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FIRST CYCLE DEGREE (B.SC.) IN ANIMAL CARE

Towards an evaluation of the quality of the cow maternal interactions with the calf in the early hours postpartum

Supervisor:

Prof.ssa Marta Brščić

Cosupervisor:

Dott.ssa Claudia Manfrè

Submitted by:

Aurora Bosco

Student n.

1222092

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Abstract

Nowadays, in dairy farms, the most common practice in the cow-calf management is the early separation right after birth or within the 24h after parturition. The reasons for the implementation of this practice are found in the increase of saleable milk (and thus profit) and the avoidance of the separation stress that would derive from the abrupt late separation. The growing concern for farm animal welfare, along with the rising demand for "animal-friendly" dairy product, is driving increased interest in cow-calf contact systems (CCC), that consist of keeping cow and calf together in various degrees and for different amounts of time. Both cow and calf benefits from CCC systems in terms of health, behavior and productivity, if the right debonding strategies are applied. This thesis aims at investigating cows' behavior during the critical stage of pre and post parturition, focusing on the evaluation of the quality of the cow-driven interactions towards the calf. Behaviors of a sample of 8 cows of the Pezzata Rossa Italiana breed were observed through video-recordings that started one hour before parturition and lasted at four hours postpartum, for a total of 5 hours. Behaviors were recorded with the Instantaneous Sampling technique, with intervals of 30 seconds between each scan. Results showed that all cows provided strong maternal care by grooming the calf and keeping physical contact with it, especially in the first hour after giving birth, with a gradual decrease of the expression of these behaviors over the course of hours. Although these affiliative behaviors decreased during time, agonistic behaviors were not recorded, suggesting that even if cows progressively diminish the time dedicated to the calf, the quality of this interactions remains positive. Time of no contact with the calf was indeed spent in activities such as resting, sniffing the litter and eating, that are common behaviors and suggest the absence of distress when the calf is still nearby.

Introduction

1.1 Conventional management of cow and calf on dairy farms.

Relying on data from 2023, in Italy there are 24.094 dairy farms, mostly located in the North of the country, for a total of 2.618.026 animals raised in them (De Vincenzi, 2023). Over the course of the last 15 years, the number of dairy livestock has been more or less constant, but what changed was the distribution of this livestock on farms with different dimensions: the number of animals raised on medium and small farms (<500 individuals) is decreasing, while livestock raised on large farms is increasing (Vignone, 2024). Both in small and large farms, one of the most critical issues is the management of the dairy cows in the peripartum period and the subsequent management of the newborn calves. The first stage of this delicate phase is the transition of the cow from lactating to non-lactating, thus allowing a moment of pause from milking before starting the new lactation after giving birth, mimicking the natural decrease of the suckling behavior of the calf when weaning. This practice is referred to as “dry-off”, it starts at about 60 days from the expected due date and have an important role to preserve cow’s udder health and to optimize milk production in the following lactation (Vilar et al., 2020). There are two main methods for drying-off lactating cows: the abrupt one, that consist of stopping the milking on a set day, and the gradual one, in which the milking is decreased gradually. In the period before completing dry-off, cows are fed less amount of food to decrease milk production and are given antibiotics to avoid infections due to the clogging of the milking duct.

During the transition time, defined as the period between the 3 weeks before and the 3 weeks after calving (Grummer, 1995), cows go through physiological and behavioral changes. Calving time can be predicted by observing different signs occurring in the cow: hormonal signs, such as the abrupt decrease of oestradiol-17β and progesterone concentration in blood circulation (Shah et al., 2006), clinical signs, such as relaxation of the pelvic ligament and decrease in body temperature (Aoki et al., 2006), and behavioral signs, such as increasing standing/lying transitions and tail raising (Saint-Dizier et al., 2015). When calving is imminent,

the cow is moved to a designated calving area. In order to allow the periparturient cows to get used to the new environment, food and, if present, social companions, it is advised to move them 1-2 days before parturition. Moreover, moving the cows too late in the birth process could represent a stressor capable of altering the ability of calving normally (Matamala et al, 2021).

Under normal conditions, cows give birth by themselves, but in some cases, to ensure the health of both the mother and the offspring(s), operators or veterinarians can help in the calving process. The causes of dystocia can be both maternal, such as pelvic canal abnormalities, uterine inertia or neoplasm of the vagina, and fetal, such as oversize fetus or wrong disposition in the birth canal (Mekonnen et al., 2016), and it is crucial to detect signs of this abnormalities as soon as possible to intervene.

Once the calf (or calves in cases of twin births) is born, the management of both the dams and their offsprings change among farms. The most common practice is the early cow-calf separation within the first 24-hours postpartum, in order to increase the quantity of the saleable milk and to avoid the formation of a strong cow-calf bond that, with the increase of the amount of time spent together, will be more difficult to break (Flower et al., 2003). Moreover, it has been shown that early separation can prevent the transmission of diseases and parasites from the mother to the calf (Faubert et al., 2000), although literature does not support early cow-calf separation on the basis of cow or calf health (Beaver et al., 2019). Alternatives to this early separation will be discussed later in this thesis. After separation, calves are moved in the calf pen, where they can be housed singularly (for a maximum of 8 weeks) or with other calves. The European Directive 119/2008 sets the standards for the calf pen dimension (that depends on calves' number and size), bedding, temperature, ventilation, and other parameters to ensure calves welfare. The most important management factor in determining health and survival of the newborn calf is the intake of high-quality colostrum (Godden, 2008). Bovine colostrum consists of the mixture of lacteal secretions and constituents of blood serum, such as immunoglobulins and other serum proteins, that accumulate in the mammary gland during the prepartum dry period and can be harvested immediately following parturition, in the first six postpartum milkings (Foley et al., 1978). The

ingestion of the colostrum from the calf is essential for its nutritional values, since it is a concentrated source of nutrients with 1.85 times the DM, 4.52 times more protein, 1.68 times more fat, and greater concentrations of minerals and vitamins than whole milk (Foley et al., 1978), but most importantly for the development of the calf immune system through passive transfer. Calves are indeed born with an immature immune system, and totally depend on the colostrum to receive essential immunoglobulins. It is important that the colostrum is fed as soon as possible, since IgG's intestinal absorption is a temporary ability that decreases with time, until it completely stops at about 24-hours after birth (Lopez et al., 2022). To ensure the immediate intake of high-quality colostrum, it is discouraged to rely only on the suckling activity of the calf towards the mother, since it is not possible to evaluate the exact quantity that has been consumed. Moreover, in cases such as the early cow-calf separation, the calf has not access to the mother, so an alternative colostrum delivering method must be used. The most efficient methods to deliver colostrum are buckets, nipple bottles or esophageal feeders. Due to the critical importance of colostrum to newborn calves, every dairy farm must have an adequate supply of high quality, disease-free colostrum available (Lopez et al., 2022). This supply can consist of the excess colostrum produced by the dams housed in the farm and must be stored correctly to ensure its quality. The best way to store colostrum is by freezing it, in order to avoid bacterial proliferation and to maintain its nutritional values and immunoglobulins levels.

