was video recorded by an experimenter located outside the arena from the time of removing the halter for 15 min. Behaviours were recorded using an Everio HD memory camcorder. The experimenter, blinded to the treatment, scored the behaviour with the software package INTERACT (Mangold International GmbH, 2011, Version 14, Graf-von-Deym-Straße 5, 94424 Arnstorf, Germany). Behaviour of the category 'playing' was never observed in the open field test and only four calves showed play behaviour in the novel object test. Thus, playing was only analysed in the confrontation test.

Saliva collection for the analysis of the adrenocortical reactivity

Before the confrontation test, a halter was attached to the calf and a saliva sample was taken for the cortisol analysis. Another saliva sample was taken 5 min after completion of the test. We also collected saliva samples before and after each open field test. However, due to initial problems with the collection of saliva samples, saliva samples from the open field test were not analysed. Saliva samples were collected as described by Pieler *et al.* (2013). A cotton swab (Salivette; Sarstedt AG & Co., Sarstedtstraße 1, 51588 Nümbrecht-Rommelsdorf, Germany)

was placed into the mouth of a calf until it was well soaked and was then centrifuged for 15 min at $4000 \times g$. Saliva was then frozen at -20°C until the analysis. Saliva samples were analysed in duplicates within one lot of reagents. A commercial enzyme-immuno-assay was used (IBL International GmbH, Flughafenstraße 52a, 22335 Hamburg, Germany) and analyses were completely automatically processed on an analyser (BEP2000, Siemens Healthcare GmbH, Henkestr. 127, 91052 Erlangen, Germany). Intra-assay coefficient of variation was below 3% and inter-assay below 5%.

Statistical analysis

The statistical analysis was done in Python programming language using SciPy stack-Scientific Computing Tools for Python (version 3.6.5, Python Software Foundation, 9450 SW Gemini Dr, ECM# 90772, Beaverton, OR 97008, USA). Residuals were examined to verify normality and homogeneity of variances. Due to non-normality and heterogeneity of variances, all data were analysed with the Mann-Whitney-*U*-test to determine differences between Maternal Contact and Motherless calves. Treatment was the only independent variable. Behaviour, cortisol level change, and calves' general activity were dependent variables.

Predictors were considered significant at $P < 0.05$, however, as this study had partly an exploratory character and multiple statistical models were tested, we recommend interpreting *P*-values against a Bonferroni corrected threshold level α' which was calculated for each test. The Bonferroni corrected threshold levels α' were 0.008, 0.006, and 0.004 for the open field test ($k = 6$), novel object test ($k = 9$), and the confrontation test ($k = 12$), respectively. For each general activity level, the Bonferroni corrected threshold level α' was 0.01 ($k = 5$).

General activity

We imputed the missing values for the general activity of one calf (Motherless treatment) for its 3rd and 4th day of age by calculating the mean value of all seven other Motherless calves for days three and four, respectively. The missing values had to be imputed due to technical issues with the sensor. We performed five Mann-Whitney-*U*-tests for each type of general activity data: (1) differences between Maternal Contact and Motherless calves during the 3rd and 27th day of age, (2) differences in general activity between treatments in the period of the 3rd to 13th day of age (period A, i.e. before separation of Maternal Contact calves from the dams), and (3) differences in general activity between treatments during the 14th to 27th day of age (period B). (4 and 5) compare periods A and B within each treatment. This was done because general activity might be affected by calves' age or the separation of Maternal Contact calves from the dams.

Results and discussion

General activity

Data for locomotion are depicted in Fig. 1 and detailed in online Supplementary Table S3. Locomotion in the recorded 24-d period was greater in Motherless calves than in Maternal Contact calves (Fig. 1a), which was mainly due to the treatment difference in period B (i.e. older calves, Fig. 1b). Motherless calves exhibited greater locomotion than Maternal Contact calves also before Maternal Contact calves were separated from the dams (period

