Study of the relational network of the dairy calf raised under a nurse, before weaning

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Master's Thesis

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AMF = Automatic Milk Feeder

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Introduction

Dairy farming has to face multiple challenges and constraints: environmental and sanitary regulations and increased workload [1]. The latter is directly linked to the increase in herd size and must cope with the decrease in labor force [2]. Farmers have therefore tried to adapt by developing automated milk feeding systems and by standardizing the rearing of dairy calves in individual pens [3]. In the first case, the Automatic Milk Feeder (AMF) theoretically ensures good growth of the calves, saves time and provides more flexibility to the farmers in their daily work [4]. In the second, according to Anne-Marie Bouma et al. the rearing of calves up to eight weeks of age in individual pens limits the spread of disease [5]. However, these patterns lead to stereotyped behaviors [6], defined by Masson (2006) as "repetitive behaviors induced by frustration, repeated attempts to cope and/or central nervous system dysfunction" [7]. Furthermore, this early separation generates emotional health problems in the calf, with the development of significant chronic stress [8]. This chronic stress in turn affects the physiological health of the calf with the erosion of the abomasum and the formation of ulcers [9], which greatly diminishes the welfare of the calf. This is why society is increasingly questioning these rearing practices. It demands a more sustainable industry: that is, productive, flexible, respectful of the environment, of the welfare and health of humans and animals [1]. In particular, the question of animal welfare is socially very much associated with the debate on the status of the animal and the legitimacy of breeding conditions or even of breeding at all. Establishing a definition of animal welfare is complex, since it must respond to several considerations. The definition must be broad enough to be applied to the various contexts in which animals are affected by humans. It must also be precise to allow for application in the field, and finally, it must provide a solid foundation on which regulations can be based [10]. ANSES proposed in 2018 the definition presented in Box 1:

Box 1. Definition of animal welfare (Anses, 2018): « The welfare of an animal is the positive mental and physical state related to the satisfaction of its physiological and behavioral needs, as well as its expectations. This state varies according to the animal's perception of the situation ».

It leads to questioning the quality of mental life of animals and the means to improve them. But in a more global way, the evaluation and the respect of the 4 principles developed in a more ancient way by the Welfare Quality protocol (2009) is today the reference: good housing conditions, an adapted food, a good health and an appropriate behavior of the animals.

To meet this societal demand on dairy farming systems, the European regulation (EC n°834/2007) has for example required that calves raised in organic farming must be fed, during the first three months of life, with milk preferably from the mother. In addition, Annex III of the EU Regulation No. 889/2008 stipulates that calves older than seven days must be kept in pens that can accommodate several animals. And even if there is no constraint on the method of milk distribution, generally done with the AMF or the bucket (with or without teat), rearing methods considered more "natural", such as considered more "natural", such as suckling cows, are reappearing in a marginal way in northern European countries [3]. This practice often consists of using so-called "unproductive" cows due to mastitis, lameness or reproductive problems to raise replacement heifers. This involves the adoption of two or three calves per cow until weaning (~6 months).

Initial surveys of farmers who practice “natural” suckling (as opposed to artificial suckling such as AMF or bucket) point to a reduction in workload, they report that diarrhea has decreased on their farms, that the calf mortality rate has also decreased and that the calves are healthier and heavier at weaning [**11, 12**]. Beyond the zootechnical aspects, there are many ethological questions concerning the quality of the relationship between the nurse cow, who acts as a mother figure, and the calf. Indeed, this nurse cow is in no way biologically linked to the calf. Adoption is often carried out far from calving and the description of the relationship between the nurse and the calves, between the calves themselves and the rest of the herd are, to our knowledge, only rarely described in the scientific literature.

This is the context of the INRAE-SEBEA project. The objective of this two-year project is to develop a guideline for farmers who wish to introduce a system of nurse cows on their farm. The aim is to provide scientific insight into the well-being and health of cows and calves. Part of this project is to study, over time, the construction of the relational network of the calf before weaning. By relational network, we mean the different actors with which the calf will interact from birth to weaning, *i.e.*: his nurse cow, his “sister” calves, the other cows and calves in the herd, but also humans.

The present work carried out during my end-of-study internship took place on the experimental farm of Mirecourt (INRAE-Aster) where the nurse cow system started in 2016 and was coordinated by INRAE Theix (UMRH). **It was an exploratory study on the evolution of proximity relationships and the possible existence of a privileged bond, between calves and their nurse in the months following adoption as she is not their biological mother. I have also been interested in the evolution of the reaction to humans, an essential partner of animals in breeding.** First, I will present a literature review on the mother-calf attachment relationship and on the docility of the heifer. Then, I will present the experimental protocol developed over three periods (May-August-October) to carry out the observations of the animals on pasture as well as the behavioral tests (human avoidance test, choice/separation test and new object test). Third, the results of the data collection during the observations and behavioral tests were analyzed and discussed before the conclusion.