1.2 Importance of the cow-calf bonding.

In nature, the bond that mammalian species form between mother and their offsprings is fundamental for the survival of the latter, since the mother is the most important social contact for the newborn during the first months and, in addition to feeding and care, she provides the offspring with the acquisition of important information in relation to the physical and social environment (Orihuela et al., 2021). The phase in which the bond develops is known as the 'sensitive period,' characterized by a limited window of time dependent on the early reception of

sensory stimuli of sight, touch, smell, and hearing (Figure 1) naturally exchanged with the mother or surrounding environment in the neonatal stage. In particular, in ungulates, olfactory cues are fundamental in the first 1-2 hours, where maternal care is exclusive to select against the opportunity of the calf to suckle from other cows present in the flock (Broad et al., 2006). In dairy cows, there is evidence that 5-min contact with a calf immediately postpartum is sufficient for the formation of a strong, specific maternal bond with that calf (Hudson et al., 1977). In these minutes, the dam starts licking and grooming the calf, thus stimulating its activity and circulation as well as its ability to breathe, urinate and defecate (von Keyserlingk et Weary, 2007). The importance of the maternal filial bond is essential even for behavioral development to adulthood, since a poor bond quality can lead the calf to an increased aggressiveness and an impaired ability to aggregate with conspecifics (Damian et al., 2018).

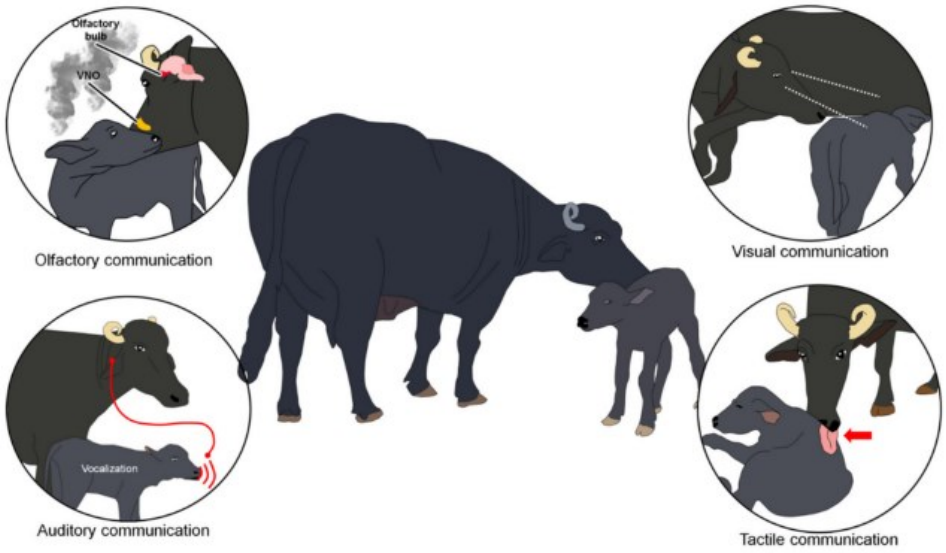

Figure 1: Senses and site of action in the brain during the imprinting process (Orihuela et al., 2021)

1.3 Pros and cons of alternatives to the early cow-calf separation

Nowadays, in dairy farms, the most common practice to manage the cow and its calf after birth is to separate them as soon as possible, or at least within the 24 hours after parturition. The advantages of this practice are many and well described by Beaver et al. (2019) and can be grouped into three main categories: economical, sanitary and ethical. Starting from the economical one, it is well known that allowing the calf to suckle directly from the mother will result in a decrease of the amount of saleable milk, thus decreasing also the farm's profits. From the health point of view, both cow and calf could benefit from the early separation. Firstly, artificially feeding the colostrum to the calf allows a control over the quantity and quality of it, that would be difficult in the case of natural feeding, and consequently ensuring a better development of the calf's immune system, as previously stated. Moreover, keeping the calf in the calving area could result in the increase of transmission of infectious diseases, since it has been demonstrated that the cow's fecal coliform count peaks in the periparturient period (Pelan-Mattocks et al., 2000). From the ethical point of view, many studies concluded that keeping the cow and calf together (even for only 5 minutes after birth) will result in the formation of a bond, that will then be stressful for both the dam and the calf to break later in life (Hudson and Mullord, 1977; Lidfors, 1996; Weary and Chua, 2000; Flower and Weary,2003).

On the other hand, in recent years the public concern about the cow-calf early separation practice is increasing (Busch et al, 2017), and it is precisely ethical, health and productivity considerations that are driving growing attention and interest in the research and implementation of alternative systems to early separation. These alternative systems, also called cow-calf contact systems (CCC), allow the expression of natural behaviors between the dam and its calf. Cattle are social animals that, in natural environments, live in groups. Before giving birth, the cow temporarily separates from the group and isolates her calf for the first few days, after which she reintroduces it to the group. From around two weeks of age, cows may begin leaving their calves for longer intervals, with gradual weaning occurring at approximately 6–8 months of age. In this period, the mother can provide maternal

