Evaluation of bonding and maternal behaviours in cattle

– Swedish dairy cows in a cow-calf contact system

Utvärdering av bonding och moders beteende hos nötkreatur – Svenska mjölkkor i ko-kalv kontakt system

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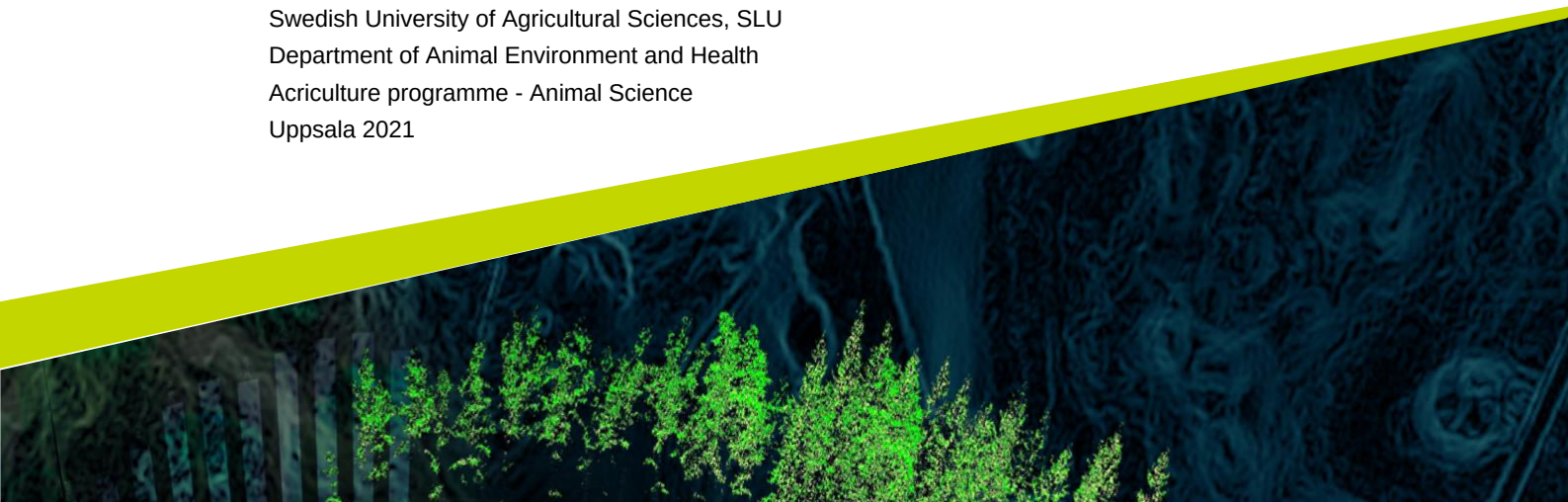

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Abstract

In today's commercial dairy production, it is common practice to separate the cow and calf a few hours after parturition. There is, however, increased interest from both farmers and consumers, to keep the cow and calf together for a longer period while still maintaining dairy production, because it is perceived as more animal friendly. This puts a higher demand on the cow to rear the calf and it is required that the cow show maternal behaviours and can bond with and take care of the calf. Since the dairy cow has been separated from the calf for generations, the selection for maternal behaviours may have been less rigid.

This study aimed to investigate the maternal behaviours of modern dairy cows and assess bonding behaviours between cow and calf as well as to investigate breed and parity differences in the expression of maternal behaviours. A total of 10 Swedish Red (SR) and 9 Swedish Holstein (SH) cows with either a female (n = 15) or male calf (n = 4) were included in the study. The animals were housed in a cow-calf contact system from 48 hours after birth. Maternal-filial interactions were observed at the maternity pen when the calves were one day old. A preference test was used at 4 weeks of age to assess the time that calves spent in proximity of the mother cow in comparison to an unfamiliar cow (calf test) and to assess the preference of the mother for her calf over an unfamiliar calf (cow test). A neophobia test to determine the calves' use of the mother as a secure base to explore was performed at 5 weeks of age. Data were analysed in R using Generalized Linear Models (count data) and Generalized Linear Mixed Models (continuous data).

On the first day after birth, SR cows sniffed their calves more than SH cows (Deviance 54.992, $p<0.01$), and multiparous (MP) cows vocalized more than primiparous (PP) cows (Deviance 138.078, $p<0.05$). In the calf preference test, calves of PP cows sniffed the unfamiliar cow more often than calves of MP cows (Deviance 3.9359, $p<0.05$). SR calves vocalized more than SH calves (Deviance 21.0147, $p<0.01$), and male calves vocalized more than female calves (Deviance 22.768, $p<0.01$). Also, SR calves were closer to their mothers for a longer period ($F_{1,14}=5.3897$, $p<0.05$). In the cow preference test, the SR cows were sniffed more often by the unfamiliar calves in the choice pen than the SH cows, i.e the unfamiliar calves interacted more with the tested cow when SR cows were tested (Deviance 8.2290, $p<0.01$). MP cows were sniffed more often by their calves compared to PP cows (Deviance 5.0111, $p<0.05$). Males calves were also more vocal during the cow test (Deviance 26.942, $p<0.05$). Cows with female calves were faster to leave the start box ($F_{1,12} = 5.4634$, $p<0.05$). During the neophobia test, cows with a male calf spent more time in the buckets ($F_{1,14}=8.0770$, $p<0.05$), ate more concentrate ($F_{1,13} = 6.2011$, $p<0.05$) and kept a longer distance to their calves, compared to female pairs ($F_{1,14}=8.4190$, $p<0.05$). Furthermore, the calves of PP cows explored more and spent more time in the buckets ($F_{1,14}=5.7627$, $p<0.05$).

After studying the maternal behaviours and bonding in the modern dairy cows it was found that SR cows and calves displayed a stronger filial bond and that cow parity also played a role in the expression of bonding behaviours. Due to inbalanced number of male and female calves no conclusion can be made regarding sex effects.

Further studies on bonding behaviour development in cow-calf systems and its impact on later social behaviours are encouraged.

Keywords: cow-calf contact, maternal behaviour, bonding, dairy production

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Abbreviations

MP	Multiparous
PP	Primiparous
SH	Swedish Holstein
SR	Swedish Red
Week 1	Test week one when the calf is one week of age
Week 4	Test week four when the calf is four weeks of age

1. Introduction

In commercial dairy production with *Bos Taurus,* the cow and calf have been separated shortly after birth for centuries due to many reasons. The common practice today is to separate the calf from the mother within 24 hours after birth (Agenäs, 2020) and then house the calf in a separated location where it is fed artificially with whole milk or milk replacer (Busch et al., 2017). With modern techniques such as the milking robot and the high milk yield of the cow, the interest for keeping the cow and calf together for a longer period in a cow-calf system has increased both by farmers and consumers (Agenäs, 2017). One of the main concerns of consumers about commercial dairy production is the early separation between cow and calf, and there is an increased demand for developing a management system that allows having the cow and calf together and still maintaining high dairy production (Agenäs, 2017). Keeping cows and calves together puts a higher demand on the cows to rear their calf and requires that the cows express maternal behaviours and can bond with and take care of their offspring.

This master thesis was part of the project Sustainable dairy production with cow and calf together at The Swedish University of Agricultural Science. The purpose of this study was to investigate bonding development between high producing dairy cows and their calves and describe maternal behaviours in modern dairy cows that were kept with their calves, as well as investigating breed and parity differences in maternal behaviours between cows. Further, we aimed to describe specific behaviours shown by the cows and calves that might indicate a bond between the cow and calf by performing a preference test and a neophobia test.

2. Literature

In previous studies, when cows of dairy breeds were kept together with their calves, problems have been observed where the cows also licked alien calves and allowed them to nurse (Edwards, 1983). Feral cattle have been observed to rarely nurse or lick alien calves and it may be due to a stronger mother-offspring bond (Vitale et al., 1986). Adopting behaviours by dairy cows may be caused by weakened maternal behaviours due to genetic changes during the decades when maternal behaviours have not been a priority in breeding (Rørvang et al., 2018). Though, in studies with other domesticated animals such as pigs, results have suggested that maternal behaviour have been preserved during domestication (Jensen, 1986; Nowak et al., 2000).

2.1. Bonding

Maternal bonding and maternal behaviours between modern dairy cows and their calves during the period around parturition are well described in literature (Edwards & Broom, 1982; Edwards, 1983; Weary & Chua, 2000; Stěhulová et al., 2008; Kent, 2020). The maternal bonding and parent-offspring relationship at a later stage in lactation are however not that well studied when it comes to modern dairy cows, since they are often separated from their calves soon after birth. A recent study showed that a week of nightly cow-calf contact and the calf being allowed to suckle positively affected the cows' motivation to reunite with their calves compared to cows with cow-calf contact but not being suckled by the calf and cows with no cow-calf contact at all (Wenker et al., 2020). Maternal behaviours and bonding are thought well described in literature when it comes to beef cattle (Buddenberg et al., 1986; Lidfors & Jensen, 1988; Le Neindre, 1989; von Keyserlingk & Weary, 2007; Hoppe et al., 2008; Stěhulová et al., 2013) and buffalos (Orihuela et al., 2021) that often are raised with their calves for longer periods. Bonding and attachment studies have also been performed in humans for decades and the development of a theory of attachment has been made by John Bowlby (1969) where other researchers later have contributed to that work and extended it to several other species (Ainsworth,

1969; Gubernick, 1981). The bond between mother and infant does not exist at birth but develops during the first year of life (Nowak & Boivin, 2015).

Social bonding is defined as the mutual, affiliative relationship between two individuals (i.e. between mother and infant) that lasts for a relatively long time and survives temporary separation (Gubernick, 1981; Newberry & Swanson, 2008; Nowak & Boivin, 2015). This bond is characterized by maintaining proximity, performing synchronized activities, and expressing affiliative behaviours like allogrooming, nursing and provision of warmth and protection (Gubernick, 1981; Newberry & Swanson, 2008). When individuals who are bonded were reunited after being separated they exhibited reinstatement and greeting behaviours (Newberry & Swanson, 2008). Keeping close proximity between the mother and young increased the opportunities for social transmission of information about food sources and predators (Thorhallsdottir et al., 1990; Newberry & Swanson, 2008). Measure and study bonding development in animals has its challenges since they verbally cannot express their emotions (Gubernick, 1981; Nowak & Boivin, 2015).

2.2. Behaviours between cow and calf

There is a possibility that dairy cattle have less strong maternal behaviours compared to beef cows (Kiley-Worthington & de la Plain, 1983). Since dairy cows have been separated from their calves for generations, the selection for maternal behaviours might have been absent and become less rigid (Kiley-Worthington & de la Plain, 1983). Beef cows are normally reared together with their calves, on the other hand, and are likely to have been selected for better maternal behaviours (Kiley-Worthington & de la Plain, 1983). Two important characteristics of maternal behaviour are early calf recognition and the possibility to create a strong bond between cow and calf (Kiley-Worthington & de la Plain, 1983).