Bibliographic synthesis

Farmed ruminants are gregarious animals. Their social organization is partly based on dominance/subordination relationships, but it is affinity relationships that ensure cohesion, especially since their need for social experiences has been demonstrated [13]. Indeed, individual recognition can lead to a form of affiliation between related or unrelated individuals [14]. These affinities may impact other behaviors such as feeding behavior. For example, an experiment by Scott et al. (1995) showed that group feeding behaviors were modulated not only by the food preferences of individuals but also by the degree of familiarity between animals in the social group [15]. Furthermore, group living provides the animal with social models that allow it to acquire new activities more efficiently than through individual learning [16]. During the learning process, animals will be influenced by the behavior of their fellows and this process will be at the origin of food preferences and avoidance [17]. This was shown by Thorhallsdottir et al [18], where the feed given to the ewes is also the feed that the lambs consume the most. Calves suckled by their mothers learn to eat roughage at a younger age [19].

The formation of a “social core” at an early age and its maintenance during the various stages of the animal's life facilitates future social relationships and makes it possible to improve mutual tolerance and thus reduce the frequency of aggressive interactions [20]. In fact, it is with the mother that the young animal forms a preferential bond in the hours following birth [21]. In most ungulates, almost immediately after parturition, a period of intense reciprocal stimulation between the mother and the young has been observed [22]. At birth, the cow removes the amniotic fluids from her calf by licking it; this behavior is stimulated by the presence of the amniotic fluids [23]. The newborn emits odors and vocalizations while the mother presents the young with a variety of stimuli: tactile, thermal, auditory and visual, which would allow recognition between individuals [24]. This complex exchange associated with the postpartum period, would be the basis for the establishment of a stable bond between the mother and the newborn [25]. Indeed, several studies have suggested that there is an optimal period for the development of this stable and specific bond between the offspring and the mother [22]. This “critical period” may vary from one species to another and from one individual to another: from a few minutes post-parturition to several days [26]. In fact, two types of animals can be distinguished: “followers” and “hiders”. The tendency for “follower” animals, such as sheep, is to follow their mothers after birth and suckling. Whereas “hiding” species like the newborn kid that has been suckled will lie down and sleep [27]. In the case of Adams and Klopfer's (1964) results [28], the first five minutes avoids rejection from the mother even if the newborn was subsequently removed for up to three hours. Whereas an immediate separation after parturition but for only one hour leads to maternal rejection. This mechanism may explain the behavior of ruminants to isolate themselves from the group before parturition: to avoid herd members sniffing or touching the newborn, at the risk of confusing the attachment between the young and the mother [29]. Subsequently, young ruminants develop a preferential relationship with their mother, which is reflected in the search for spatial proximity to the mother [21]. According to a study by Nowak (1990) [30] using a choice test, the young can perfectly recognize their mother from a foreign ewe from three days of age. This success rate increases due to postnatal learning that becomes more refined with age.

It seems essential for the calf to be attached to his mother to ensure his survival, since the mother provides him first and foremost with his food needs and security in relation to the outside world. Psychiatrist John Bowlby (1969) and psychologist Mary Ainsworth (1973) conceptualize attachment behaviors in Box 2:

Box 2: « Attachment is a deep and lasting emotional bond that links one person to another in time and space. In the presence of his mother, the young feels secure thanks to the comforting gestures of the adult, but their separation induces anxiety » [31, 32 ,33].

His basic principle is that a young person needs to develop an attachment relationship with at least one person in a stable manner in order to experience normal social and emotional development. The infantile behavior associated with attachment is, according to Susana Terono [34], the search for or the maintenance of proximity with this preferred individual and Bowlby adds that it is the same when a stressful situation occurs [33]. Indeed, Bowlby emphasizes the notion of a "secure base" that the attachment figure represents for the young person: he can thus explore his environment while knowing that he can find refuge quickly [32].

The young ruminant, which therefore focuses its attention only on its mother, will, over time, gradually expand its social environment as it integrates the group and thus develop its own network of relationships. In the case of multiple births, the family group develops from the links that are formed between the animals of the same litter. The young will synchronize their activities to graze or rest [21]. Furthermore, if the presence of a familiar conspecific decreases the distress response (characterized by vocalizations and elevated plasma corticosteroid levels [35] in three-week-old lambs when separated from their mothers, the calming effect is even more pronounced with the presence of the twin. As they are reared together, they develop a mutual familiarity resulting from direct contact [36]. In general, Ligout et al. showed that lambs recognized their unrelated but reared mates within the same small group. Using a choice test, they showed that lambs spent more time in proximity to a familiar pen mate than to a stranger lamb [37]. In addition, after being separated from their mothers, lambs emit fewer distress bleats when associated with a familiar individual rather than a previously unencountered lamb [38].