care to its offspring and the latter can choose when and how much to suckle. It has been demonstrated that the growth rate at 6-8 weeks of calves that are fed at libitum is higher than that the one of calves that are fed artificially following recommendations (fed 10% of their body weight twice a day) (Appleby et al., 2001; Grøndahl et al., 2007) without consequences on the health of the calves. Moreover, Shamay et al. (2005) also demonstrated that a higher milk intake during the nursing period leads to an higher amount of milk produced in the heifers first lactation, validating the hypothesis that, despite the profit loss due to the decrease of the saleable milk in farms that don't apply the early-separation practice, there could be an economic benefit also for CCC systems. Over the course of the years, different CCC systems have been developed, and Johnsen et al. (2016) well described the advantages and disadvantages of four types of them: Free cow-calf contact, half day cow-calf contact, restricted suckling contact, and foster cow system.
In free cow-calf contact, the dam and its calf are kept together 24h a day for an extended period (6 to 12 weeks) in which they are free to interact and nurse at any time. One of the main benefits of this system is allowing self-regulation on nursing bouts and the expression of natural affiliative behaviors, such as licking and rubbing, that will result in a decrease of abnormal behaviors in the calf, such as tongue rolling and cross suckling, that are associated with the unsatisfied motivation to suck (de Passillè, 2001) or hunger (Herskin, 2010). As stated before, calves benefit from natural suckling also in terms of nutrition and growth rate as well as amount of milk produced in the first lactation. In this type of CCC system, however, the cow and its calf are abruptly separated at 8-12 weeks of age, due to the high amount of milk needed to feed the calf, that result in a consistent profit loss for farmers. This sudden separation is thought to be deleterious also for the cow and calf themselves, because breaking the strong bond that they established result in an increase of stress indicators, such as high pitch vocalization of the calves (Johnsen et al., 2015).

In the half day cow-calf contact system, where cows are kept together with their calf for 12h/d, calves are shown to have high weight gains both pre-weaning (due to the higher milk consumption during natural milking) and post-weaning (because calves are accustomed to being separated from the mother, thus less dependent on

feeding). Despite the high amount of time being separated, the cow and its calf form a strong bond, that however is less stressful to break than the one formed in full CCC (Wenker et al., 2022). One disadvantage that could arise from this system is the labor needed to move the calf back and forward to the mother's pen. This labor intensity can be even higher for the restricted suckling contact system, in which calves are allowed to stay with the mother and suckle only for short periods, such as 15, 30 or 60 minutes twice a day. Despite the ability to suckle high amounts of milk in short periods, the weight gains of calves in this system are highly variable, probably because the studies conducted on the matter are variable in terms of time spent together, the timing of nursing, breed and the amount of milk produced by the cow (Johnsen et al., 2016). Another CCC system is the foster cow system, that consists of keeping together 2-4 calves with one cow, that may or may not be the mother of one of them, and allowing them to perform suckling behavior. The advantage of this system is that calves are socially grouped, and this is beneficial in terms of weight gain, health and post-weaning social skills (Warnick et al., 1977), other than all the benefits deriving from the natural suckling described before. A problem that could arise from this CCC system is when the foster cow rejects the cow, or when she performs less affiliative behavior, although this usually does not happen.

In all of the cow-calf contact systems described, the main challenge for the welfare of the animals is the increase of stress after separation, since allowing more contact means forming a stronger bond that is difficult to break. For this reason, it is fundamental that gradual debonding strategies are adopted, such as gradually decreasing the time the dam and its calf spend together rather than abruptly separate them or fitting calves with nose-flaps in order to stop the suckling behavior but still allowing the contact with the mother (or foster mother).

1.4 Legislative and stakeholder's perspective on cow-calf management

Article 3 of the European directive 98/58/EC, also known as "General Farm Animals Directive," states that "Member States shall make provision to ensure that the owners or keepers take all reasonable steps to ensure the welfare of animals under their care and to ensure that those animals are not caused any unnecessary pain, suffering or injury". However, this Directive adopts a broad language and does not include species-specific regulations for dairy cattle welfare, nor regulations regarding the crucial topic of cow-calf separation (Nalon and Stevenson, 2019). Indeed, except for the Council Directive 2008/119/EC, that lays down minimum standards for the protection of calves, European dairy cattle's welfare is not regulated by any species-specific normative. This is not true for other farm animals such as poultry or pigs, the last of which are objects of clear legislation even regarding the separation of piglets from their mother, that cannot happen within three weeks after being born. This exclusion of dairy cattle from legislative protection could be because, compared to the pork and poultry industries, dairy has a more positive public image than the others due to marketing strategies and poor information (Busch et al., 2017; M. Placzek et al., 2021; Christoph-Schulz et al., 2015). However, over the course of years, the public interest in products from animal-friendly husbandry systems and consequently in the welfare of animals housed in these systems is increasing. In a review on the public attitude towards early-separation practice, Placzek et al. (2021), analyze multiple surveys conducted on people from UE and USA that investigate their acceptance or rejection of the practice. The results enlighten two main issues: most consumers are unaware of this practice and, when aware, they are not in favor of it, mainly because it is unnatural and stressful for the animals in question. However, the same perspective is not shared by farmers that, by adopting alternative methods to the cow-calf early separation, would decrease the amount of saleable milk they produce and, therefore, would face a profit decrease (Alvåsen et al. 2023). Moreover, different CCC systems, such as the restricted-suckling contact system, imply an increased amount of labor required to move the calf to the mother's pen multiple times a day, other than specifically designed structures intended to accommodate both cow and calf that must be kept meticulously clean to avoid the transmission of pathogen to the

calf, that has an undeveloped immune system in respect of adult cows. However, some farmers argue that keeping the calf with the mother for a prolonged period of time will result in calmer and more confident animals, thus easier to manage (Vaarst et al, 2020). Moreover, the increasing demand of "animal-friendly" dairy products and the willingness of consumers to pay more for products that come from farms that respect these measures (Risius and Hamm, 2017) is convincing more farmers to try to adopt CCC. To summarize, consumers, when aware of it, have an overall negative opinion on the early-separation practice, while farmers tend to support the reasons for which it has been adopted for the past decades. The scientific community, despite agreeing on the fact that an early separation is beneficial for the decrease of disease transmission and the avoidance of the post-separation stress, is increasingly highlighting evidence that the benefits of adopting CCC systems outweigh the disadvantages from the animal welfare point of view.
The 2023 EFSA's Scientific Opinion entitled "Welfare of Calves" stated that calves should stay with their mother for a minimum of 24h and that "from a welfare point of view, prolonged cow–calf contact should increasingly be implemented due to the benefits for calf and cow, so that in the future, calves should have contact with the dam during the whole pre-weaning period" (EFSA, 2023). This statement clearly supports the importance of adopting alternatives to early separation to increase dairy cattle's welfare but, as stated by the EFSA report itself, more research is needed to identify how to implement these alternatives.