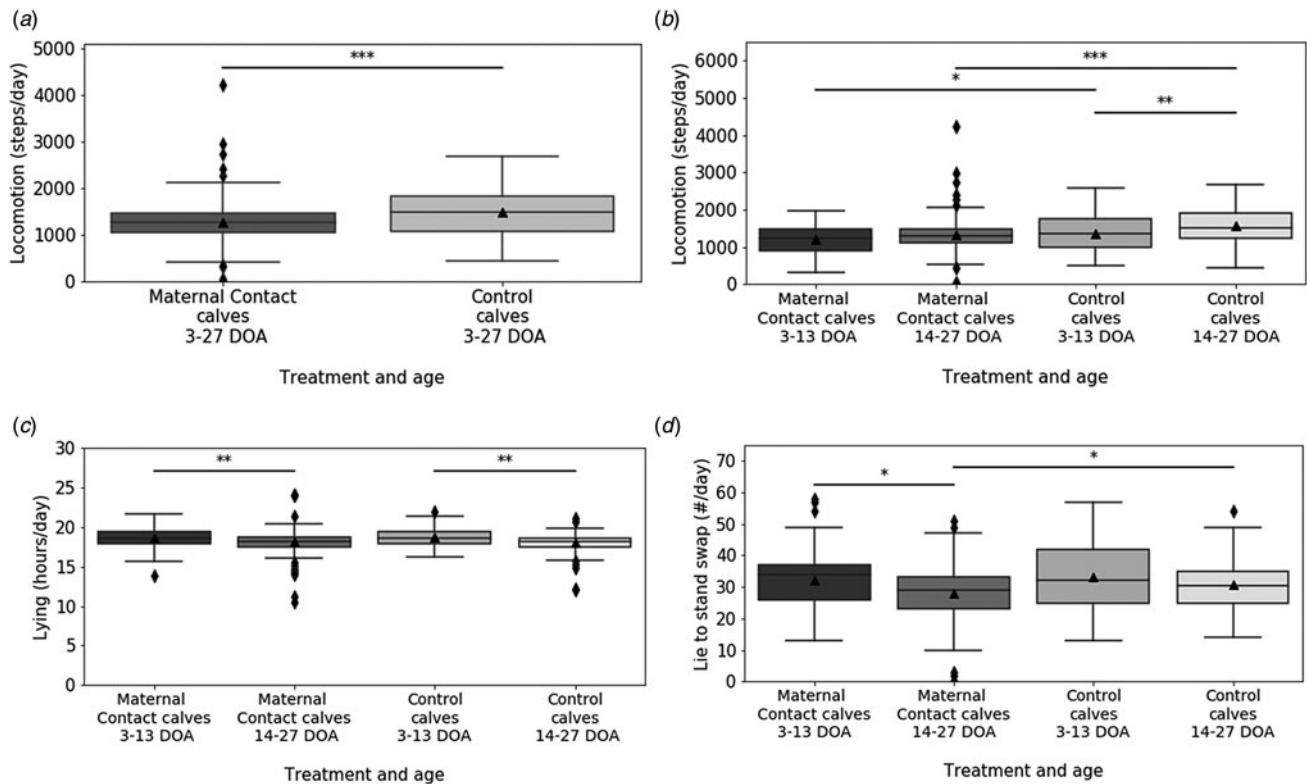


Fig. 1. (a) shows the number of steps per day (steps/day) for Maternal Contact and Motherless calves during the calves' third to 27th day of age. Locomotion per day (steps/day) (b), the duration of lying per day (hours/day) (c) and the daily number of swapping events between lying and standing (#/day) (d) during the calves' third to 13th and 14th to 27th day of age for Maternal Contact and Motherless calves. Box-and-whisker-plots represent the data, with boxes depicting the inter-quartile range with the central bar as the median. The whiskers mark regular data, where everything beyond is an outlier. Outliers are represented as diamonds and the mean values as triangles. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

A, Fig. 1b), but this difference was no longer significant after the Bonferroni correction. As Motherless calves were separated from the dams before bonding with the dams fully developed (Flower and Weary, 2001), increased locomotion in Motherless calves probably indicated calves' need for social contact. If there is no mother, as in the case of early separation, calves tend to search social contact in other animals (Stěhulová *et al.*, 2008), which might explain the increased locomotion in Motherless calves. Social contact to cows has survival benefits for the calves (Von Keyserlingk and Weary, 2007). This motivation to be mothered can be satisfied by providing foster cows (Le Neindre and Garel, 1979). In our study, we only observed behavioural interactions among animals on day 1 of age (online Supplementary Table S2). While in the first day of life all but one Motherless calf had physical contact to cows other than their dam as well as other calves, only two Maternal Contact calves contacted other cows and calves. Data of social interactions supports our prediction that Motherless calves might have searched social contact, however, observations of social interactions over many days will be needed to investigate that. Further, while Motherless calves expressed greater locomotion in period B than in period A, age did not affect locomotion in Maternal Contact calves (Fig. 1b). This result surprised us, as increased locomotion is an indicator of separation distress in calves (Flower and Weary, 2001) and was thus expected to increase in Maternal Contact calves after permanent separation from the dams. However, as Flower and Weary (2001) showed, locomotion after separation increases for some hours and then decreases. Thus, an increased locomotion due to separation could not be seen over the two-week period.