In dairy farming, the mother is often absent with animals raised on artificial milk. Interactions between humans and their animals are daily. The young are artificially fed by the farmer from their first days of life. Early and regular gentle contact reduces the animals' fear of humans [39]. In his studies, Rushen et al. define gentle handling as physical contact such as petting or brushing as well as food rewards. In two studies by Hemsworth et al. (1989), experimenters stayed close to the heifer for several minutes at the time of her first calving. The results showed that milking was facilitated and therefore less labor was required to manage the herd during this activity. In addition, the time to approach the experimenter was also reduced suggesting a decrease in the level of fear [40; 41]. Similarly, Boissy and Boissou (1998) showed a decrease in the flight distance to humans and an increase in the amount of food ingested in the presence of a human when the heifers in the experiment had been handled more during their first nine months of life [42]. Thus, it appears that early contact with humans during the first weeks of life is very effective. This is also the case in goats, for example [43]. This was demonstrated by Boivin and Braastad with animals that were isolated and handled for the first ten days after weaning.

The latter approached and interacted with humans much more than control animals (which did not receive additional handling). Their results also showed that the animals were more immobile with a moving human than the control kids. In the moving human test, the control kids were initially not interested in approaching the human but also showed signs of avoidance.

Several studies show that there seem to have several key phases where human intervention is effective in improving ruminant docility: mainly at birth and weaning. However, the presence of the mother figure can negatively affect the development of social responsiveness of young ruminants to humans (lambs **44**; calves **45**). Lyons [**46**] and Krohn [**47**] explain that young herbivores living permanently with their mothers during the first months of life are more fearful of humans than those raised artificially.

According to the literature described above, the preferential, even exclusive, and strong bond between the young and the mother is created from birth [**48**]. Mothers can be very selective and refuse any other young. These studies, beyond their scientific interest and often on lactating breeds, are justified from a practical point of view by the difficulty of getting the new mother to adopt another young animal if the young animal has lost its mother or if she does not want it. This is why, in the case of an adoption where the animals are not biologically linked, several works mention the reunion of a young animal that has just been born with a freshly calved cow in order not to miss this "critical period" [**48**]. On the other hand, some articles rely on the deployment of lures to facilitate this adoption process in sheep [**49**].

The practice of rearing calves under nurse has not been described in the literature from an ethological point of view, and the behavior of the animals undergoing it is not well known. Here, the aim was to adopt several calves several days old from a cow that calved several days or even months ago. This work will therefore answer the central question: **How do the calf's pre-weaning social interactions evolve over time in a pasture-based cow-calf system?**

Several underlying questions will follow:

1. Can the reunion of a young calf and a cow outside of the “sensitive period” around calving generate motivation in the cow towards this calf and thus allow for calf adoption?
2. In the case of a successful adoption, is there a preferential bond that develops between the nurse cow and the calf?
3. How will the relationships with the nurse cow, other calves and humans change with age?

The limited literature on what could be called "late" adoption without luring does not allow us to ensure the success of calf adoption. Furthermore, the motivation of the cow towards the calf remains to be qualified, but we suppose that criteria such as observation directed towards the calf can allow to qualify the motivation of the cow towards the calf.

Table 2- Summary table of foster cows on the Mirecourt farm that have adopted as of January 2021.

Family group no.	ID Cow	Last date of delivery	Adoption date	Number of adopted calves	ID adopted calves	Breed cow	Average amount of milk produced before adoption (L)
1	3004	03/10/2019	12/01/2021	2	1001 1002	Prim'Holstein	8,4
2	2352	30/01/2020	08/02/2021	2	1003 1007	Prim'Holstein	6,7
3	2367	30/10/2020	16/02/2021	3	1009 1010 1019	Prim'Holstein	17,1
4	803	08/02/2021	26/02/2021	3	1018 1020 1023	Montbeliarde	15,9
5	175	09/02/2021	12/03/2021	3	1028 1034 1035	Montbéliarde	19,5
6	5087	30/10/2020	22/03/2021	3	1037 1040 1041	Montbeliarde	15,1
7	5078	25/02/2021	25/03/2021	3	1043 1045 1046	Prim'Holstein	19,7
8	3002	12/02/2021	07/04/2021	3	1047 1049 1050	Prim'Holstein	17,2
9	2316	02/03/2021	19/05/2021	3	1052 1053 1055	Montbeliarde	16,95