2. Aim

The aim of this thesis is to evaluate the cow-calf interactions in the first four hours postpartum, focusing also on the cow's position in the pen, on the type of contact with the calf and on the cow's posture, analyzing how these variables change according to the time from parturition. To understand what activities are performed by cows when they are not in physical or visual contact with the calf, also common and situation-related behaviors will be observed. Individual variability has also been taken into consideration to understand if the quality of maternal interactions recorded is due to individual temper of the cow. This study is part of a wider project that aims at improving cow and calf welfare on farms by promoting best practices in high quality management of the CCC system.

3. Materials and methods

3.1. Quantitative behavioral evaluation

3.1.1. Description of the context and the cows involved in the study

This study was conducted on a sample of 8 cows raised at the "Lucio Toniolo" Experimental Agricultural Company, an autonomous management center within the University of Padua, whose mission is to support the educational activities of the four Agripolis departments (BCA, DAFNAE, MAPS, TESAF) as well as the research and experiments initiatives of the University (www.aziendagraria.unipd.it).

The cows in question are of the Pezzata Rossa Italiana (PRI) breed and raised in the didactic dairy cattle barn, where at the moment of the study there were 36 lactating cows, 5 dry cows, 11 heifers, 8 yearling heifers and 8 calves. At the Lucio Toniolo company, cows are dried off 60 days before the expected calving date, and 4 days before parturition they are moved in the "calving room" that consists of a pen of 40 m^2 provided with litter (figure 2a). In the ideal situation, calves are early separated from their mother 15 minutes after being born and transferred to a calf littered pen (2.10 m x 1.60 m), where they are bottle fed with usually 3L of colostrum, that was milked right before from the mother or taken from the colostrum bank available in the farm, where the surplus colostrum is stored. However, when cows give birth at night, early separation is not always possible, since operators are not always available. This means that cows have the possibility to spend more time with the calf and are separated as soon as an operator is available.

The 8 cows analyzed for this study (Table 1) gave birth in the period between February and June 2021. All of them gave birth during the night (where "night" is considered as the time between sunset and sunrise in that particular day), a part for one cow that gave birth during the day (Figure 2b). Only one cow was primiparous, while the others had a parity between 2 and 7. All parturitions were unassisted, apart from a twin calving that required the presence and help of two operators (Figure 2c). Since most of the parturitions happened late at night, early separation was not possible, and the time spent with the calf varied among the dams from 2.30 hours

to 7.25 hours. Often, another pregnant and close to calving cow was present in the calving pen.

Figure 2a-c. Photograms of the analyzed videos in different situations: a) dam in the "calving pen";b) dam and calf right after parturition; c) dam giving birth assisted by two operators.

Cow ID number	Day of parturition	Night/day	Time with calf	Parity	Assisted parturition
1	08/02/2021	Night	7h25m	7	no
2	30/03/2021	Night	4h35m	7	no
3	28/03/2021	Night	2h25m	5	yes (twins)
4	23/07/2021	Night	4h47m	4	no
5	01/06/2021	Night	4h20m	3	no
6	25/06/2021	Day	2h27m	2	no
7	13/02/2021	Night	6h	1	no
8	03/05/2021	Night	6h	4	no

Table 1: Information about the 8 parturitions selected for the quantitative behavioral assessment.

3.1.2. Description of the observation techniques

The cows subject to the study were recorded with surveillance cameras from one hour before the parturition to 4 hours after. The videos were analyzed using Behavioral Observation Research Interactive Software (BORIS, version 8.20.4, Torino, Italy) with the Instantaneous sampling technique, combined with Focal Animal one, applying 30 seconds intervals. This technique was chosen instead of the Continuous sampling one because previous studies confirmed that both quantitative behavioral observation methods are reliable to detect maternal behavior in the early hours postpartum, demonstrating also that both techniques can differentiate the behaviors exhibited based on the time the cows spend with their

calves (Manfrè et al., 2024). However, Instantaneous sampling technique can underestimate some behaviors, especially those that have a short duration, such as vocalizations or short interactions with the calf (sniffing, horning, etc.), especially with larger time intervals; hence in this study a short interval between the scans of 30 seconds has been set, to minimize this problem as much as possible. Each video has been analyzed from one hour before the parturition to four hours after, setting the time of parturition as the moment of calf expulsion, for a total of 600 (120 scans x 5 hours) scans for each cow.

3.1.3. Ethogram

The working ethogram used in this study (Annex A) was adapted from previous studies (Manfrè et al., 2024) and is divided into behavioral categories that take into consideration common cow's behaviors, such as eating and resting, as well as specific behaviors that can be exhibited both before and after the parturition, such as restlessness or placenta ingestion. Behaviors towards the calf and towards the other cow present in the pen were divided into two categories: "affiliative" and "agonistic" for both the subjects of the interaction. Since some cows didn't spend all of the recording time with their calf, the ethogram included a specific category of "calf searching" behaviors, such as looking out of the pen or sniffing the litter. Other than behaviors, a list of modifiers was taken into consideration for each observation, including the posture of the cow (standing/lying), the position of the cow in the pen (front/back), the type of contact with the calf (physical/visual/no contact), and the locked condition in the feeding rack or to the pen. The video recordings didn't include the sound, so acoustic communication couldn't be analyzed into detail.

3.2 Statistical analysis

The data extracted from BORIS were inserted in an Excel (Microsoft Office, Microsoft Corporation, Redmond, WA) spreadsheet where the experimental unit was the single animal and then were converted from absolute frequencies of scans into percentages of scans in which the animal was observed engaged in a specific behavior or modifier, based on the total number of scans for each time interval. Descriptive statistic was calculated in order to obtain the mean percentage and standard deviation. Normally distributed data of interaction with the calf and modifiers with a prevalence of at least 10% were submitted to analysis of variance with the statistical software SAS (SAS Inst. Inc., Cary, NC, USA), using a mixed model that included the effect of time from parturition as a fixed effect and the cow as random effect. Statistical significance was implemented for P values < 0.05.