The maternal behaviours performed by the cows especially in the beginning after parturition can be affected by the rearing conditions when the cows themself are young (Le Neindre, 1989). During the first four hours after birth, non-mothered primiparous (PP) cows took longer to start licking their calves after birth but spent more time licking than mothered PP cows did, this difference was non-existing during observations one month later (Le Neindre, 1989). Calves to non-mothered PP cows required fewer attempts before the first successful suckling after birth but at one month of age, the calves to mothered PP cows suckled more than calves to non-mothered PP cows (Le Neindre, 1989). There was also a difference in how long the cows let an alien calf suckle; non-mothered Friesians were more accepting than mothered Friesians to accept an alien young (Le Neindre, 1989). In the study, it was

shown that the Friesian cows were able to rear their own calves but the cows seemed to have trouble with preventing alien calves to suckle (Le Neindre, 1989). However, Lidfors *et al.,* (1994b) observed a few occasions where cows of either Swedish Red (SR) or Swedish Holstein (SH) sniffed on, pushed or threatened alien calves.

Licking and sniffing immediately after birth are typical behaviours of ungulates, which facilitate the mother to recognize the odour and features of the young so that further parental investment is directed to her offspring (Alexander & Shillito, 1977). Hudson & Mullord, (1977) noticed that the first hours after birth were most important for the bond between the cow and calf, since the bond was not formed in 50% of the animals if no contact was allowed in the first 5h directly after birth.

Maternal behaviours have in previous studies been observed to differ between breeds, both between dairy breeds (Lidfors, 1996; Loberg & Lidfors, 2001) and between dairy and beef breeds (Selman et al., 1970; Le Neindre, 1989; Geburt et al., 2015). SR has been observed to show more maternal behaviours when compared to SH (Lidfors, 1996; Loberg & Lidfors, 2001). Some maternal behaviours have also been observed in previous studies to be affected by parity (Edwards & Broom, 1982; Le Neindre & D'Hour, 1989; Lidfors, 1996; Stěhulová et al., 2013). Multiparous (MP) cows have been observed to lick unfamiliar calves more than PP cows (Edwards, 1983). The sex of the calf has also been observed to affect the maternal care, where male calves were given more protective care from the cows than female calves (Stěhulová et al., 2013). Lidfors & Jensen (1988) did however not find any difference between the sex and maternal behaviours directed towards the cows or the calves in beef cattle. One-month-old male calves have been observed to be licked more often than females but one-year-old female calves were licked longer than males (Le Neindre, 1989).

2.2.1. Licking

Licking is an important maternal behaviour and cows spend a lot of time licking the calves, especially during the first hours after birth (von Keyserlingk & Weary, 2007). MP cows tended to lick their calves for a longer period compared to PP cows during 5 minutes observations (Le Neindre & D'Hour, 1989) respectively mean duration of 117 minutes observations (Lidfors et al., 1994b). However, MP cows tended to spend more time licking during the first hour, while PP cows compensated with more licking during the second hour (Edwards & Broom, 1982).

For establishing the bonding between the cow and calf, licking of the newborn is considered essential and lack of licking can result in a breakdown of maternal behaviour of cows since the cows do not learn the calf's odour (von Keyserlingk &

Weary, 2007). During the critical bonding period immediately after birth, it is important that the cows do not come in contact with other calves because licking alien calves might interfere with the bonding process between the mother and young (von Keyserlingk & Weary, 2007). In nature, the cows often separate from the herd before calving and there are speculations that one reason for this is to reduce the risk of both the calves and cows coming in contact with other animals, and so avoid bonding with alien calves (von Keyserlingk & Weary, 2007). Therefore, the housing of the cows right after calving can affect the bonding process and cows that are housed nearby others might have a higher incidence of failed bonding but further research needs to be done (von Keyserlingk & Weary, 2007).

2.2.2. Vocalization

When keeping the cows and calves together in a maternity pen, the cows tended to be more vocal during the first hours after birth compared to when the calves were 24 hours or older (Weary & Chua, 2000). The call rate went from 60 calls/h when the calves were 6 h of age to 0.3 calls/h at 72h of age (Weary & Chua, 2000; von Keyserlingk & Weary, 2007). Barfield *et al.*, (1994) showed that the calves could recognize the vocalizations of the mothers even if the mothers were not visible and could even distinguish between vocalizations from the mothers and alien cows. However, they only tested the calves' recognition of the mother between three to five weeks of age, and there was no major difference between the weeks (Barfield et al., 1994).

2.2.3. Nursing

Nursing is one of the most important maternal behaviours in cattle and within a few hours after birth, the cows would let the calves suckle (von Keyserlingk & Weary, 2007). During the first week of life, the mothers initiated a lot of the nursing events but this then decreased over time (Lidfors et al., 1994a). Often, the suckling bouts were initiated by the calves especially after day seven and it increased by age (Lidfors et al., 1994a).

2.3. Measuring bonding

Since bonding is defined as a relationship involving affections and emotions between two individuals, it is not possible to directly measure the strength of the bond with just the behavioural responses, it also requires verbal support of perceived emotions (Nowak & Boivin, 2015). In animals, it is only possible to determine whether animals are bonded by measuring the behavioural and

physiological criteria since they lack verbal language that is possible for us to understand (Nowak & Boivin, 2015). Therefore, the occurrence of a bond between two animals is based on attachment behaviours, but these behaviours may differ depending on the situation and the motivation of the individual (Gubernick, 1981; Nowak & Boivin, 2015). To measure bonding when lack of verbal support, some criteria have been used in previous studies on both humans and animals (Gubernick, 1981; Nowak & Boivin, 2015). The criteria used are: the preference for one individual over another; seeking and maintenance of proximity to the bonded individual; response to separation from the presumed attachment individual; response to a reunion and the use of the attachment individual as a secure base to explore the environment (Gubernick, 1981; Nowak & Boivin, 2015). These five criteria should be used in combination to determine if individuals are bonded or not (Nowak & Boivin, 2015).

Preference for one individual over another

To test the preference, the tested individual is given a choice between a familiar individual and a matched individual that is unfamiliar to the tested individual (Gubernick, 1981; Nowak & Boivin, 2015). The choice between the familiar and unfamiliar individual can either be presented simultaneous or sequentially (Gubernick, 1981; Nowak & Boivin, 2015). The test can both be done in non-human mammals or humans (Nowak & Boivin, 2015). Preference for an individual is determined by the difference in certain behaviours displayed in presence of or directed towards the assumed attached figure, compared to the same behaviour directed towards an unfamiliar individual (Gubernick, 1981; Nowak & Boivin, 2015). If the tested individual can interact physically with the unfamiliar or familiar individual, it is important to bear in mind that their behaviours in return might affect the behaviour of the tested animal (Gubernick, 1981; Nowak & Boivin, 2015). The tested animal is expected to approach or spend more time in the proximity of the presumed attached individual than the unfamiliar individual if they recognise and prefer the attached individual (Gubernick, 1981; Nowak & Boivin, 2015). But it can also be that they spend time in close proximity of the presumed attached individual if the unfamiliar avoids or threatens the tested individual or if the presumed attached individual recognises the test animal and interacts with it (Gubernick, 1981; Nowak & Boivin, 2015). It can also be that the tested animal does not show any preference for the unfamiliar or attached familiar individual or spend more time in close proximity of the unfamiliar even though they are attached to the presumed individual but feel the security from that individual and explore the environment (Gubernick, 1981; Nowak & Boivin, 2015). Then to distinguish these effects, a comparison of the infant's behavioural response to the mother with the

behavioural responses to the unfamiliar and another familiar individual should be done (Gubernick, 1981).

Seeking and maintenance of proximity

To measure bonding, proximity by itself is not enough for concluding bonding between individuals (Nowak & Boivin, 2015). The absence of seeking and maintaining proximity cannot by itself function as evidence for the lack of preference for an individual or attachment to the individual (Gubernick, 1981). Information about the preference must be taken into account when indicating attachment from proximity data (Nowak & Boivin, 2015). There is a challenge in measuring proximity when the animals are moving freely since the behavioural expression change with age (Nowak & Boivin, 2015). During the development of the young, the behaviours change from proximal forms of contact such as clinging to a distal form with more of observing and communicating vocally with the attached individual instead (Gubernick, 1981; Nowak & Boivin, 2015). The challenge is therefore to identify what constitutes seeking and maintenance of proximity with the attachment individual when the young develops (Gubernick, 1981; Nowak & Boivin, 2015).

Response to separation and reunion

Attached individuals typically display distress when separated from each other (Gubernick, 1981; Nowak & Boivin, 2015). The reactions to separation may, however, vary because they might be influenced by several factors such as the social context, environment at the separation place and what type of relationship the attached individuals have (Gubernick, 1981; Nowak & Boivin, 2015). Change of behaviour upon separation might indicate attachment and lack of reaction might indicate an absence of attachment (Gubernick, 1981). Separation studies themselves might not provide enough evidence to detect and argue for the presence of attachment and other methods to verify attachment such as preference test need to be run independently from the separation test (Gubernick, 1981). When reunited the attached individuals may make immediate and sustained contact with each other but the responses might be influenced by some factors such as duration of separation, the environment where the separation and reunion take place and the relationship between mother and infant before the reunion (Gubernick, 1981; Nowak & Boivin, 2015).

The use of an individual as a secure base to explore

Prior studies have indicated that infants are more probable to explore a new environment and object if an attached figure is present such as a mother or caregiver (Gubernick, 1981). When presented to a new environment the infant might stay in

close proximity of the mother but the infant can also leave the close proximity and explore the new object (Gubernick, 1981). The same difficulties as the proximity measurements are applicable here, and it must be shown that the infants react in response to the presence of the presumed attached figure and not another familiar figure (Gubernick, 1981).

2.4. Knowledge gap

2.4.1. Maternal behaviour

Several studies have been made previously looking at maternal behaviour and the relationship between the cow and calf. However, no recent studies are available where maternal behaviours are investigated in today's dairy cows. Le Neindre (1989) studied maternal behaviour and the mother-calf social relationship in the Friesian cows that were either mothered or non-mothered. That study was however run 30 years ago and the dairy cows have since then been continued selected against maternal behaviour. Lidfors *et al.,* (1994b) looked at maternal behaviour before and after calving in SR and SH with a focus on the choice of calving site when group-housed. Other papers have been published on the topic of maternal behaviour more recently, but those studies did look at the behaviours of cows from beef breeds, breeds that have been selected for early calf recognition and stronger maternal behaviours and therefore might differ from the highly selected dairy cow breeds. Only a few behavioural studies have been conducted on dairy cows but with a focus on the separation between cows and calves. Therefore, there is a need to improve our understanding of how modern dairy cows can raise their own calves when kept in a cow-calf contact system.

2.4.2. Bonding and attachment in cattle

An attachment theory has been developed during decades around what is needed to be measured to assume that attachment and a bond has taken place between two figures. This theory has primarily been used for studies in humans and other non-human primates (Maestripieri, 2001) but the theory has been adapted to non-primate animal research and methods have been developed to measure the bonding in species such as sheep (Nowak et al., 2000; Hernandez et al., 2009; Nowak & Boivin, 2015). Very few studies are available today on attachment and bonding in cattle and there are no fully developed methods for measuring mother-young bonding in cattle. Therefore, there is a need to develop and test methods that can measure the bonding in cattle.