Material and Method

1. Animals, housing and management

The study was conducted from January to August 2021 with 25 female calves (Prim'Holstein, Montbeliarde and crossbred dairy breeds) and nine nurse cows (Prim'Holstein and Montbeliarde), at the Mirecourt - ASTER experimental unit in the Vosges region. The latter has been in Organic Agriculture since 2006 and the animals are exclusively fed with hay and grass. The animals are placed in buildings from November to April. This year, they were taken in the pastures in mid-April. During the period in the building, the cows received an average of 20.5 kg of DM/day including 4.3 kg of DM of Alfalfa-Grass regain (2nd cut) as well as 16.2 kg (of Dry Matter) of permanent meadow hay. The nurse calved between October 2019 and May 2021. The study calves were born between January 9th, 2021 and April 30th, 2021, and remained with their biological mothers for 48 h to assimilate colostrum. They were then placed in a collective nursery, on straw, for a minimum of three days. When two or three female calves were old enough to be adopted and a cow meeting the technicians' criteria was also available (i.e., subjectively considered docile and that showing mastitis or lameness), then the adoption could take place. Once the family group was formed (i.e. the nurse cow and her adopted calves), (***Table 1)*** the animals were kept together for a minimum of 15 days in a 7*5m stall, half of which was strawed and not the other half (scraped area). Water was available *ad libitum*, the building was open to the outside, there was no temperature control or artificial light. The family group then joined a second family group for a minimum of 15 days, in a barn identical to the previous one. The nurses grazed for five hours a day (between 11:00 a.m. and 4:00 p.m.), without their calves and according to the weather conditions in order to allow a dietary transition for one week before grazing. Then, the different groups were taken to pasture to form a single herd, composed first of six nurse cows and their respective 16 calves. The pastures were between de 0,64 to 1,41 hectares in size, and had no shelter, but trees lined the sides of the paddocks, providing shade and shelter for the animals. Water was also available ad libitum. The adoptions that took place after mid-April joined the herd on pasture following the same type of procedure (three nurse cows and nine calves). At no time were the cows milked during the study.

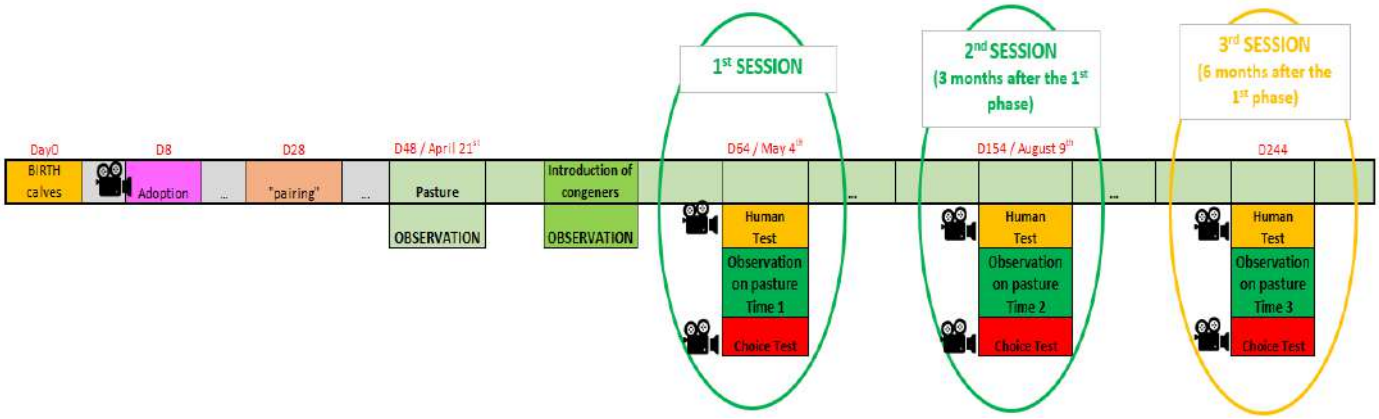


Figure 1- Timeline of the study's calves for the year 2021

Legend:

« Pairing » — *= Meeting of two-family groups*

Pasture — *= First grazing of the first 6 family groups*

Introduction of congeners — *= Introduction of family groups n°7 and n°8 to pasture (with the first 6 family groups)*

= Filmed test

2. Experimental design

The purpose of this longitudinal study was to analyze, over time, the calf's interactions with its nurse cow in particular, and in part with other calves and cows as well as with humans.

We started with three important time points: adoption, introduction to pasture and introduction of new conspecifics (***Fig.1***).

- The first one: adoption. This test consisted of presenting two or three calves to a cow and analyzing the behavior of each animal through video observation and in particular the motivation of the cow towards the calves (the analysis of the behavior of the calves will not be discussed here).
- Then, the objective of the grazing introduction was to analyze the reaction of the calves to a new environment and to new partners. In a stressful situation, does the calf get closer to its nurse? This part will not be treated here.
- The last point: the introduction of new conspecifics in the herd. This test allowed once again to observe the reaction of the calves during a new situation, in this case the introduction of new nurse cows and calves. This part will not be treated here

After these three steps, three key repeatable sessions were determined to analyze the animals' behaviors over time (***Fig.1***). The first session was two weeks after the grazing introduction (in May); the second session was three months after this grazing introduction (in August); and the third session was six months later, before entering the barn and before weaning (in October, not discussed here). These key sessions followed a specific pattern of behavioral tests and observations: the human test, conducted first to avoid habituation bias; the choice/separation test; and the new object test, which involved analyzing the reactions of a family group to a new object (but which will not be discussed here). These key sessions were also punctuated by several observations, made by experienced observers, to estimate the calves' preferred individuals.