4. Results

The results of the descriptive statistic analysis are shown in Table 2, expressed as percentages of scans (mean ± standard deviation) recorded for each behavior listed in the working ethogram (Annex 1), for each hour from parturition. Further statistical analysis focused on how the time spent on cow-calf interactions and the modifiers of posture, position on the pen and type of contact with the calf changed according to time from parturition. Since all the agonistic behaviors towards the calf were not observed or were observed in very low percentages, they were not submitted to further statistical analysis. The only cow-calf interactive behavior that had enough prevalence of scans to be included in the analysis was the grooming behavior. The levels of significance of the grooming behavior and the modifiers included in the analysis of variance are listed in Table 3. The levels of individual cow variability for each significant behavior (p-value<0,05) are reported in Table 4. In table 5, a more accurate analysis of how the percentages of time spent in grooming the calf and in the significant modifier conditions change according to time from parturition is shown.

From now on, for convenience, percentages of scans for each time interval will be referred as amount of time spent in a specific behavior or modifier in that interval, since it has been demonstrated that there is a strong correlation between results of behavioral observations obtained from Instantaneous Sampling technique and results obtained from Continuous Sampling technique when the interval between each scan is 30 seconds, as in the case of this study (Manfrè et al., 2024).

	-1	1	2	3	4
Standing	45,3 ± 13,9	93,6 ± 10,7	85,7 ± 17,3	69,9 ± 20,8	67,6 ± 17,6
Lying sternal	38,6 ± 20,5	3,9 ± 10,6	14,2 ± 17,4	30,1 ± 20,8	29,1 ± 18,1
Lying lateral	14,5 ± 20,8	1,7 ± 4,1	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Transition	0,0 ± 0,0	0,1 ± 0,3	0,0 ± 0,0	0,0 ± 0,0	0,1 ± 0,3
Physical contact	0,0 ± 0,0	92,4 ± 10,8	80,0 ± 21,0	58,6 ± 37,5	53,3 ± 45,0
Visual contact	0,0 ± 0,0	6,4 ± 11,3	12,7 ± 19,5	12,3 ± 24,3	4,3 ± 8,0
No contact	0,0 ± 0,0	1,3 ± 2,7	7,3 ± 11,8	29,1 ± 39,4	35,6 ± 42,1
Front	48,2 ± 30,0	46,8 ± 44,1	56,6 ± 44,9	54,5 ± 38,3	37,6 ± 38,6
Back	50,2 ± 30,6	52,5 ± 43,9	43,3 ± 45,0	45,5 ± 38,3	59,2 ± 39,7
Locked	3,2 ± 9,1	0,4 ± 1,2	0,7 ± 1,8	5,5 ± 11,1	0,0 ± 0,0
Not locked	95,2 ± 8,7	99,6 ± 1,2	99,3 ± 1,8	94,5 ± 11,1	96,8 ± 4,8
Sniffing	0,0 ± 0,0	2,3 ± 6,5	2,7 ± 7,7	0,4 ± 1,2	0,4 ± 1,2
Nudging	0,0 ± 0,0	1,1 ± 1,2	2,3 ± 3,9	0,9 ± 0,9	0,5 ± 1,0
Nursing	0,0 ± 0,0	0,0 ± 0,0	1,7 ± 4,1	1,5 ± 3,2	1,0 ± 2,9
Grooming calf	0,0 ± 0,0	73,3 ± 13,2	34,0 ± 15,4	18,3 ± 11,6	10,8 ± 12,5
Horning calf	0,0 ± 0,0	0,0 ± 0,0	0,1 ± 0,3	0,0 ± 0,0	0,1 ± 0,3
Friendly cow	0,4 ± 0,6	0,1 ± 0,3	0,4 ± 0,8	0,5 ± 0,8	0,0 ± 0,0
Allogrooming	0,0 ± 0,0	0,0 ± 0,0	0,4 ± 1,2	0,0 ± 0,0	0,6 ± 1,5
Displacing cow	0,0 ± 0,0	0,0 ± 0,0	0,1 ± 0,3	0,1 ± 0,3	0,1 ± 0,3
Horning cow	0,0 ± 0,0	0,0 ± 0,0	0,1 ± 0,3	0,0 ± 0,0	0,0 ± 0,0
Sniffing litter	14,5 ± 4,0	4,9 ± 5,6	13,0 ± 7,8	16,7 ± 6,3	13,1 ± 5,6
Looking out	0,1 ± 0,3	0,2 ± 0,4	2,2 ± 4,2	5,8 ± 7,7	5,2 ± 11,0
Agitation	0,3 ± 0,6	0,5 ± 0,8	0,2 ± 0,6	0,6 ± 1,2	0,2 ± 0,6
Vigilance	5,2 ± 3,8	3,2 ± 3,2	11,4 ± 7,0	5,4 ± 4,0	3,1 ± 3,1
Exploration	0,0 ± 0,0	0,2 ± 0,6	0,8 ± 1,4	1,5 ± 1,1	0,8 ± 0,9
Placenta ing.	0,0 ± 0,0	0,9 ± 1,3	1,1 ± 2,2	1,4 ± 1,5	14,9 ± 23,1
Birth fluids ing.	4,4 ± 6,5	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Partum	30,0 ± 14,4	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Partum contr.	7,1 ± 3,4	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Secondment	0,0 ± 0,0	0,8 ± 1,3	4,0 ± 6,2	0,2 ± 0,4	0,3 ± 0,6
Restlessness	10,5 ± 4,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
Vocalization	0,4 ± 0,6	0,6 ± 1,0	1,0 ± 1,1	1,5 ± 1,8	0,9 ± 1,2
Locomotion	0,4 ± 0,6	0,1 ± 0,3	0,5 ± 0,6	1,7 ± 2,5	0,8 ± 1,2
Resting	1,5 ± 2,3	1,5 ± 4,1	3,3 ± 5,7	16,9 ± 18,7	13,9 ± 18,3
Inactivity	10,0 ± 8,9	2,2 ± 3,1	8,3 ± 5,9	7,9 ± 6,5	5,8 ± 5,0
Self-grooming	4,5 ± 4,0	1,3 ± 1,9	3,3 ± 5,8	2,2 ± 3,7	3,8 ± 4,7
Stereotypy	0,7 ± 1,8	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,3 ± 0,9
Eating	0,5 ± 0,9	0,6 ± 1,8	1,3 ± 2,1	4,4 ± 5,0	3,4 ± 5,6
Rumination	1,9 ± 3,2	0,0 ± 0,0	0,0 ± 0,0	4,4 ± 7,2	7,2 ± 10,9
Not visible	5,9 ± 7,4	5,9 ± 6,7	7,4 ± 8,5	6,7 ± 6,4	8,9 ± 8,2
Other	0,1 ± 0,3	0,1 ± 0,3	0,2 ± 0,4	1,1 ± 1,7	0,3 ± 0,9

Table 2: Percentages of scans (mean±standard deviation) recorded for each behavior of the ethogram (Annex A) in the hour before and in the four hours from parturition.