2.5. Hypothesis

Based on this literature review the hypothesis in this study is that there should be a variation between animals in maternal behaviour, both between breed where the Swedish Red (SR) should show more maternal behaviour, between parity where cows in later parity should show more maternal behaviour and between the sex of the calves where cows to a male calf will show more maternal behaviours.

3. Material and method

3.1. Material

The study was carried out between March and May 2020 at the Lövsta research station outside Uppsala, Sweden. The animals used in this study were a part of the bigger project titles: “Sustainable dairy production with cow and calf together” studying cow-calf contact systems. For this study, the animals were only used for behavioural observations and no additional treatment was added, they were however moved, and cows and calves were separated during parts of the test, which can be questioned from an ethical point. Therefore, this study was run under the ethical approval for the project Sustainable Dairy Production with Cow and Calf Together with the ID number 5.8.18-18138/2019.

Nineteen pairs of cows and calves were used in the study, of these were ten pairs from the breed SR and nine pairs from the breed SH. Four of the calves were males and 15 were females. The calves were born from the first week of March 2020 until the first week of April 2020. The calves were born in maternity pens indoors and were kept there with the mother until approximately 48 hours of age. After 48 hours from birth, the pair was relocated to a special area within a loose housing system where the cow-calf contact system was equipped with cow-driven smart gates and the cows were milked in a DeLaval VMS (Voluntary Milking System) robot. We followed the calves during their first five weeks of life. During the preference test, 26 randomly selected cows (unfamiliar cows) and 20 calves (unfamiliar calves) were used in addition to the 19 cow-calf pairs studied.

3.2. Method

3.2.1. Direct observations at maternity pen

When the calves were between 24 to 48 hours of age, direct observations in the maternity pens were performed for 4 hours per pair. Early behaviours of both cow and calf were registered according to the ethogram in Table 1. Performed behaviours were counted, pauses of 5 seconds or more in the performance of behaviour before continuing again were seen as a break and therefore the behaviour was counted as two separate occasions.

Table 1. Ethogram for the direct observations at maternity pen

Behaviour	Definition	Reference
Vocalization	Every single vocalisation with inhalation between two occurrences	Lidfors, 1996
Sniffing	Muzzle in contact with or close proximity of any part of the other's body (<10cm)	Jensen, 2012
Licking	Tongue in contact with any part of the other's body	Jensen, 2011
Nursing	Cow letting the calf suckle	Ventorp & Michanek, 1991
Suckling	Calf with teat in the mouth and suckling	

3.2.2. Preference test

To test the preference of the cows and calves for each other, a preference test was performed in an outside arena that consisted of a triangular-shaped area (Figure 1) and was built with 1.7 m high movable metal gates units customized for cattle. The arena was adapted to cattle from a similar test previously used in sheep to measure maternal-filial bonding (Nowak & Boivin, 2015). At one side of the arena, two holding pens were built with the size $8m^2$ respectively $7m^2$ (choice pen, Figure 1). Opposite of the choice pens was a 25 m^2 holding pen (start box, Figure 1) with a gate out to the arena (Figure 2). The area in the triangle outside of the pens was divided into four zones with pink marking on the ground, a contact zone within the area 1,5 m out from the choice pens and an outer zone in the rest of the area separated in two equal big sides with a line vertically through the arena (Figure 1). In the middle of the arena, a square of $3m^2$ was painted as an alternative to the start box (neutral zone, Figure 1) where the calves were moved if the calves after the given 5 minutes did not leave the start box. Four cameras were used to cover the

whole arena, one camera recording the right pen and contact area, one camera recording the left pen and contact area, one camera recording the start box and one camera recording part of the outer zones and gate out from the start box

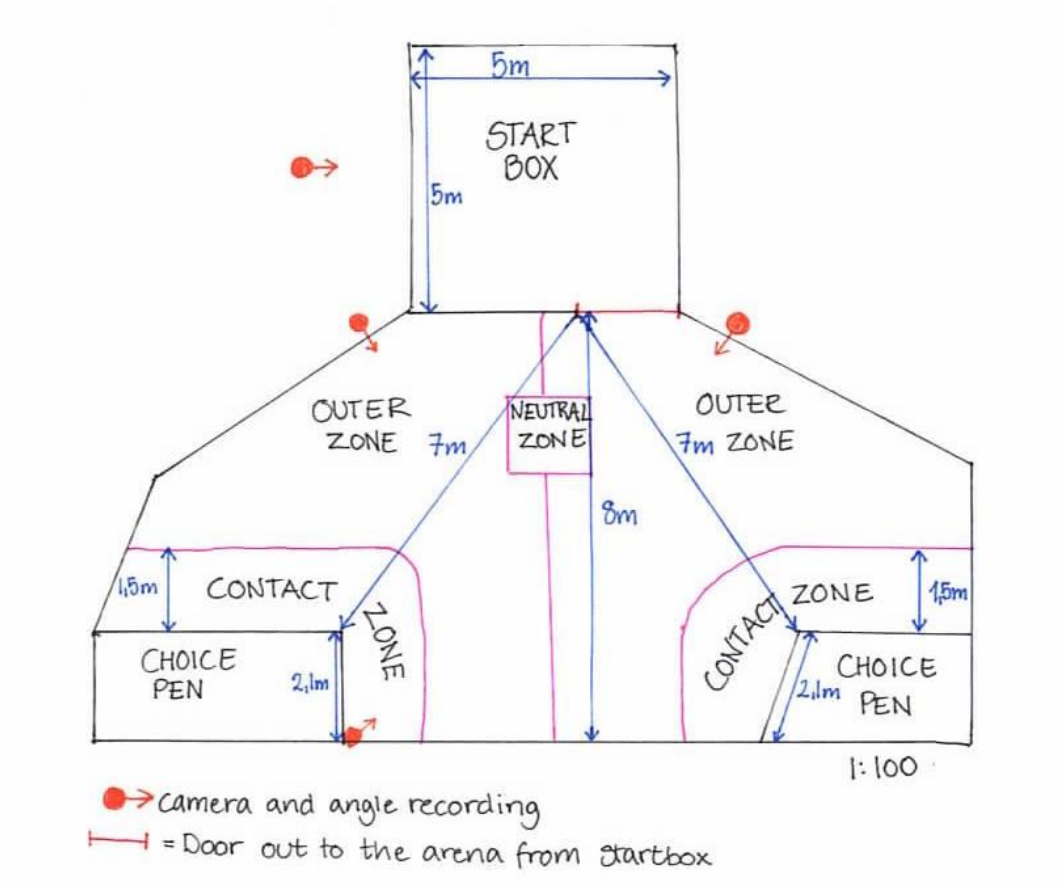

Figure 1. Sketch of the arena used for preference testing

Figure 2. Picture of the gate out to the test area from the start box

Figure 3. Picture of the right choice pen with a door into the stable. A camera was placed on the left pillar at the same height as the ventilation openings

Figure 4. Picture of the left choice pen with a door into the stable.

Figure 5. Picture of the start box from the test area view.

The tests were done when the calves were one and four weeks of age and consisted of two phases. In phase 1, the calves were tested with the mother (familiar cow) and an unfamiliar cow, from now on referred to as the calf test, and in phase 2, the cow was tested with her calf (familiar calf) and an unfamiliar calf from now on referred to as the cow test. In between the phases, there was a 15-30 minute break to move and relocate the animals. Before phase 1 started, a randomly selected unfamiliar cow and the mother of the tested calf was moved to their positions in either the left or right choice pen, which was randomly selected but balanced for the breed. The tested calf was moved in a wagon and when the calf was placed in the start box, recordings of behaviour and vocalization were made for three minutes (Table 2). After the three minutes, the gate out to the test area was opened and the test started and lasted for five minutes with recordings of behaviours and time spent in the different zones (Table 2). If the calf did not leave the start box during these five minutes, the experimenter guided and placed the calf in the neutral zone and the test continued for five more minutes. After five minutes, the calf was let into the mother's pen during the break and the unfamiliar cow was moved back. A randomly selected unfamiliar calf was then taken to the arena and placed in the choice pen that previously kept the unfamiliar cow. The mother cow was then let out to the test area and guided to the start box, some cows needed their calf to be moved with them (the calf was then later moved back to the choice pen). When the cow was in the start box, the cow test started and recordings of behaviour and vocalization for three minutes began (Table 2). After the three minutes of recordings, the gate out to the test area was opened and recordings of behaviour, vocalization and time spent in the different zones for 5 minutes started (Table 2). After five minutes, the test was finished even if the cow did not leave the start box and the animals were moved back to their home place.

Table 2. Ethogram for the preference test observations, both cow and calf

Behaviour	Definition
Number of cow vocalizations	Every single vocalisation with inhalation between two occurrences that could be localized to the test (familiar) cow
Number of calf vocalizations	Every single vocalisation with inhalation between two occurrences that could be localized to the test (familiar) calf
Number of unfamiliar cow/calf vocalizations	Every single vocalisation with inhalation between two occurrences that could be localized to the unfamiliar individual
Number of unidentified vocalizations	Every single vocalisation with inhalation between two occurrences that could not be localized to any individual
Total number of vocalizations	All vocalizations observed during a test, Cow vocalizations + calf vocalizations + unfamiliar cow/calf vocalizations + unidentified vocalizations
Number of touches between familiar calf and familiar cow	Every touch between familiar individuals
Number of touches between familiar calf/cow and unfamiliar cow/calf	Every touch between unfamiliar individuals
Number of times the cow sniffed the calf	Cow exploring another individual (familiar or unfamiliar) without touching
Number of times the calf sniffed a cow	Calf exploring another individual (familiar or unfamiliar) without touching
Number of times the unfamiliar cow (calf) sniffed the tested calf (cow)	Unfamiliar animal exploring the test animal without touching
Latency to leave the start box (s)	The latency between opening the gate and the test animal leaving the start box
Time spent in the unfamiliar contact zone (s)	Time spent in the contact zone on unfamiliar animal site
Time spent in the familiar contact zone (s)	Time spent in the contact zone on familiar animal site
Time spent in the unfamiliar outer zone (s)	Time spent in the outer zone on unfamiliar animal site
Time spent in the familiar outer zone (s)	Time spent in the other zone on familiar animal site
Time spent out of sight in the unfamiliar site of the arena (s)	Time the animal was not visible on unfamiliar site

Time spent out of sight in the familiar site of the arena (s)	Time the animal was not visible on the familiar site
Total time spent in the unfamiliar site	Total time spent in the unfamiliar animal site of the arena, contact zone unfamiliar + outer zone unfamiliar + out of sight unfamiliar site
Total time spent in the familiar site	Total time spent in the familiar animal site of the arena, contact zone familiar + outer zone familiar + out of sight familiar site

3.2.3. Neophobia test

To observe how the calves used the mother as a secure base to explore the environment, a neophobia test was done when the calves were 5 weeks of age. The test arena was the start box from the preference test (Figure 6). In the start box, there were six white 10 l buckets, two that were empty, two with concentrate and two with carrots that were a new food for the calves (Figure 7). The choice of carrot as a novel food was adapted from Costa *et al.*, (2014) where carrots were used as a novel food in a neophobia test in calves. The empty buckets were to determine if the animals were afraid of the buckets.