Concerning the animals of the study, we first had six nurses with their 16 calves until April 28th, then we added to the herd three nurse and their nine calves.

3. Observation of animals

3.1. Adoption

The objective of this test was to characterize the motivation of the cow during her meeting with the calves. It took place in a 7*5m adoption box (***Fig.2***), which included in its first half (the scraped area) a trough with a feed fence, as well as an *ad libitum* water dispenser. The second part of the stall was strawed. A camera positioned in height allowed to film the whole experiment.

The calves were adopted by pair or three, with an average of 14.9 days old (+/- 12.7 days) at the time of adoption. Prior to the actual adoption, some prerequisites were necessary: the calves were placed in a group for a minimum of three days in the nursery and fed with milkbar. The adoption was done at the end of the morning between 11am and 12pm. The calves were then fasting from the night before. The nurse was placed at least 24 hours before the adoption in the adoption box order to be familiarized with her environment.

There were at least three phases during this test.

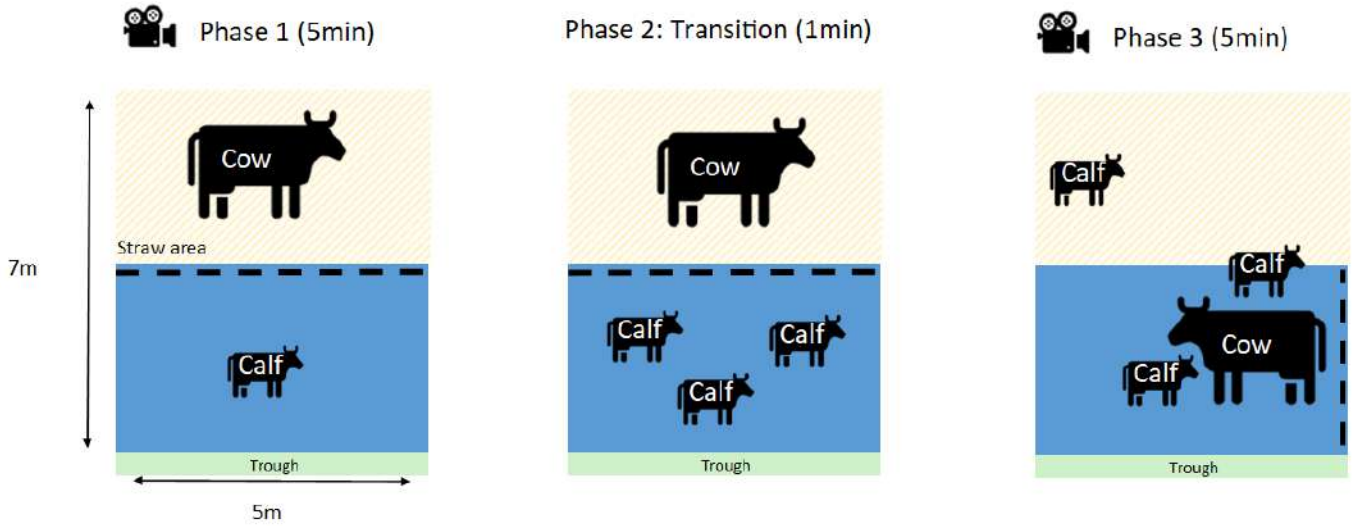


Figure 2- Design of the adoption test in 3 phases, 2 of which are filmed

Legend:

= open-air fence

1) First, the nurse was pushed into the straw area of the box and the door (allowing contact) was closed. The first calf was placed in the scraped area for five minutes.
2) The other calves were then brought in (up to a maximum of 3). After the last calf was introduced, there was a minute of acclimation before the center gate was opened to allow the calves to join the cow.
3) Once the center gate was opened, the calves and nurse were able to interact. There was no human intervention during this phase (unless there was a danger to the calves). This third phase lasted 5 minutes and was filmed.
4) At the end of these five minutes, two possibilities were considered:
    A. The human did not have to intervene since the nurse spontaneously let the calves suckle. The observations stopped when all the calves were fed and gave up suckling on their own.
    B. The man had to intervene to help the calves suckle under two conditions:
        i. Either because the nurse did not let her do it
        ii. Or because the calves did not know how to suckle
5) If a technician had to intervene, he would bring the nurse cow to the feed fence, where she was blocked. The technician would then show each calf how to suckle (until the calf had absorbed the information and was able to resume suckling). Technicians could use restraints if the cow proved to be aggressive and would not let herself be suckled. The fixed (overhead) camera continued to record during the lunch period to capture "post-feeding" interactions between the nurse and the calves.