Further, our calves were prevented from suckling the dams and nutritional independence from the mother has been shown to reduce separation distress in calves (Johnsen *et al.*, 2018).

Data for lying behaviour are in online Supplementary Table S3 and Fig. 1c. There was no difference between the treatments regarding the total duration of lying in the recorded period of 24 d or in either period A or B. Thus, despite an increased locomotion in Motherless calves, calves in both treatments were able to rest. This result is contra to previous reports (Kerr and Wood-Gush, 1987), where calves with maternal contact showed longer lying behaviour than early separated calves. In the study from Kerr and Wood-Gush (1987), calves with maternal contact, and not calves separated from the mother, could be active on the pasture, which might lead to an increased need to rest, though locomotion was not recorded to support this assumption. In our study, calves of both treatments had similar space allowance, which might result in no treatment difference in the lying duration in the 24-d period. Similar to previous reports (Swanson and Harris, 1958; Kerr and Wood-Gush, 1987), in our study, the duration of lying decreased in older calves in both treatments (Fig. 1c) which indicates that calves were developing appropriately for their species.

Swapping between lying and standing did not differ between the treatments in the observed 24-d period or period A, but in period B Motherless calves swapped more often than Maternal Contact calves (online Supplementary Table S3, Fig. 1d), but the difference was no longer significant after the Bonferroni correction. There was no difference in swapping between periods A and B in Motherless calves. Maternal Contact calves

exhibited less swapping in period B than in period A (Fig. 1d), but the difference was no longer significant after the Bonferroni correction.

Behaviour patterns indicative of emotionality

Data pertaining to the behavioural tests are shown in Table 1. Compared to Motherless calves, Maternal Contact calves expressed fewer vocalisations in the open field test and were immobile for a shorter period when confronted with an unknown cow. This provides some evidence that Maternal Contact calves might have been less fearful during behaviour tests than Motherless calves. However, other behaviours in the tests did not differ between treatments. Furthermore, after the Bonferroni correction, the treatment differences in vocalisation and immobility were no longer evident. Our results are thus in line with the results from Buchli *et al.* (2017) who also showed no difference in fearfulness in calves reared with or without their mothers. While others showed some indications of reduced fearfulness in calves reared with mothers, those effects were minimal and no corrections for large numbers of tests were considered (Wagner *et al.*, 2012, 2013, 2015).

In our study, Maternal Contact calves were more vigilant than Motherless calves when confronted with an unknown cow. It was previously indicated that calves reared with their mothers have greater social skills than calves separated from the dams soon after birth (Buchli *et al.*, 2017). It has been proposed that during early social interactions communicative skills are developed (Bekoff, 1972). Specifically, Bekoff (1972) suggests that one very important skill that must be acquired early in life is to differentiate signals indicative of prosocial and agonistic intentions of the social companions. Our results indicate that calves start to develop social skills immediately after birth. However, social skills probably keep developing as animals mature. For instance, while we saw no treatment difference in submissive behaviour, Wagner *et al.* (2012) and Buchli *et al.* (2017) who tested calves with contact to either dams or foster mothers for a minimum of 30 d (and up to 180 d) after birth showed that calves reared with either their dams or foster mothers showed more submissive behaviour than those raised without a mother. In contrast to other studies in which only calves that were reared with the dams had contact to other cows, we allowed equal opportunities for social contact (to adult and young companions) in calves of both treatments. While calves gain early social skills from the dam, individuals other than the dam are likely important at a greater age. In semi-natural conditions, calves spend their first days with the mother isolated from the herd and are brought to the herd once the cow-calf bond is established (Reinhardt, 1980). Thus, calves in semi-natural environment obtain first social skills from the dam.