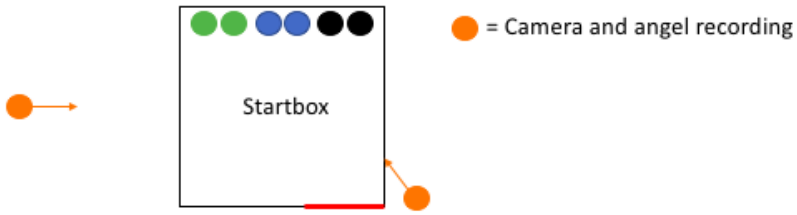

Figure 6. Sketch of the arena for the neophobia test

Figure 7. Picture of the buckets in the back of the start box

Before the test started, the buckets were weighed and contained 500 g of feed per bucket. The mother and calf then got 15 minutes in the start box with access to all 6 buckets at the same time. After 15 minutes, the buckets were weighed again to see how much was eaten. The test was recorded with two video cameras and the video material was then later analysed for feeding behaviour (Table 3).

Table 3. Ethogram for neophobia test observations

Behaviour	Definition
Amount concentrate eaten	The amount of concentrate eaten (g) during the test by the cow and calf together
Amount carrots eaten	The amount of carrots eaten (g) during the test by the cow and calf together
Time the calf spent with head in the carrot bucket	Total time (s) the calf spent with the head inside one of the buckets with carrots
Time the calf spent with head in the concentrate bucket	Total time (s) the calf spent with the head inside one of the buckets with concentrate
Time the calf spent with head in the empty bucket	Total time (s) the calf spent with the head inside one of the empty buckets
Total time the calf spent with head in any of the buckets	Total time (s) the calf spent with the head inside any of the buckets, time spent in carrot bucket + time spent in concentrate bucket + time spent in the empty bucket
Time the cow spent with head in the carrot bucket	Total time (s) the cow spent with the head inside one of the buckets with carrots

Time the cow spent with head in the concentrate bucket	Total time (s) the cow spent with the head inside one of the buckets with concentrate
Time the cow spent with head in the empty bucket	Total time (s) the cow spent with the head inside one of the empty buckets
Total time the cow spent with head in any of the buckets	Total time (s) the cow spent with the head inside any of the buckets, time spent in carrot buckets + time spent in concentrate buckets + time spent in the empty buckets
Chewing	Chewing after the head had been in any bucket
Time the calf spent exploring the buckets	Total time (s) the calf sniffed and explored the bucket without having the head inside any bucket
Time the cow spent exploring the buckets	Total time (s) the cow sniffed and explored the bucket without having the head inside any bucket
Time spent in away distance	Total time the calf stands or moves with more than one cow length distance to the mother
Time spent in close distance	Total time the calf stands or moves with between a calf and cow length distance to the mother
Time spent in contact distance	Total time the calf stands or moves within a calf-length distance to the mother

3.2.4. Video analysis

The four videos from each preference test were synchronized to start at the same time using the trim function in QuickTime Player (Apple Inc). The same was done with the two camera angles from each neophobia test. The video material was then later behaviour coded in open source software BORIS (version 7.9.15) with the possibility to run all videos from each test simultaneous (Friard & Gamba, 2016). For the analysis of the preference test, behaviours were coded according to the ethogram in Table 2. A code Out of sight needed to be added due to that some cameras were placed in angels that resulted in not full coverage of the test arena. However, it was still possible to detect whether the test animal was on the unfamiliar or familiar site of the arena. For the analysis of the neophobia test behaviours were coded according to the ethogram in Table 3.

3.3. Statistical analysis

The behaviour observations data were summarized in Excel and information about the animals regarding breed, sex of the calf and parity (PP or MP cow) were added. Measurements of the amount of food eaten were also added to the neophobia test data. Statistical analysis to test the distribution of observed behaviours depending on breed, parity and sex of the calf was done in the open-source software R (version 4.0.2) (The R Foundation for Statistical Computing) and RStudio (version 1.3.1056) (RStudio Team, 2020). The means and Standard Error of Mean (sem) for breed, parity and sex of the calf were calculated (packages: Rmisc). Fitted GLM with a Poisson link was used for the count data (package: stats) and it was checked for overdispersion (package: AER) and if there was an overdispersion, a fitted GLM with quasi-Poisson were used instead (package: stats). In the data from the early maternal behaviours overdispersion was found in the following data: the number of vocalizations by the cow, vocalizations by the calf, licking occasions on the calf by the cow, licking occasions on the cow by the calf, sniffing occasions on the calf by the cow, sniffing occasions on the cow by the calf, nursing events and suckling occasions by the calf and for this data, a fitted GLM with quasi-Poisson was then used. In the data from the preference test of the calf, overdispersion was found in the following data: the number of times familiar cow sniffed the calf, familiar cow vocalization, calf vocalization, unfamiliar cow vocalization, unidentified vocalization and the total number of vocalization and for this data, a fitted GLM with quasi-Poisson was then used. In the data from the preference test of the cow overdispersion was found in the following data: the number of cow vocalization, familiar calf vocalization, unfamiliar calf vocalization, unidentified vocalization and the total number of vocalization and for this data, a fitted GLM with quasi-Poisson was then used. The GLM output was then put into an ANOVA analysis of deviance (package: stats). An ANOVA analysis of variance was used for the duration data and the assumption of normality was checked with a Shapiro-Wilk normality test (package: stats). If the data was not normal distributed a log-transformation or, if we had 0 in the dataset, a square root transformation was tried (package: base). In the data from the preference test of the calf following data was not normal distributed and contained 0 in the dataset; Latency to leave the start box and time spent in the unfamiliar contact zone and for this data, a square root transformation was tried. In the data from the preference test of the cow following data was not normal distributed and contained 0 in the dataset; Latency to leave the start box and time spent in the unfamiliar contact zone and for this data, a square root transformation was tried. In the data from the neophobia test following data was not normal distributed and contained 0 in the dataset; time the calf spent with head in the empty bucket and for this data a square root transformation was tried.

If data could not be normalized, outliers were visually selected from a Normal Q-Q plot and then excluded from the analysis. This only occurred for the latency to leave the start box data from the preference test of the cow where two cows that did not leave the start box were seen as outliners and excluded from the analysis. The results are presented as observed means and standard errors of the mean, count data is presented with Deviance, Degrees of freedom and p-value, duration data is presented with F values ($df_{effects}$, df_{error}), and p-values. The test results were considered statistically significant when the p-value was <0.05.

4. Result

4.1. Early maternal behaviour

Several behaviours were recorded during the behavioural observations at the maternity pen, we have, however, only focused on the vocalizations, licking, sniffing and nursing/suckling since these are social behaviours and the behaviours that are most relevant for the bonding between cow and calf. One observation occasion has missing data on vocalization since the observation situation did not allow us to hear the cow and calf, only see them. The effect of breed on the behaviours observed at the maternity pen is shown in Table 4. The statistical analysis showed that SR cows sniffed their calves more than the SH cows (Deviance 54.992, P <0.01, Table 4). No other breed differences were found from these observations (Table 6)

Table 4: The effect of breed on mean ± sem behavioural variables performed in the maternity pen for 4 hours.

Behaviours	Breed	
	SR	SH
Number of vocalizations by the cow	56.7 ± 18.4	33.0 ± 8.6
Number of vocalizations by the calf	1.3 ± 0.5	1.3 ± 0.4
Number of lickings occasions on the calf by the cow	27.6 ± 7.1	26.8 ± 5.8
Number of lickings occasions on the cow by the calf	0.4 ± 0.2	0.3 ± 0.2
Number of sniffing occasions on the calf by the cow	31.9 ± 5.0 [A]	15.4 ± 2.4 [B]
Number of sniffing occasions on the cow by the calf	7.8 ± 2.2	6.7 ± 1.1
Number of nursing events	22.6 ± 6.3	11.4 ± 3.1
Number of suckling occasions by the calf	23.1 ± 6.0	11.8 ± 3.2

[A, B] Values within a row with different superscripts are significantly different ($p < 0.01$)

The effect of parity on the behaviours observed at the maternity pen is shown in (Table 5). It was shown that the MP cows vocalized more compared to the PP cows (Deviance 138.078, $P < 0.05$, Table 5). No other parity difference was found from these observations (Table 5)

Table 5: The effect of parity on mean ± sem behavioural variables performed in the maternity pen for 4 hours.

Behaviours	Parity	
	PP	MP
Number of vocalizations by the cow	34.6 ± 8.5 [a]	69.3 ± 27.6 [b]
Number of vocalizations by the calf	1.4 ± 0.4	1.0 ± 0.5
Number of lickings occasions on the calf by the cow	29.3 ± 7.0	23.7 ± 3.0
Number of lickings occasions on the cow by the calf	0.4 ± 0.2	0.2 ± 0.3
Number of sniffing occasions on the calf by the cow	25.4 ± 3.8	21.9 ± 6.8
Number of sniffing occasions on the cow by the calf	7.3 ± 1.4	7.3 ± 2.6
Number of nursing events	16.5 ± 4.9	18.7 ± 6.4
Number of suckling occasions by the calf	17.1 ± 4.6	18.9 ± 6.4

[a, b] Values within a row with different superscripts are significantly different ($p < 0.05$)

The effect of the sex of the calf on the behaviours observed at the maternity pen is shown in (Table 17, Appendix 1) No difference was found that depended on the sex of the calf from these observations (Table 17, Appendix 1).

4.2. Descriptive statistics of the preference test

Preference tests were done when the calves were one and four weeks of age but since we were not able to get the arena in place until the first calf was four weeks of age, we have missing data from week one. Only 8 out of 19 pairs were tested both at one week of age (Week 1) and four weeks of age (Week 4). Since the low number of animals with data from both test weeks, the comparison between weeks will be excluded from the statistical analysis. The data from the tests at week 1 will however be used and compared with the data from week 4 from the eight animals that were tested during both occasions. The comparison will be done by looking at the means and sem values and will not be statistically evaluated.

A summary of the data from the calf test for the eight pairs that were tested both at week 1 and 4 is shown in Table 6. In the data, some vocalizations were not possible to connect to an animal since it was not possible to visually detect which animal that vocalized on the video, these were registered as unidentified vocalizations. There was a higher number of vocalizations and sniffs and calves took longer to leave the start box at one week compared to four weeks of age (Table 6). However, two calves did not leave the start box in week one and were guided to the neutral zone after five minutes, which most likely explains this difference in the time to leave the start box and spent in the familiar site of the arena. Calves spent more time on the familiar site during week one compared to week 4, but the time spent on the unfamiliar site of the arena is the same between week 1 and 4 (Table 6).