A follow-up of the adoption success was made: it was a 15min film without human intervention. Thus, if several days were needed to consider the adoption successful, there was a film each day. Successful adoption is defined here as acceptance of suckling with the manifestation of maternal care directed toward the calf such as licking [**50**]. If no human intervention was noted for 24 hours, the adoption was considered successful.

If the cow refused contact with the calves or would not allow herself to be suckled, then restraint was necessary. If this situation persisted beyond seven days, the adoption was considered a failure. In this case, the calves are brought back to the milkbar and waited for in groups until another nurse is available.

Observations of the proximity or distance between two animals are part of the tools used to describe the social relations of animals living in groups [**51**]. The interactions and their quality can also be noted.

- The use of the "**scan sampling method**" (SCAN) has been used here. Scan sampling essentially consists of recording what the subject is doing at a given moment (equivalent to a photo). Here, scan sampling was used to record the position of the cow every 15sec in relation to the barrier (phase 1) and in relation to the nearest calf (phase 3).
- Morever, a "**semi continuous sampling method"** (FOCUS) of the cow was carried out. This consists of a complete sampling of the behaviors of a given subject during a determined period of time (equivalent to a movie), here two times five minutes (phases 1 and 3). Various behaviors were recorded such as locomotion and interaction with the calf(s) as well as the subject of observation (calf(s) or other).

Figure 3 - Photo of a calf with its identification necklace.

The question here is whether the cow was attracted to the calf, whether the calf attracted her attention (phase 1) and whether she sought to interact with the calf (phase 3).

- We determined that the "contact" area was defined when the cow was located between direct contact with the barrier and 50cm (visible marker on the video). We transferred this surface to the surface of the box, which gave us a theoretical value of 7%.
- The "far" area was defined when the cow was in the second half of the pen. The theoretical value was therefore 50%.
- Finally, the "near" zone is the difference of the two previous zones, which is 43%.

Other components such as the observation of the cow directed towards the side where the calf is or the interactions (positive and negative) are also reported.

The selected variables from the adoption test are: Calf_obs_P1; Contact_P1; Threat_Kick_P2; Day_required_successful_adoption.

- Calf_obs_P1 = observation directed towards the calf in phase 1
- Contact_P1 = contact at the fence during phase 1
- Threat_Kick_P3 = threat and kick directed at the calf in phase 2
- Day_required_successful_adoption = number of days required for a successful adoption

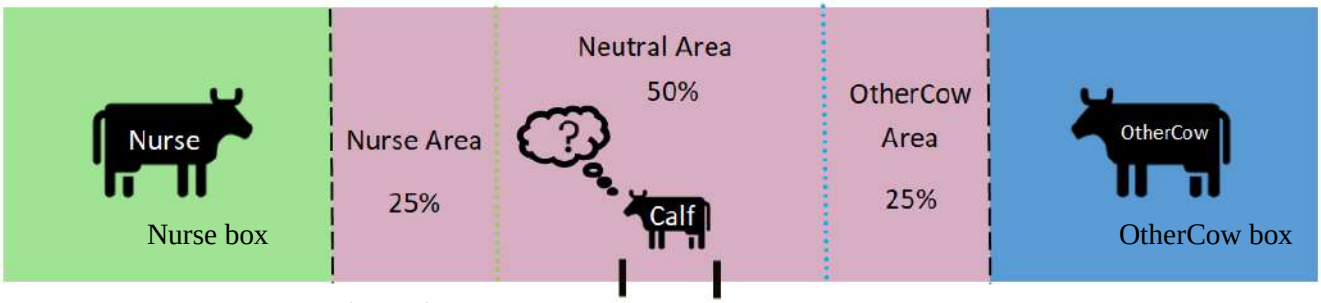
3.2. *In situ* observation of the grazing introduction

The purpose of this test was to evaluate whether the nurse cow was a secure base for the calf. The scan sampling method was used. Every six minutes, each cow was "scanned" to determine her position in relation to a calf (contact, proximity or far) as well as her interaction behavior. Cows were identified by numbers painted on each side of their body. We were able to identify the calves from afar thank to colored necklace (*Fig 3*). Only one observer was needed for this observation. The observer was placed in an observation tower in the middle of the pasture, three meters above the ground, so as not to disturb the animals. These observations took place at the time of grazing, around 11:00 am, then in the evening around 7:00 pm, for 2 hours each session (equal to 20 scan samplings). The observation period was repeated the next day at the same time. Observations began when all the animals were in the pasture.