Behavior	P-value
% Physical contact	**<0.001**
% Visual contact	0,256
% No contact	**0,0091**
% Standing	**<0.001**
% Lying sternal	**0,0027**
% Lying lateral	**0,013**
% Front	0,7297
% Back	0,8512
% Grooming calf	**<0.001**

Table 3:Results of the analysis of variance on the effect of time on expression of behaviors and modifiers.

Behavior	Individual Variability
Physical contact	36%
No contact	24%
Standing	5%
Lying sternal	8%
Lying lateral	8%
Grooming calf	5%

Table 4: Individual variability of cows for each behavior and modifier that resulted significant in the analysis of variance.

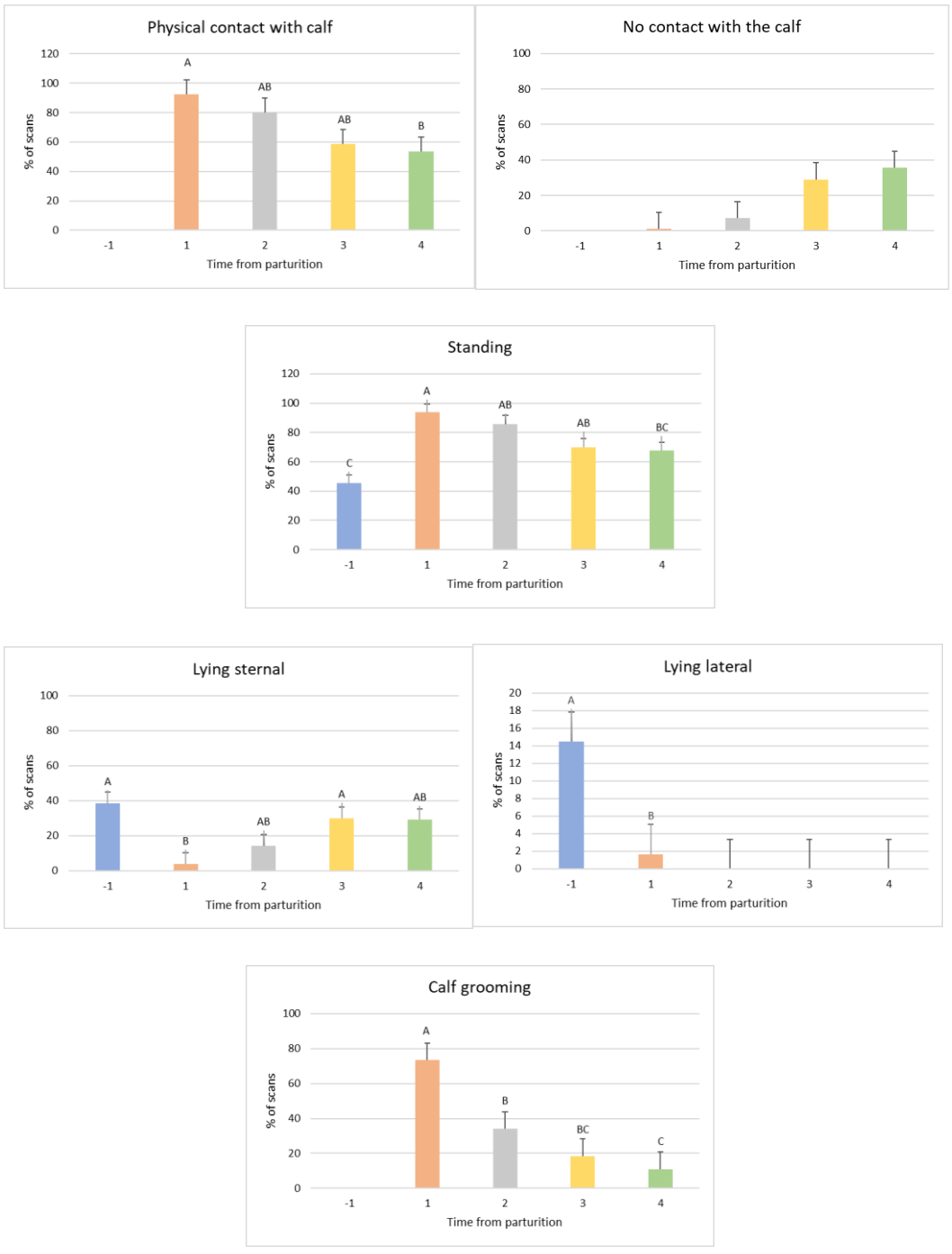

Table 5: Percentages of scans of behaviors and modifiers that statistically differ according to the hour from parturition. Different letters indicate statistical difference (p-value<0,5).