Table 6. Mean ± sem number or duration of behaviours performed in the calf preference test at one and four weeks of age in 8 cow-calf pairs.

Behaviours	Week 1 Mean ± sem	Week 4 Mean ± sem
Number of times the cow sniffed the calf	2.4 ± 0.5	0.4 ± 0.2
Number of times the calf sniffed familiar or unfamiliar cow	1.0 ± 0.3	0.5 ± 0.2
Number of times the unfamiliar cow sniffed the calf	1.0 ± 0.6	0.1 ± 0.1
Number of cow vocalizations	46.3 ± 10.6	28.3 ± 5.0
Number of calf vocalizations	1.8 ± 1.1	0.8 ± 0.6
Number of unfamiliar cow vocalizations	1.9 ± 1.3	1.5 ± 0.7
Number of unidentified vocalizations	24.9 ± 7.9	3.4 ± 0.9
Total number of vocalizations	74.8 ± 11.7	33.9 ± 4.4
Number of touches between calf and unfamiliar cow	0.3 ± 0.3	0.0 ± 0.0
Number of touches between calf and familiar cow	0.5 ± 0.3	0.1 ± 0.1
Latency (s) to leave the start box	123.1 ± 44.7	89.0 ± 31.1
Time spent (s) in the familiar outer zone	77.1 ± 14.0	51.8 ± 11.6
Time spent (s) in the familiar contact zone	132.4 ± 19.3	83.6 ± 23.0
Time spent (s) out of sight in familiar site of the arena	18.8 ± 11.3	26.7 ± 12.2
Total time spent (s) in the familiar site	228.4 ± 23.6	162.1 ± 29.0
Time spent (s) in the unfamiliar outer zone	33.2 ± 16.0	30.5 ± 8.0
Time spent (s) in the unfamiliar contact zone	29.8 ± 10.6	40.4 ± 14.7
Time spent (s) out of sight in unfamiliar site of the arena	9.9 ± 4.8	1.7 ± 1.7
Total time spent (s) in the unfamiliar site	72.8 ± 22.5	72.6 ± 20.4

A summary of data from the cow test for the eight pairs that were tested both at week 1 and 4 is shown in Table 7. The cows vocalized more, and the total number of vocalizations was also higher during week 1 compared to week 4 (Table 7). The other categories of performed behaviours did not differ in numbers between the weeks (Table 7). The mean time for the cow to leave the start box was higher during week 4 (Table 7) The cows, in general, spent more time in the familiar site of the

arena during week 1 compared to week 4 (Table 7) The cows spent less time close to the unfamiliar calf during week 1 compared to week 4 (Table 7).

Table 7. Mean ± sem number or duration of behaviours performed in the cow preference test at one and four weeks of age in 8 cow-calf pairs.

Behaviours	Week 1 Mean ± sem	Week 4 Mean ± sem
Number of times the cow sniffed the familiar or unfamiliar calf	0.6 ± 0.3	0.8 ± 0.3
Number of times the calf sniffed the cow	0.0 ± 0.0	0.1 ± 0.1
Number of times the unfamiliar calf sniffed the cow	0.3 ± 0.2	0.4 ± 0.3
Number of cow vocalizations	34.3 ± 1.8	12.4 ± 3.9
Number of calf vocalizations	0.1 ± 0.1	0.5 ± 0.4
Number of unfamiliar calf vocalizations	3.4 ± 2.7	7.8 ± 5.7
Number of unidentified vocalizations	6.5 ± 3.0	6.0 ± 4.0
Total number of vocalizations	44.3 ± 4.2	26.6 ± 7.1
Number of touches between cow and unfamiliar calf	0.3 ± 0.2	0.0 ± 0.0
Number of touches between cow and familiar calf	0.0 ± 0.0	0.1 ± 0.1
Latency (s) to leave the start box	19.2 ± 9.0	26.3 ± 14.1
Time spent (s) in the familiar outer zone	70.1 ± 10.3	64.4 ± 15.2
Time spent (s) in the familiar contact zone	157.2 ± 15.2	128.6 ± 30.6
Time spent (s) out of sight in familiar site of the arena	19.3 ± 12.9	0.0 ± 0.0
Total time spent (s) in the familiar site	246.6 ± 13.6	193.0 ± 31.5
Time spent (s) in the unfamiliar outer zone	32.4 ± 10.2	60.2 ± 20.6
Time spent (s) in the unfamiliar contact zone	15.7 ± 5.3	55.2 ± 16.7
Time spent (s) out of sight in unfamiliar site of the arena	7.2 ± 4.6	0.0 ± 0.0
Total time spent (s) in the unfamiliar site	60.4 ± 13.5	115.4 ± 33.6

4.3. Statistical analysis of the preference test

For the statistical analysis, we only used the data from test week four. Here we had data from 18 out of 19 pairs since one cow needed to be kept in the sick stable during the test occasion and could not be moved.

During the calf test, calves born to PP cows sniffed more than calves born to MP cows (Deviance 3.9359, $P < 0.05$, Table 8). The unfamiliar cows sniffed more when calves from PP cows were tested (Deviance 4.0900, $P < 0.05$, Table 8). No other parity differences were found from these observations (Table 8).

Table 8: The effect of parity of the cow on mean ± sem behavioural variables during the preference test of the calf

Behaviour	Parity	
	PP	MP
Number of times the calf sniffed the familiar or unfamiliar cow	1.1 ± 0.4[a]	0.1 ± 0.1[b]
Number of times the familiar cow sniffed the calf	1.4 ± 0.7	0.1 ± 0.1
Number of times the unfamiliar cow sniffed the tested calf	0.5 ± 0.2[a]	0.0 ± 0.0[b]
Number of familiar cow vocalizations	23.3 ± 3.7	27.1 ± 6.3
Number of calf vocalizations	2.8 ± 1.4	0.4 ± 0.3
Number of unfamiliar cow vocalizations	4.1 ± 1.9	3.0 ± 2.2
Number of unidentified vocalizations	6.2 ± 1.5	9.9 ± 3.4
Total number of vocalizations	36.4 ± 4.4	40.4 ± 4.3
Number of touches between calf and unfamiliar cow	0.1 ± 0.1	0.0 ± 0.0
Number of touches between calf and familiar cow	0.3 ± 0.1	0.0 ± 0.0
Latency (s) to leave the start box	50.7 ± 13.9	92.0 ± 35.5
Time spent (s) in the familiar outer zone	59.6 ± 7.6	71.4 ± 16.7
Time spent (s) in the familiar contact zone	96.4 ± 16.1	57.7 ± 17.7
Total time spent (s) in the familiar site of the arena	173.6 ± 18.1	149.2 ± 29.4
Time spent (s) in the unfamiliar outer zone	44.6 ± 11.5	49.8 ± 8.7
Time spent (s) in the unfamiliar contact zone	52.6 ± 15.5	31.1 ± 9.3
Total time spent (s) in unfamiliar site of the arena	104.5 ± 23.0	80.9 ± 13.4

[a,b] Values within a row with different superscripts are significant different ($p < 0.05$)

During the calf test, male calves were vocalizing more than female calves (Deviance 22.768, $P < 0.01$, Table 9). No other differences depending on the sex of the calf were found from these observations (Table 9).

Table 9: The effect of sex of the calf on mean ± sem behavioural variables during the preference test of the calf

Behaviour	Sex	
	Female	Male
Number of times the calf sniffed the familiar or unfamiliar cow	0.5 ± 0.2	1.5 ± 0.9
Number of times the familiar cow sniffed the calf	0.9 ± 0.6	0.8 ± 0.5
Number of times the unfamiliar cow sniffed the tested calf	0.3 ± 0.2	0.5 ± 0.3
Number of familiar cow vocalizations	26.5 ± 4.0	18.8 ± 3.3
Number of calf vocalizations [1]	0.8 ± 0.4^{A}	5.8 ± 3.3^{B}
Number of unfamiliar cow vocalizations	4.1 ± 1.7	2.0 ± 2.0
Number of unidentified vocalizations	6.7 ± 1.8	10.8 ± 3.6
Total number of vocalizations	38.1 ± 4.0	37.3 ± 2.6
Number of touches between calf and unfamiliar cow	0.1 ± 0.1	0.0 ± 0.0
Number of touches between calf and familiar cow	0.1 ± 0.1	0.3 ± 0.3
Latency (s) to leave the start box	77.5 ± 20.0	29.1 ± 10.0
Time spent (s) in the familiar outer zone	65.8 ± 8.4	58.6 ± 22.1
Time spent (s) in the familiar contact zone	75.3 ± 14.3	102.6 ± 26.3
Total time spent (s) in the familiar site of the arena	162.1 ± 18.2	171.2 ± 34.5
Time spent (s) in the unfamiliar outer zone	44.1 ± 7.8	55.6 ± 22.6
Time spent (s) in the unfamiliar contact zone	36.5 ± 10.2	71.1 ± 28.2
Total time spent (s) in unfamiliar site of the arena	86.1 ± 13.7	127.7 ± 49.0

[A,B] Values within a row with different superscripts are significant different ($p < 0.01$)

During the calf test, calves of the breed SR vocalized more than SH calves (Deviance 21.0147, P <0.01, Table 10). The SR calves spent more time in the familiar contact zone compared to the SH calves ($F_{1,14}$=5.4, P < 0.05, Table 10). No other differences depending on the breed were found in these observations (Table 10).

Table 10: The effect of breed on mean ± sem behavioural variables during the preference test of the calf

Behaviour	Breed	
	SR	SH
Number of times the calf sniffed the familiar or unfamiliar cow	1.0 ± 0.4	0.4 ± 0.3
Number of times the familiar cow sniffed the calf	1.3 ± 0.8	0.4 ± 0.2
Number of times the unfamiliar cow sniffed the tested calf	0.5 ± 0.2	0.1 ± 0.1
Number of familiar cow vocalizations	20.0 ± 3.1	30.8 ± 5.8
Number of calf vocalizations	3.1 ± 1.5[a]	0.4 ± 0.3[b]
Number of unfamiliar cow vocalizations	5.4 ± 2.4	1.5 ± 0.5
Number of unidentified vocalizations	8.5 ± 1.9	6.5 ± 2.8
Total number of vocalizations	37.0 ± 4.9	39.1 ± 3.7
Number of touches between calf and unfamiliar cow	0.1 ± 0.1	0.0 ± 0.0
Number of touches between calf and familiar cow	0.3 ± 0.2	0.0 ± 0.0
Latency (s) to leave the start box	48.8 ± 17.8	89.3 ± 28.4
Time spent (s) in the familiar outer zone	57.1 ± 10.7	73.1 ± 11.4
Time spent (s) in the familiar contact zone	109.0 ± 15.0[c]	46.8 ± 13.8[d]
Total time spent (s) in the familiar site of the arena	183.6 ± 20.3	139.7 ± 22.8
Time spent (s) in the unfamiliar outer zone	41.2 ± 11.7	53.3 ± 9.3
Time spent (s) in the unfamiliar contact zone	50.5 ± 16.2	36.4 ± 11.6
Total time spent (s) in unfamiliar site of the arena	94.0 ± 24.4	97.0 ± 16.3

[a,b] Values within a row with different superscripts are significant different ($p < 0.05$)

During the cow test, calves of MP cows sniffed more compared to calves of PP cows (Deviance 5.0111, P <0.05, Table 11). The touches between the cows and the unfamiliar calves were higher during tests with PP cows compared to MP cows (Deviance 5.5527, P <0.05, Table 11) No other differences were found between parity from these observations (Table 11).