3.3. *In situ* observations of the relationship in three sequences between the nurse and the calf

The objective of these *in situ* observations, under stable conditions, was to characterize the relationship between the nurse and her foster calves. These repeated observations took place in May, August and the last part will take place in October. Scanning samples (all six minutes) were used to record the relative position between members of a family group and their social interactions. Two observers were required, one to scan the cow, the other to scan the calves. A third observer used the focus observation method to detail the calves' behaviors. The order of the observations of the calves was decided in the field according to the individuals in activity, in order not to observe inactive animals. These observations took place in the morning, around 7:30a.m., and at the end of the afternoon, around 6p.m., in order to observe a maximum of interactions between the animals [**53**], always during 2 hours.

Step 1: Choice test (5min)



Step 2: Separation test (2min)

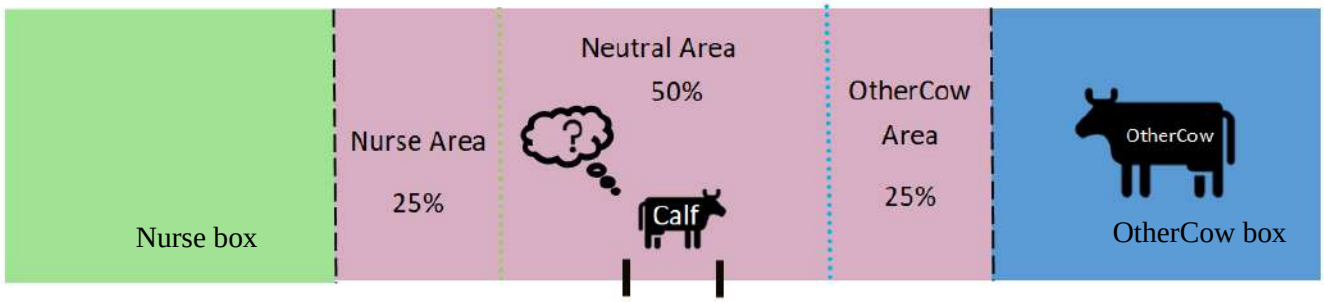


Figure 4 : Choice/Seprataion test design

The data on mutual interactions of the calves was intended to determine the identity of the calf's primary social partner (respective nurse cow or not) and thus determine a potential attachment of the calf to its nurse and vice versa.

Variables selected during the pasture observations to qualify the relationship between the calf and the nurse and compare it with the variables of the adoption test:

- ContactNurseS1 = percentage of SCAN where the calf is seen in contact with its nurse in session 1 (S1)
- ContactNurseS2 = percentage of SCAN where the calf is seen in contact with its nurse in session 2 (S2)

4. Behavioral tests

4.1. Choice/Separation test(Fig. 4)

A "choice" test [52] was used to quantitatively assess the selectivity (behavior preferentially oriented towards the nurse cow) of the calves towards the cows. The session 1 involved the six cows that joined the pasture simultaneously. The session 2 involved the nine cows of the experiment that had time to adapt to their conspecifics. The order of the animals was set to minimize the age difference between the calves and not to put animals that had been together for the "pairing" in the same passage group. Calves ranged in age from one and a half months for the youngest to four months for the oldest. Prior to the test, the calves were separated from their nurse for one hour. In order to comply with the ethical charter, the calves were separated from their nurse for a maximum of 2 hours. The age of the calves for the second test session varied from 4 to 7 months. In both sessions, the choice test was conducted during the day, from 9:00 am to 1:00 pm. Each calf was introduced for five minutes in this setup (closed pen with openings allowing contact between animals, 10 m * 7 m) where the legitimate nurse and another cow from the herd were simultaneously present. Cows were placed in adjacent open pens, also 5 m * 7 m. A final step was the separation test: the removal of the nurse for two minutes. Several criteria related to the calves' behavior were recorded during the test: the respective times spent with the two cow zones (zones were delimited to facilitate observation) and the number of zone changes, the frequency of interactions with the other cow (sniffing, contact) and the number of vocalizations. As for the cows, their vocalizations were recorded as well as their lighting and licking behavior with the calf and their contact with the grid.

Variables retained in the choice test to qualify the relationship between the calf and the nurse and compare it with the variables of the adoption test:

- Time_Nurse_S1 = Percentage of time spent by the calf in the area close to its nurse in Session 1
- Time_Nurse_S2 = Percentage of time spent by the calf in the area close to the nurse in Session 2

To facilitate the understanding of the time spent in each area, a preference index has been used as follows:

Preference index = ((time spent in feeder area * 1) + (time spent in other cow's area * (-1)))/100.

The values were distributed from -1 when the calf was mostly in the other cow's area to +1 when the calf spent a large part of its time in the nurse's area.