Adrenocortical reactivity

There was no treatment difference before the test in the baseline value of cortisol ($\mu \pm \sigma = 0.13 \pm 0.06 \mu\text{g/dl}$, $U = 55.5$, $P = 0.178$) indicating that there was no difference in the stress levels between Maternal Contact and Motherless calves. Wagner *et al.* (2015) showed that calves with permanent contact to dams had reduced basal levels of cortisol one month after separation from the mothers compared to calves with two 15-min maternal visits or early-separated calves. As our calves received maternal contact only during night hours, this might not have been sufficient to lead to long-lasting reduction in the baseline cortisol level. To

Table 1. Mean (\pm sd) duration (D , in seconds/15 min) and number of events (E , in #/15 min) of displayed behaviours in the open field (OF, $n = 24$), novel object (NO, $n = 24$), and confrontation test with an adult cow (CO, $n = 24$)

Behaviour	Results		
	Mean \pm sd	U	P
Open field test			
Grooming (D)	13.3 \pm 19.0	53.5	0.147
Immobility (D)	297.0 \pm 127.8	59.5	0.244
Elimination (E)	1.0 \pm 1.0	70.5	0.475
Ambulation (E)	179.0 \pm 142.0	63.0	0.312
Vocalisation (E)		43.0	0.044*
Motherless	4.8 \pm 8.5		
Maternal contact	13.2 \pm 15.8		
Exploration (D)	235.1 \pm 117.8	59.5	0.244
Novel object test			
Grooming (D)	14.2 \pm 19.9	63.5	0.312
Immobility (D)	168.9 \pm 128.3	69.0	0.443
Elimination (E)	1.0 \pm 1.0	68.0	0.412
Ambulation (E)	157.0 \pm 100.0	70.0	0.465
Vocalisation (E)	6.0 \pm 10.0	60.5	0.248
Exploration (D)	317.2 \pm 165.8	63.0	0.312
Latency to first contact with the novel object (seconds)	69.2 \pm 84.8	55.0	0.170
Contact with the novel object (D)	36.0 \pm 35.0	48.5	0.092
Ambulation near the novel object (E)	31.0 \pm 16.0	65.5	0.364
Confrontation test			
Grooming (D)	8.4 \pm 11.5	44.5	0.057
Immobility (D)		40.5	0.037*
Motherless	328.1 \pm 151.0		
Maternal contact	236.4 \pm 145.0		
Elimination (E)	1.0 \pm 1.0	49.0	0.076
Ambulation (E)	144.0 \pm 74.0	58.5	0.226
Vocalisation (E)	2.0 \pm 6.0	54.5	0.129
Exploration (D)	266.2 \pm 168.9	53.0	0.413
Playing ^a (D)	4.5 \pm 9.3	54.0	0.134
Sniffing unfamiliar cow (D)	0.3 \pm 1.1	66.0	0.375
Physical contact with unfamiliar cow (D)	1.4 \pm 2.4	67.0	0.388
Submission (D)	2.8 \pm 3.1	59.0	0.230
Vigilance (D)		30.5	0.008**
Motherless	3.8 \pm 1.5		
Maternal contact	16 \pm 17.1		
Received agonistic behaviour (D)	10.8 \pm 6.2	65.0	0.353

^aPlaying was never observed in the OF and only four calves showed play behaviour in the NO. Thus, playing was only analysed in the CO. * indicates levels of significance with * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

better understand the mechanism of establishment of basal cortisol levels, future effort should measure basal cortisol level repeatedly at various ages in calves with and without maternal contact.

Furthermore, for the saliva sampling, our calves had to be restrained. As calves were not yet used to being restrained, the restraint itself might have affected the basal cortisol level. However, since cortisol reaches saliva *via* passive diffusion, there is a time-lag of 10 min between peak cortisol levels in blood and in saliva (Bozovic *et al.*, 2013; Hernandez *et al.*, 2014), so that the potential influence should be minimal. In our study, we also did not observe differences between Maternal Contact and Motherless calves in the change in cortisol levels from before to after the confrontation test ($\mu \pm \sigma = 0.13 \pm 0.06$ $\mu\text{g/dl}$, $U = 65.0$, $P = 0.354$), which is in line with the results by Wagner *et al.* (2015) and the behavioural data of our study, which indicated no treatment effect on fearfulness.

In conclusion, there was a weak indication that Maternal Contact calves were less fearful than Motherless calves, though the results should be considered with caution. Maternal contact in the first 14 d of age seems to be of importance for the development of calves' social competence. Although our Motherless calves were likely seeking social contact as seen by increased locomotion compared to Maternal Contact calves, the received social interactions were likely not advantageous for social skill learning. Further effort should investigate the effects of maternal and other social contacts on development of social skills with increasing age.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029920000527>

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