Table 11: The effect of parity of the cow on mean ± sem behavioural variables during the preference test of the cow

Behaviour	Parity	
	PP	MP
Number of times the cow sniffed the familiar or unfamiliar calf	1.0 ± 0.3	0.7 ± 0.4
Number of times the familiar calf sniffed the cow	0.1 ± 0.1[a]	0.3 ± 0.2[b]
Number of times the unfamiliar calf sniffed the cow	0.3 ± 0.2	0.6 ± 0.4
Number of cow vocalizations	14.5 ±2.2	13.3 ± 4.4
Number of familiar calf vocalizations	1.7 ± 0.8	2.0 ± 1.3
Number of unfamiliar calf vocalizations	5.2 ± 4.1	4.9 ± 2.4
Number of unidentified vocalizations	8.7 ± 1.9	10.0 ± 3.1
Total number of vocalizations	30.2 ± 4.1	30.1 ± 6.9
Number of touches between cow and unfamiliar calf	0.5 ± 0.2[a]	0.0 ± 0.0[b]
Number of touches between cow and familiar calf	0.1 ± 0.1	0.0 ± 0.0
Latency (s) to leave the start box	27.5 ± 9.2	46.2 ± 17.4
Time spent (s) in the familiar outer zone	61.1 ± 15.1	48.0 ± 14.0
Time spent (s) in the familiar contact zone	59.7 ± 19.8	127.2 ± 36.5
Total time spent (s) in familiar site of the arena	124.6 ± 27.9	175.2 ± 39.7
Time spent (s) in unfamiliar outer zone	71.9 ± 15.7	33.9 ± 16.4
Time spent (s) in unfamiliar contact zone	75.2 ± 17.5	36.6 ± 14.4
Total time spent (s) in unfamiliar site of the arena	148.3 ± 29.4	70.5 ± 28.1

[a,b] Values within a category with different superscripts are significant different ($p < 0.05$)

During the cow test, unfamiliar calves sniffed more on SR mothers than on the SH mothers (Deviance 8.2290, P <0.01, Table 12). No other differences were found between breeds from these observations (Table 12).

Table 12: The effect of breed, on mean ± sem behavioural variables during the preference test of the cow

Behaviour	Breed	
	SR	SH
Number of times the cow sniffed the familiar or unfamiliar calf	1.2 ± 0.3	0.5 ± 0.3
Number of times the familiar calf sniffed the cow	0.3 ± 0.2	0.0 ± 0.0
Number of times the unfamiliar calf sniffed the cow	0.7 ± 0.3^{A}	0.0 ± 0.0^{B}
Number of cow vocalizations	14.6 ± 2.5	13.4 ± 3.7
Number of familiar calf vocalizations	1.9 ± 0.9	1.8 ± 1.1
Number of unfamiliar calf vocalizations	5.8 ± 4.4	4.1 ± 2.1
Number of unidentified vocalizations	8.7 ± 2.2	9.9 ± 2.6
Total number of vocalizations	31.0 ± 4.3	29.1 ± 6.2
Number of touches between cow and unfamiliar calf	0.3 ± 0.2	0.3 ± 0.3
Number of touches between cow and familiar calf	0.1 ± 0.1	0.0 ± 0.0
Latency (s) to leave the start box	26.6 ± 10.4	44.7 ± 14.6
Time spent (s) in the familiar outer zone	57.8 ± 16.6	53.8 ± 12.8
Time spent (s) in the familiar contact zone	75.7 ± 22.1	98.8 ± 35.9
Total time spent (s) in familiar site of arena	136.7 ± 31.2	153.9 ± 36.4
Time spent (s) in unfamiliar outer zone	60.0 ± 15.3	53.4 ± 20.5
Time spent (s) in unfamiliar contact zone	74.9 ± 18.4	41.8 ± 15.4
Total time spent (s) in unfamiliar site of arena	134.9 ± 30.9	97.0 ± 33.4

^{A,B} Values within a row with different superscripts are significant different ($p < 0.01$)

During the cow test, male calves vocalized more compared to female calves (Deviance 26.9423, P <0.05, Table 13). Also, the number of unidentified vocalizations was higher when a mother to a male calf was tested (Deviance 27.3302, P <0.05, Table 13). Mothers of female calves were faster to leave the start box than mothers of male calves ($F_{1,12} = 5.4634$ $p<0.05$, Table 13). Two mothers of female calves did however not leave the start box during the five minutes and were seen as outliers and excluded since they had no data available for that parameter. No other differences were found to be dependent on the sex of the calf from these observations (Table 13).

Table 13: The effect of sex of the calf on mean ± sem behavioural variables during the preference test of the cow

Behaviour	Sex	
	Female	Male
Number of times the cow sniffed the familiar or unfamiliar calf	0.7 ± 0.2	1.5 ± 0.3
Number of times the familiar calf sniffed the cow	0.1 ± 0.1	0.3 ± 0.3
Number of times the unfamiliar calf sniffed the cow	0.4 ± 0.3	0.3 ± 0.3
Number of cow vocalizations	14.9 ± 2.6	11.3 ± 1.8
Number of familiar calf vocalizations	0.9 ± 0.5^{a}	5.3 ± 1.9^{b}
Number of unfamiliar calf vocalizations	5.1 ± 4.1	4.8 ± 2.2
Number of unidentified vocalizations	7.2 ± 1.4^{a}	16.3 ± 4.2^{b}
Total number of vocalizations	28.1 ± 4.1	37.5 ± 7.0
Number of touches between cow and unfamiliar calf	0.2 ± 0.2	0.5 ± 0.5
Number of touches between cow and familiar calf	0.1 ± 0.1	0.0 ± 0.0
Latency (s) to leave the start box	26.5 ± 9.5^{a}	58.7 ± 15.6^{b}
Time spent (s) in the familiar outer zone	60.3 ± 12.6	41.2 ± 18.6
Time spent (s) in the familiar contact zone	92.3 ± 23.2	63.7 ± 38.8
Total time spent (s) in familiar site of arena	153.3 ± 27.9	112.8 ± 36.5
Time spent (s) in unfamiliar outer zone	53.4 ± 14.3	69.8 ± 23.5
Time spent (s) in unfamiliar contact zone	53.1 ± 14.7	85.2 ± 22.7
Total time spent (s) in unfamiliar site of arena	107.5 ± 26.3	154.9 ± 42.2

^{A,B} Values within a row with different superscripts are significant different ($p < 0.01$)

4.4. Neophobia test

The neophobia test was performed when the calves were five weeks old and we had data from 18 out of 19 pairs since one cow needed to be kept in the sick stable during the test occasion and could not be moved. In one of the 18 pairs, we have missing data on how much feed was eaten by the cow and calf.

During the neophobia test, pairs with male calves ate more concentrate compared to pairs with a female calf ($F_{1,13}$ = 6.2011, P <0.05, Table 14). Cows with male calves spent more time in the concentrate bucket ($F_{1,14}$=8.0770, P <0.05) and empty bucket ($F_{1,14}$=19.463 P <0.001) compared to mothers of female calves (Table 14). The total time spent in the buckets is also higher in mothers of male calves compared to mothers of female calves ($F_{1,14}$=8.6108, P <0.05, Table 14). The male calves spent more time away from the mothers compared to the female calves

($F_{1,14}$=8.4190, P <0.05, Table 14). No other differences depending on the sex of the calves were observed during these observations (Table 14).

Table 14: The effect of sex of the calf on mean ± sem behavioural variables during the neophobia test

Behaviour	Sex	
	Female	Male
Amount (g) concentrate eaten	414.9 ± 89.6[a]	901.2 ± 28.8[b]
Amount (g) carrots eaten	9.7 ± 2.5	17.9 ± 9.2
Time (s) the calf spent with head in the carrot buckets	11.7 ± 4.5	25.3 ± 19.9
Time (s) the calf spent with head in the concentrate buckets	20.5 ± 8.5	31.0 ± 12.7
Time (s) the calf spent with head in the empty buckets	5.6 ± 2.7	14.1 ± 4.6
Total time (s) the calf spent with the head in any of the buckets	37.8 ± 12.1	70.4 ± 23.3
Time (s) the cow spent with head in the carrot buckets	3.4 ± 1.8	12.8 ± 7.7
Time (s) the cow spent with head in the concentrate buckets	80.6 ± 17.5[a]	220.4 ± 76.7[b]
Time (s) the cow spent with head in the empty buckets	1.0 ± 0.4[A]	6.7 ± 2.1[B]
Total time (s) the cow spent with the head in any of the buckets	84.9 ± 17.8[a]	239.9 ± 81.2[b]
Time (s) the calf spent exploring the buckets	48.1 ± 7.8	63.2 ± 11.9
Time (s) the cow spent exploring the buckets	36.6 ± 6.4	46.9 ± 11.7
Time spent (s) in contact distance	644.0 ± 48.6	496.8 ± 36.5
Time spent (s) in close distance	206.7 ± 26.1	229.2 ± 27.6
Time spent (s) in away distance	84.1 ± 23.6[a]	199.0 ± 19.6[b]

[A,B] Values within a category with different superscripts are significant different ($p < 0.001$)
[a,b] Values within a category with different superscripts are significant different ($p < 0.05$)

During the neophobia test, calves from PP cows spent more time in the buckets in total ($F_{1,14}$=5.7627, P <0.05, Table 15) and they also spent more time sniffing and exploring the buckets ($F_{1,14}$=5.5975, P <0.05, Table 15) compared to calves from MP cows. No other differences were observed between parity from these observations (Table 15)

Table 15:The effect of parity of the cow on mean ± sem behavioural variables during the neophobia test

Behaviour	Parity	
	PP	MP
Amount (g) concentrate eaten	448.5 ± 116.9	575.2 ± 133.9
Amount (g) carrots eaten	11.7 ± 3.9	10.4 ± 3.1
Time (s) the calf spent with head in the carrot buckets	21.8 ± 8.3	3.6 ± 1.6
Time (s) the calf spent with head in the concentrate buckets	30.8 ± 10.9	10.3 ± 3.9
Time (s) the calf spent with head in the empty buckets	10.4 ± 3.7	2.9 ± 1.4
Total time (s) the calf spent with the head in any of the buckets	63.1 ± 15.3[a]	16.8 ± 6.1[b]
Time (s) the cow spent with head in the carrot buckets	8.6 ± 3.4	0.5 ± 0.5
Time (s) the cow spent with head in the concentrate buckets	102.3 ± 29.7	126.3 ± 45.7
Time (s) the cow spent with head in the empty buckets	2.5 ± 0.9	1.8 ± 1.6
Total time (s) the cow spent with the head in any of the buckets	113.5 ± 32.7	128.6 ± 47.1
Time (s) the calf spent exploring the buckets	63.0 ± 8.6[a]	33.2 ± 6.4[b]
Time (s) the cow spent exploring the buckets	39.8 ± 7.6	37.4 ± 8.4
Time (s) spent in contact distance	573.8 ± 54.7	670.1 ± 58.6
Time (s) spent in close distance	223.0 ± 30.5	194.0 ± 26.2
Time (s) spent in away distance	129.2 ± 26.9	78.8 ± 36.7

[a,b] Values within a category with different superscripts are significant different ($p < 0.05$)

During the neophobia test, SR cows and calves spent more time within a calf-length distance to each other compared to SH cows and calves ($F_{1,14}$=6.3662, P <0.05, Table 16). The SH cows and calves on the other hand spent more time with a calf-length distance to each other compared to SR cows and calves ($F_{1,14}$=5.3538, P <0.05, Table 16). No other differences were observed between breeds from these observations (Table 16)

Table 16: The effect of breed on mean ± sem behavioural variables during the neophobia test.