5. Discussion of results

During the five hours of observation, cows expressed a variety of behaviors that are specific for the calving period, listed in the ethogram as "Situation related behaviors" (Annex A). Cows were restless during the hour before partum due to the physiological pain and discomfort typical of this stage, and spent most of the time actively pushing the calf out of the birthing canal in the lying position. In the hour before parturition, cows were also observed licking birth fluids, and in the hours after, especially in the 4th, they expressed placentophagy behavior (7 out of 8 cows expelled the placenta in the observation interval, out of which 6 ate it). The attraction to amniotic fluids and placenta occurs usually from 12 hours before and up until 24 hours after parturition respectively (Machado et al., 1997a), and is thought to occur due to the analgesic properties of these fluids, that lower sensitivity to pain and allow a rapid recover in order for the cow to promptly provide maternal care to its offspring (Machado et al., 1997b). Interestingly, cows expressed behaviors that are commonly associated with the "calf searching" activity, such as sniffing the litter or looking out of the pen, even though for the majority of them (6 out of 8) the calf was always present for the observation time. Sniffing the litter is however a common behavior at this stage, since birth fluids are scattered all over the pen and cows are prone to sniff and lick them. Agitation and vigilance were observed in very low percentages (apart from the vigilance behavior in the 2nd hour postpartum), meaning that cows felt comfortable in their pen with their calf, probably also because 7 out of 8 parturitions took place in nighttime, so there were not noises or sudden movements that could alert the cows. A good indicator of the emotional status of cows while interacting with the calf are vocalizations (Green et al., 2021), but since the video recordings weren't provided with audio, the incidence of this behavior could be underestimated and the nature of the vocalizations couldn't be studied.

In accordance with the results found in Edwards and Broom's (1982) and Jensen's (2012) studies, this analysis shows that the preferred posture in the five observed hours was the standing one, that peaked in the first hour postpartum and gradually decreased in the subsequent hours (Table 5). Since the majority of cows give birth in recumbent position unless assisted by humans (Edwards and Broom,1982), it was

expected that a good amount of the hour before calf expulsion was spent in lying positions, both sternal and lateral. Incidence of lateral position significantly decreased in the first hour after calving and was not observed in the subsequent ones (Table 5), suggesting that it is a situation-related posture showed in proximity to calving. Laterality has not been taken into consideration in this study, but previous research shows that periparturient cows prefer to lay on the left side, probably because of the discomfort caused by the enlarged uterus that pushes the rumen further from the left (Spinka, 2009). Coinciding with the reduction of the time spent standing, in the hours after parturition cows spent increasing amount of time lying in the sternal position, especially in the 3rd and 4th hour, due to the increase of resting behavior observed in those hours. For what it concerns the position in the pen, cows spent almost equal amount of time in the front and back of the pen, and no significance was found in relation to the time from parturition. Studies have shown that dairy cows kept indoors prefer to calve in a shelter (thus in a more isolated place, that could be identified as the back of the pen), when they give birth during the day and when housed individually at the time of parturition (Proudfoot et al., 2014). The fact that 7 out of 8 of the cows included in the study gave birth during the night and most of them were housed with another periparturient cow could explain why there isn't a significant difference in the time spent in the front or the back of the pen in the hour before calf expulsion. The locked condition was observed in the hour prior to calving, since one cow had a twin parturition and was tied to the pen to receive assistance by operators. Locked condition was also observed in the third hour postpartum where cows were locked in the feeding rack.

In the four hours after parturition, the time spent by the cow keeping a physical contact with the calf peaked in the first hour and then gradually decreased (Table 5). Two out of the 8 cows object of the study were separated from their calf 2,5 hours after parturition (Table 1), so results about the interactions with the calf and the distribution among time of the three types of contact could be underestimated, but individual variability was low (Table 4), so the results can still be considered reliable. Moreover, the decrease of cow-calf physical contact during the hours after parturition is well documented in literature (von Keyserligk and Weary, 2007;

Edwards and Brooms, 1982) and is strictly related to the progressive decrease of the licking behavior (Edwards and Broom, 1982; Lidfors and Jensen, 1988; Illmann and Spinka, 1993). As expected, grooming is indeed the main behavior observed in the four hours post-partum, and its duration peaks in the first hour postpartum and steadily decrease in the subsequent hours (Table 5). Licking behavior is fundamental to stimulate calf activity and is thought to stimulate also breathing, circulation, urination and defecation. It is also important to clean and dry-off the calf's coat to reduce evaporative heat loss (von Keyserligk and Weary, 2007) and is considered to be one of the main factors that contribute to the cow-calf maternal bond formation. This intense maternal behavior explains why in the first hours after parturition, cows did not prioritize eating and resting behaviors, that however increased over the course of hours, in accordance with Jensen (2012). Other than the licking behavior, the ethogram used for the quantitative behavioral analysis also included other affiliative behaviors: sniffing, nudging and nursing. All of these behaviors were observed in very low percentages and showed no significance in relation to the time from parturition. The failure of the calves to suckle doesn't necessarily mean that the cows object of the study have poor maternal instincts. Indeed, according to literature dairy calves usually take up to 6 hours to start suckling (von Keyserligk and Weary, 2007), and this study only covered 4 hours after parturition, thus the behavior might have been expressed in the following hours. Moreover, many factors contribute to the duration of the interval between birth and first suckle, as for example udder conformation and calf's teat seeking drive (Ventorp and Michanek, 1991). The amount of time spent by the cows lying sternal could also have influenced the low expression of the nursing behavior, since it is impossible for calves to suckle from a cow that is not in standing position. Interestingly, no agonistic behavior towards the calf of the three listed in the ethogram ("horning", "displacing" and "biting") was recorded, apart from the "horning" behavior that was observed only twice, respectively in the 2nd and 4th hour postpartum, and expressed by two different cows. Le Neindre and D'Hour (1988) recorded a very low expression of aggressive behaviors by cows towards their own calf if separated right after birth and brought back after 1 hour, but the study was conducted on beef cows. In respect to the high prevalence of affiliative

ones, agonistic behaviors were not recorded, thus the ratio between affiliative and agonistic behaviors couldn't be calculated but the maternal interactions towards the calf, however, can still be considered of good quality. In the time interval with increased amount of time spent without physical or visual contact with the calf (the 3rd and 4th hours after parturition) the behaviors expressed with most frequency were "resting", "eating the placenta" and "sniffing the litter". Previously on this thesis it has already been discussed the nature of the placentophagy and litter sniffing behaviors. The reason for the increased prevalence of the resting behavior during the hours postpartum must be found in the enormous amount of energy spent for the parturition and for the subsequent prompt maternal care, that occupied almost exclusively the first hour. The increase of resting behavior is thus a physiological response to the high energy-demanding activities that the parturition phase implies and must not be misinterpreted as a loss of interest towards the calf. The individual variability for each behavior analyzed in the statistical analysis was low (<60%), meaning that factors such as time spent with the calf, parity and parturition time, did not affect the results. This study thus suggests that the progression of good maternal interactions during time and the absence of agonistic ones is a species-specific factor, even if it must be considered that the population analyzed was small, consisting only of 8 cows.