Behaviour	Breed	
	SR	SH
Amount (g) concentrate eaten	459.0 ± 130.3	547.6 ± 119.2
Amount (g) carrots eaten	11.5 ± 3.3	10.8 ± 4.2
Time (s) the calf spent with head in the carrot buckets	18.8 ± 8.4	9.6 ± 6.4
Time (s) the calf spent with head in the concentrate buckets	35.8 ± 11.1	6.6 ± 3.3
Time (s) the calf spent with head in the empty buckets	11.1 ± 4.0	2.9 ± 1.3
Total time (s) the calf spent with the head in any of the buckets	65.8 ± 16.3	19.2 ± 7.2
Time (s) the cow spent with head in the carrot buckets	9.2 ± 3.8	0.8 ± 0.4
Time (s) the cow spent with head in the concentrate buckets	107.6 ± 32.6	116.6 ± 40.3
Time (s) the cow spent with head in the empty buckets	2.0 ± 0.9	2.5 ± 1.4
Total time (s) the cow spent with the head in any of the buckets	118.9 ± 35.9	119.9 ± 41.3
Time (s) the calf spent exploring the buckets	57.6 ± 9.2	43.8 ± 9.6
Time (s) the cow spent exploring the buckets	37.8 ± 8.6	40.2 ± 7.0
Time (s) spent in contact distance	659.2 ± 45.1[a]	551.3 ± 70.4[b]
Time (s) spent in close distance	181.5 ± 23.2[a]	249.4 ± 34.2[b]
Time (s) spent in away distance	94.9 ± 25.3	127.9 ± 38.9

[a,b] Values within a category with different superscripts are significant different ($p < 0.05$)

5. Discussion

Our results from the early maternal behaviour observations showed that SR cows sniffed their calves more and were during the cow test also sniffed more by the unfamiliar calves than SH cows. SR calves showed more preference for and stayed in closer proximity to their mother compared to the SH calves. SR calves also vocalized more than SH calves when separated from their mothers. This suggests that SR calves had a stronger bond to their mother which confirms the hypothesis of the present study. No difference between SR and SH was found for the preference shown by the cows for their calves over unfamiliar calves. MP cows vocalized more compared to PP cows during the second day after given birth. Calves from PP cows sniffed more during the calf test and were sniffed more by the unfamiliar cows compared to calves from MP cows. Calves from PP cows also spent more time sniffing and exploring the buckets during the neophobia test. Parity was not found in our study to have any effect on the preference shown for, or the proximity kept to the mother or the calf. The results from the second day after birth and the preference test suggest that the pairs with MP cows are more bonded and shows more maternal behaviours which confirms the hypothesis. The neophobia test, however, did not follow the same pattern where the PP pairs appeared to be more bonded and is rejecting the hypothesis of the present study. Male calves were observed to vocalize more when separated from the mother during both the calf and cow tests. Mothers of female calves were, however, faster to leave the start box and stayed in closer proximity to their calves. These results are somewhat contradictory, in one hand the reaction to separation suggests that pairs with male calves are more bonded whereas the proximity measurement seems to indicate that pairs with females are more bonded. It might however just be that the males and females express the bond differently and that these measures do not necessarily mean that female or male calves are more or less bonded. However, the unbalanced number of female (n= 15) and male (n=4) calves in the present study could also have influenced the results and caution should be taken interpreting these results.

5.1. Maternal behaviour

In our study, the SR cows sniffed their calves significant more than the SH cows which was the only breed difference found from the observations at the calving pens. This result agrees with results found by Loberg & Lidfors (2001) when studying cows behaviours towards foster calves. Sniffing the calves was the only difference found between the same two Swedish breeds where the SR cows sniffed the calves more compared to SH (Loberg & Lidfors, 2001). This difference might however be explained by that the SR cows are more prone to sniff in general since Stěhulová et al., (2008) showed that cows of the SR breed tended to sniff on other animals and in the air more often than the cows of SH breed. Loberg & Lidfors (2001) speculated if the SR animals in comparison with SH had a stronger tendency to show maternal behaviours because of their observations with more maternal-young behaviours shown by the animals of the SR breed. Further, they discussed if the SR cattle just had a stronger tendency to show mother-young behaviours in general and not necessarily toward their own calves/mothers, or if they actually can be selective and only direct these behaviours towards their own young respectively mother and thereby creating a bond (Loberg & Lidfors, 2001). The difference in sniffing might therefore not just be a difference in maternal behaviour and is instead a behavioural difference between the breeds in general with or without a calf present. During the preference test of the cow, it was in our study also observed that SR cows were sniffed more by the unfamiliar calves, if this was a reaction to that the SR cows sniffed the unfamiliar calf more was however not investigated. One problem with the analysis of the calf sniffing during the calf test as well as the cow sniffing during the cow test, is that it was not shown on which animal the tested individual was sniffing. It was not registered separately for the familiar respectively unfamiliar animal. The sniffing results from the tested animals are therefore needed to be used together with the results from the animals in the choice pens which are the ones that the tested animal was interacting with. The increased sniffing on a SR cow by the unfamiliar calves might indicate that cows from the SR breed encouraged the unfamiliar calves to sniff to a greater extent than SH cows did. In other words, the SR cows might have shown more mother-young behaviours towards the unfamiliar calves than the SH cow did. This result then might support the discussion regarding that the SR cows showed more maternal behaviours in general and not necessary only towards the own calves that Loberg & Lidfors (2001) initiated in their paper. SR calves have also been observed to be more social towards other calves compared to SH calves (Loberg et al., 2008) which might add to the conclusion that SR animals show more behaviours towards other individuals in general and not necessary only towards their own mothers/young. It can however be discussed how this difference in the amount of sniffing will affect the bonding

between cow and calf and the cows' recognition of the calf since sniffing is an important component of mother-young recognition based on observations on sheep (Alexander & Shillito, 1977). If the SR cows are sniffing their calves more in this early stage it might then result that the SR cows later in the lactation will be better at recognizing their calves by their smell compared to SH cows. If and how this will affect the cows' ability to take care of and bond with the calves needs however further investigation. Following up studies need to be done looking at the cows and calves in social groups, with both other cows and calves, to see if there is any difference between the breeds on the ability to recognize the calves respectively mothers when mixed with other animals that they can freely interact with. No correlation between early licking, which also is suggested to affect the later ability to recognize the offspring's, and later interactions between cows and calves was however found in beef breeds (Lidfors & Jensen, 1988) which can indicate that at least licking does not play a role in later recognition. Sniffing in comparison with licking might however to a greater extend provide the cows with the smell of the calves which might make the sniffing a more important component for later recognition which argues for further investigations on the subject.

In our study, the MP cows had a higher mean of vocalizations the second day after birth compared to PP cows. Previous studies however did not found any difference between parity and number of vocalizations during the first 6 hours after birth in Friesians (Edwards & Broom, 1982) and during the second and fifth day after birth in beef cows (Price et al., 1986). However, our measurements were made more than 18 hours later than measurements in Edwards & Broom (1982) and during a longer period than Price et al. (1986) which may be the reason for the difference between our observations and previously observed results. The breed studied can also be a reason for the difference between our result and previous observations since a significant difference was observed by Lidfors (1996) for vocalization between parity when studying the same two breeds as used in our study. It was however not specified in that paper if it was the PP or MP cows that were observed to vocalize more (Lidfors, 1996). The number of vocalizations in dairy cows, when kept with the calves, was also observed by Weary & Chua (2000) where it was shown that the cows tended to vocalize more the younger the calves were, they had however too few PP cows to test the parity effect on vocalization. Since the calves learned the vocals of the mothers and can distinguish the vocalizations from the mothers and unfamiliar cows (Barfield et al., 1994) it is, therefore, possible that the calves of MP cows are better at distinguishing the mothers' vocalizations later in life since they have heard the cows' vocalization more than calves to PP cows. Later when the calves were four weeks old there was, however, no significant difference between parity for the number of vocalizations observed neither for vocalizations

by the cows, calves or total. Maybe the calves at this age already had learned the mothers' vocalization especially since they have spent almost 4 weeks in a group with twenty other cow-calf pairs where the need for the calves to recognize their mothers' increased and the cows does no longer feel the need to vocalize for the calves to learn their voice. It is also impossible from our observations, since there is a four weeks gap between the observations, to know if the PP cows at another period of the calf's early life increased their vocalization for these calves to also better recognize their mothers' vocalizations. Further investigations are needed to know if the increased vocalizations by the MP cows observed in our study leads to better recognition of the mothers by their calves when in a larger group.

Nursing is another important maternal behaviour since it is what provides the calves with nutrition and cows would normally let the calves suckle within the first few hours after birth (von Keyserlingk & Weary, 2007). During the four hours observations at the maternity pen, all calves beside one were observed suckling by themselves. No difference could be found between parity, breed or sex on the number of nursing events performed during the observation time. This differs from (Lidfors, 1996) where parity was observed to have a significant effect on suckling frequency, it was however not specified which cows, PP or MP, resulted in higher suckling frequency. In that study, it was also shown that parity had a significant effect on the duration of each suckling (Lidfors, 1996). In our study, we recorded neither the duration nor whether it was the mothers or the calves that initiated the nursing events. In a study made on beef cattle, it has been observed that during the first week after birth it was the cows that initiated the nursing events and then after a week it was the calves that initiated them (Lidfors et al., 1994a). This indicates that during the first week it is mainly the cows that ensure that the calves suckle, and it is therefore required that cows have the mother's instinct to encourage the calves to suckle but also let the calves suckle without kicking. The question remains after our study to determine the SR and SH cows' ability to encourage the calves to suckle and further study is needed to be done looking at duration time, the nursing position of the calves and measurements of whom initiated the events. All cow-calf pairs in our study were also observed by the staff of the farm from birth regarding the suckling and some calves were assisted by the staff to secure that they got enough milk intake. It was however not included any information in our study regarding which pairs got assistance and how much time the staff spent helping the calves suckle. The need for assistance from the caregiver should also be considered as it can be time-consuming if many calves need help to suckle at the beginning, which may affect how the cow-calf contact system would work on a large scale. It has been shown that 30 % of the dairy calves do not manage to suckle by themselves within 4 hours after birth (Lidfors, 1996). The safety aspect should also be taken

into account as the risk of injury increases if assistance is needed due to struggling cows that kick after the calves or the caregiver when assisting the calves.

5.2. Bonding between cow and calf

The preference test and neophobia test were in our study ways to test the criteria of attachment for animals proposed by Gubernick (1981). SR calves showed more preference for the familiar cows than the SH calves, but no difference was found between sex or parity. This suggests that SR pairs might be more bonded, and no evidence was found that parity and sex affect the bonding. This differs from previous results where male calves have shown a weaker preference for their mother than female calves when another cow was present (Veissier et al., 1990). Their preference tests were however done when the calves were between 8 and 9 months of age and weaned from their mothers (Veissier et al., 1990), an age close to when the bulls reach puberty at around 37-50 weeks of age (Rawlings et al., 2008). Our test was in comparison done when the calves were 4 weeks of age and our observations that the male calves' interest for the other cows did not differ from the female calves might be due to not reached puberty yet. No difference was found between breed, sex or parity and the preference shown for the familiar calf by the cow, which can question if SR pairs are more bonded. One reason for these results might however be that at this age it was the calves that needed to follow the mother and not the cow that followed the calves (Bouissou et al., 2001). Cattle are originally seen as hiders which means that the cows hide their calves to go and eat and be with the rest of the herd and only returns to the calf when it is time to feed the calf (Lidfors & Jensen, 1988; Padilla de la Torre et al., 2016). Though, in studies on modernly housed cattle without the possibility for the calves to act as hiders, the calves have behaved more as a follower of the mother which means that instead of laying hidden somewhere, the calves follows the mother and becomes a part of the rest of the herd already from the start of life (Lidfors & Jensen, 1988; Padilla de la Torre et al., 2016).

Mothers of females were quicker to leave the start box than cows of males which could suggest that mothers of female calves are more bonded to their calves. Two of the female mothers needed however to be excluded since they did not leave the start box during the given five minutes, all mothers of male calves did leave the start box. Parity and breed do not seem to affect the time to leave the start box, neither for cows nor calves. The sex did not either affect the calves leaving the start box.

SR pairs and pairs with a female calf kept closer proximity to each other than SH pairs and pairs with male calves. Female calves have previously also been observed to stay closer to the cows during a longer time than cows and male calves (Lidfors & Jensen, 1988) but opposite results are also observed where mothers of male calves kept closer proximity and initiated contact with the calves more than mothers of female calves (Stěhulová et al., 2013). Lidfors & Jensen (1988) however also showed that calves with lower weight tended to stay closer to the mother, therefore, discussed if the proximity difference between sex also is connected with the weight of the calves where male calves tended to be heavier. The weights of the calves when proximity measurements were done was however not included in our dataset which makes it impossible to say if weight was a confounding effect for the proximity between cows and calves in our study. Keeping closer and maintaining proximity are seen as a sign of attachment and individuals that do not keep that close and maintain proximity to each other are interpreted as having a weaker attachment (Gubernick, 1981). From this criterion, the SR pairs and pairs with female calves should be more bonded than the SH pairs and pairs with males. Bonded young individuals can however also use their mothers as a secure base to explore the environment and thereby extend the proximity even though they, in fact, are bonded (Gubernick, 1981). In the neophobia test, it was also tested how the calves used the mother as a secure base to explore but no difference was found that indicated that the SH and male calves executed more exploring behaviours instead of keeping closer proximity. It might also be that the SH and male calves are less fearful and therefore keep longer proximity to their mothers, The measurements of proximity, however, took place in a smaller enclosure and similar measurements might be more meaningful to run in a bigger enclosure where the animals are given more space and less affected by other factors (Wickler, 1976).

The calves from PP cows spent more time exploring or having the head in the buckets than calves from MP cows. Exploring the buckets was a way to measure the use of the mother as a secure base to explore unfamiliar objects, where calves that have a stronger bond to the mother will use the mother as a secure base to a greater extend (Gubernick, 1981). Our results should then be interpreted as PP calves having a stronger bond to the mother. In contrast, the MP cows had slightly higher mean time spent exploring and having the head in the buckets compared to PP cows. This can indicate that the MP pairs are less bonded since even though that the MP cows was approaching the buckets and showed the calves that the buckets were not harmful, the calves from MP cows spent less time in the buckets than calves from PP cows. It can however also be that calves from PP cows just are less fearful and more willing to approach the buckets and not necessarily due to the presence of the cows. Pairs with male calves ate more concentrate than pairs with

female calves, no differentiating was however done between the cows and calves for the feed eaten and the cows of male calves spent more time with the heads in the buckets. Therefore, the difference is most likely due to the cows eating more and not the calves since no difference was found between male and female calves and the time spent with their heads in the buckets. This then indicates a difference in hunger rather than bonding.

The SR calves vocalized more than the SH calves during the calf test. Breed difference between SR and SH in the number of vocalizations by calves has previously also been observed by Lidfors (1996) it was however not specified which breed the calves that vocalized more were. Vocalization is a common reaction to separation between cows and calves (Weary & Chua, 2000; Flower & Weary, 2001; Stěhulová et al., 2008; Shin-Jae, 2013) and the increased vocalizations might therefore be seen as a stronger reaction to separation by the SR calves. The vocalization was recorded during the whole preference test, where a brief separation took place, first with a distance and then later with a fence as a barrier and contact were possible. The strongest vocal reaction from calves after separation has though been observed to occur first after more than six hours post-separation (Weary & Chua, 2000; Flower & Weary, 2001) further observations are therefore encouraged to see if this observed difference between breeds also is existing during a longer separation. Increased vocalizations by the cows are also seen when separated (Weary & Chua, 2000; Flower & Weary, 2001; Stěhulová et al., 2008) and no difference was found between either breed, sex or parity and the cows' vocalizations during both the cow and calf tests in our study. Between the calf and cow tests, there was however only a short break in between, and the animals were not moved back to their home environment. The animals might then during the cow test not give the same behaviour response to separation the same way as if they were moved back to their home environment and the cow test were run during a separate occasion. Some animals might have also been more stressed during the cow test since the moving of the animals was different from what they were used to. For the calf test, the cow was moved from the cow-calf contact area and the calf, a procedure the cow was already used when eating and milking whereas for the cow test the cows were moved from the choice pen and the calf, a place where they naturally would not leave the calves alone. This might have stressed some cows which might have affected how they reacted during the actual test. Future studies with other ways to measure the reaction to separation are therefore needed to fully measure how bonded the cows and calves are.

5.3. Preference test

5.3.1. Difference between weeks

For this part since the low number of tested cow-calf pairs during week 1, there are no statistical results and only a comparison of the data. Our comparison of the calf tests showed that both cows and calves performed more behaviours during week 1 compared to week 4. The comparison of the cow tests, however, showed that the number of observed behaviours was almost similar between week 1 and week 4 besides the vocalizations by the mothers that was higher at week 1. The calves were faster to leave the start box at week 4 compared to week 1. The cows, on the other hand, was faster to leave the start box at week 1 compared to week 4. Both the cows and calves spent more time in the familiar contact zone and less time in the unfamiliar contact zone during week 1 compared to week 4 during their respective tests.

In future studies, a statistical analysis of the differences between weeks should be done to fully understand the changes in mother-young behaviours and the bonding process as the calves age.

5.4. Further studies

To fully understand the bonding in cattle and get to know if the modern dairy cows are still good mothers and possible to keep in a cow-calf contact system in a longer perspective further studies are needed to be done. One aspect that is needed to be looked at is how the rearing of the calf affects maternal behaviours. It was shown by Le Neindre (1989) that calves reared with the mothers expressed more maternal behaviours when becoming a mother themself compared to non-mothered cows. This can affect how the cow-calf system works in the next generation positive but also negative. The calves used in our study might show more maternal behaviours towards their future calves compared to their mothers, and thereby create a stronger mother – young bond and become more protective over their calves. The stronger maternal behaviour can also affect the safety of the system with increased risk of danger for the staff and thereby it is important to study the maternal behaviours for the next generation both behaviours directed to the calves but also protective behaviours.

In von Keyserlingk & Weary (2007) paper it was discussed that birth of the calves in maternity pens could affect the bonding positively and that another type of housing at calving such as group housing might affect the bonding process

negatively. This is due to that the group housing allows the cows to come in contact with other calves during the critical bonding period immediately after birth and also the calves can come in contact with other cows than the mother (von Keyserlingk & Weary, 2007). In our study, all calves were born in individual maternity pens where they stayed with the mother for at least 48h without contact with other cows and calves. This might then have affected the bonding process in a positive way, and it is unclear how the results of the maternal behaviours and bonding process would have looked like if the calves instead were born in a group housing system. Further research should compare the housing systems at calving regarding maternal behaviours and their effect on the bonding process.

6. Conclusion

After studying the maternal behaviour and bonding in the modern dairy cows it was found that SR cow and calves displayed a stronger filial bond and that cow parity also played a role in the expression of bonding behaviours. Our results show that SR cows sniffed their calves more, SR calves showed more preference for their mother, vocalized more when separated and kept closer proximity to the mothers compared to SH. It was also shown that MP cows vocalized more than PP cows, calves from PP cows sniffed more and was also sniffed more by the unfamiliar cows and spent more time sniffing and exploring the buckets compared to calves from MP cows. Furthermore, it was shown that male calves vocalized more when separated from the mother, but mothers of females were faster to leave the start box and female calves stayed in closer proximity to their mother compared to male calves. However, due to inbalanced number of male and female calves no conclusion can be made regarding sex effects. Further studies on bonding behaviour development in cow-calf systems associating it with later social behaviours in the groups are encouraged. Also, the behaviours towards the farmers in a long-term perspective when kept in a cow-calf system, and the long term effects of the cow herself being mothered during rearing, are aspects that require further study.

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8. Appendix 1

Table 17: The effect of sex of the calf on mean ± sem behavioural variables performed in the maternity pen for 4 hours.

Behaviours	Sex	
	Female	Male
Number of vocalizations by the cow	48.3 ± 13.2	35.3 ± 6.5
Number of vocalizations by the calf	1.3 ± 0.3	1.3 ± 1.3
Number of lickings occasions on the calf by the cow	28.3 ± 5.7	23.0 ± 0.7
Number of lickings occasions on the cow by the calf	0.4 ± 0.2	0.3 ± 0.3
Number of sniffing occasions on the calf by the cow	23.8 ± 3.8	25.3 ± 8.8
Number of sniffing occasions on the cow by the calf	8.1 ± 1.5	4.5 ± 0.6
Number of nursing events	17.1 ± 4.5	18.0 ± 7.2
Number of suckling occasions by the calf	17.6 ± 4.3	18.2 ± 7.3

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