6. Conclusions

This study aimed at assessing cows' maternal interactions towards the calf in the first hours postpartum. Results showed that although physical contact with the calf and the expression of affiliative behaviors decreased during time, no agonistic behavior was recorded, implying that the quality of the interactions, measured as the ratio between affiliative and agonistic behaviors, is in favor of the former. Moreover, the time in which cows had no physical nor visual contact with the calf was spent mainly resting, ingesting the placenta and sniffing the litter, that are common behaviors that might suggest an absence of distress. Cows provided strong maternal care, expressed mostly by licking the calf, that is a natural behavior fundamental for the calf's health. Nowadays, in commercial dairy farms, these behaviors and good interactions cannot be expressed, since calves are separated from their mother right after birth or within the 24h after being born. Although considering only the first four hours after parturition, the results of this thesis adds up to the increasing evidence that alternatives to early separation, the so called cow-calf contact systems, are beneficial both for the cow's and calf's welfare, as the 2023 EFSA report stated, but more research must be done to implement clear and effective legislative measures on this issue.

Annex A. Ethogram used for the observational behavioral analysis.

Category	Behavior	Code	Definition
Affiliative behaviors toward the calf	Sniffing	Sniff_Calf	Dam has the muzzle in close proximity (< 5 cm) of the calf and sniffs (e.g. inhales and exhales air through the nose in short repetitive manner) the calf in any body part
	Grooming/Licking	Gr_Calf	Dam has the muzzle in close proximity of the calf and licks (e.g. puts her tongue repetitively in contact with) the calf in any body part
	Nudging	Nudge_Calf	Dam pushes, shakes or rubs the calf by touching gently any body part with muzzle or head in an apparent effort to stimulate the calf to stand up
	Nursing	Nurse_Calf	Dam allows the calf to go with the muzzle under her belly and to suckle from the udder
Agonistic behaviors toward the calf	Biting	Bite_Calf	Dam bites (e.g. the act of opening and rapid closing of the jaws with the teeth grasping the skin) the calf in any body part
	Moving away or displacing	Displ_Calf	Dam moves away from the calf or displaces the calf by a forceful contact that results in the calf moving away
	Horning	Horn_Calf	Dam contacts the calf head-to-head or head-to-body in a forceful way
Affiliative behaviors toward conspecifics (apart from her calf)	Friendly interaction	Friendly_Other	Dam interacts with other animal(s) in the pen in a calm and friendly way; e.g. gets close (< 5 cm), sniffs or rubs gently any body part
	Allogrooming	Groom_Other	Dam has the muzzle in close proximity of the other animal(s) in the pen and licks or cleans any body part
Agonistic behaviors toward conspecifics (apart from her calf)	Biting	Bite_Other	Dam bites other animal(s) in the pen
	Moving away or displacing	Displ_Other	Dam moves away from other animal(s) in the pen or displaces the other animal(s) by a forceful contact that results in the other animal(s) moving away from her and/or the calf
	Horning	Horn_Other	Dam contacts the other animal(s) in the pen head-to-head or head-to-body in a forceful way
Calf searching	Sniffing the litter	Sniff_Litter	Dam has the muzzle in close proximity of litter and sniffs it
	Looking out of the pen	Look-Out	Dam goes to the fence and looks out of the pen
	Agitation	Agit	Dam moves restlessly in the pen, walks back and forth, doesn't lie down
	Vigilance	Vigil	Dam is alert, keeps her eyes open and looks around
Environmental interaction	Exploration and interaction with pen fixtures	Expl	Dam sniffs and/or licks the fixtures of the pen, sniffs the air (e.g in a Flehmen like behaviour)

Situation related behaviors	Placenta ingestion	Placenta	Dam ingests placenta
	Birth fluids ingestion	Birth_fluids	Dam ingests birth fluids (no calf)
	Parturition	Partum	Dam actively pushes the calf out of birthing canal.
	Partum contraction	Part_contr	Dam's uterus contacts
	Secondment	Secondm	Expulsion of placenta
	Restlessness	Restl	Dam is moving apparently agitated (prepartum)
Common behaviors	Vocalization	Vocalize	Dam emits vocalizations, hearable moos with open mouth and quiet humming with closed mouth
	Locomotion	Locom	Dam walks in the pen without showing signs of agitation
	Resting	Rest	Dam lays down or sleeps with eyes shut while recumbent
	Inactive	Inact	Dam is awake and, regardless of posture, she is not committed in any activity
	Self-grooming	Self-Gr	Dam performs body care by licking her body and wiping her nose and stretches, rubs on pen fixtures
	Stereotypies	Stereot	Dam is engaged in stereotyped behaviours e.g. tongue playing, tongue rolling and other non-nutritive oral behaviours, repeatedly nibbles/chews at the fixtures of the pen in a stereotyped way
	Eating	Eat	Dam eats, brings food to the mouth and chews in the manger
	Rumination	Ruminate	Dam ruminates
Other	Not visible	Not-Vis	Dam is out of sight being in a position that is either not visible from the camera frame, or the activity is not observable
	Other	Other	Dam is committed in behaviours other than those described which are not relevant for the purpose of the present study
Modifier: Posture	Standing	Stand	Dam is standing in the pen
	Lying sternal	Lying_stern	Dam is lying in the pen, on the litter, recumbent on sternum
	Lying lateral	Lying_later	Dam is lying in the pen, on the litter, recumbent in lateral position
	Transition	Trans	Dam is transitioning from standing to lying or viceversa, or from lying sternal to lying lateral
Modifier: Position in the pen	Front	Front	75% of the body of dam is located in the front half of the pen
	Back	Back	75% of the body of dam is located in the back half of the pen
Modifier: Locked condition	Locked	Locked	Dam is blocked with the head in the manger with the use of the feeding rack lock or tied for a short time to the pen.
	Not locked	Not locked	Dam is not locked in feeding rack
	Visual contact	Vis-Contact	Dam maintains eye contact with the calf before separation

Modifier: Type of contact with the calf	Physical contact	Phys-Contact	Dam maintains physical contact with the calf by standing or lying down in contact with the calf
	No contact	No-Contact	Dam does not maintain contact, neither physical nor eye contact with the calf

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