4.2. Avoidance test

The avoidance test inspired by Waiblinger, Menke, Fölsch [54] and Windschnurer, Schmied, Boivin, Waiblinger [55]. It consists of evaluating the distance animals flee when approached by a human while grazing. This test was performed first to avoid bias (handling habit). The experimenter, trained during the previous weeks, was alone to perform this test in order to limit inter-observer effects. This experimenter was the same in session 1 and 2.

During the session 1, all eight cows and their calves were tested (the last nurse cow had not yet given birth). For the session 2, all the animals in the study were tested, *i.e*. the nine nurses and their 25 calves. The order of the animals was randomized to approach the closest animal and thus limit herd movement. Standing animals were approached slowly from the front, by the person (at a rate of one step per second), who held it out at an angle of approximately 45° in front of his or her body. The distance between the person's hand and the animal's head was estimated at the time of the animal's withdrawal. The avoidance distance was tested on all animals in the herd on the same day, once per animal.

5. Data analysis

Statistical analyses were performed with the R software 4.1.0, using the R commander package. Exact probability tests of Fisher were used for comparing observed proportions of animal to theoretical proportion (hazard distribution following binomial distribution). The hypothesis for normal distribution was checked with Shapiro test. T-test for match-paired data was used, including comparisons between observed and theoretical values. Otherwise Wilcoxon test was used as non-parametric test in case of rejection of the Normal distribution hypothesis. Pearson or Spearman correlations were used to identify potential significant relationships between two variables depending on their hypothesis of binormality. P-values lower or equal to 0.05 were considered significant. Tendencies were considered with p-values between 0.055 and 0.1.

Due to a number of missing data in the different tests, only 12 calves have a complete data set. The sample size is explained by the fact that all the tests have been grouped together: adoption test, choice/separation test and observations. Indeed, we count 22 calves for the observation on pasture, 16 calves for the choice test (because the last 6 were not retained in session 1 because of their late arrival in the herd) and finally, the first two adoptions could not be filmed because the observation equipment was not in place.

Table 2: Adoption test variables selected to qualify the nurse relationship through a spearman correlation (r_s)

Legend: Calf_obs_P1 = observation directed at the calf in phase 1 ; Contact_P1 = contact at the gate during phase 1 ; Threat_Kick_P3 = threat and kick directed towards the calf in phase 3 Day_required_successful_adoption = number of days required for a successful adoption

	Calf_obs_P1	Contact_P1	Threat_Kick_P3	Day_required_successful_adoption
Calf_obs_P1	1	r_s =0,394 p = 0.29	r_s = -0,4 p = 0.29	r_s = - 0,037 p = 0.93
Contact_P1		1	r_s = -0,563 p = 0.13	r_s = 0 p> 0.1
Threat_Kick_P3			1	r_s = - 0,630 p = 0.13

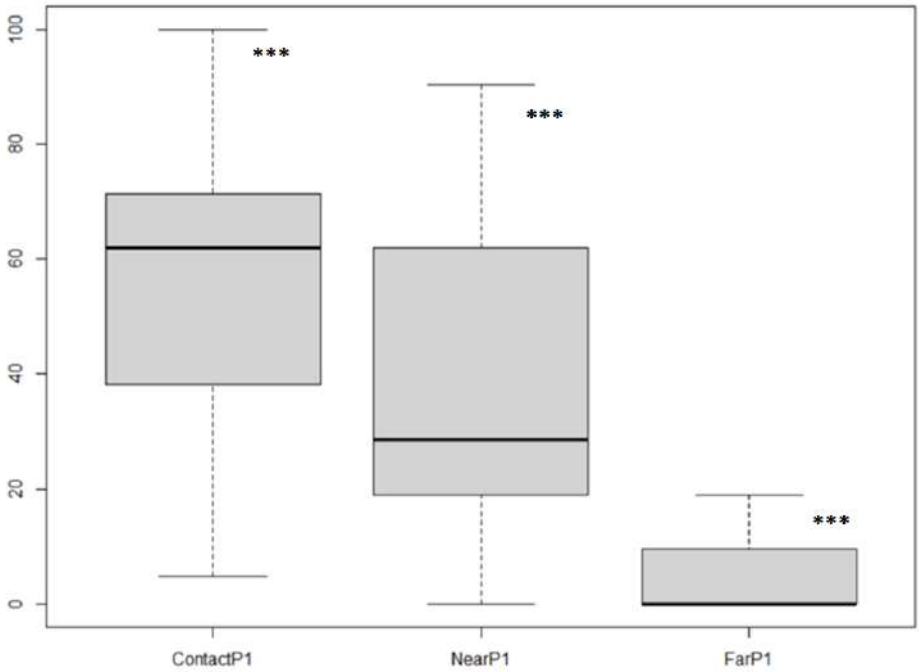


Figure 5: Spatial distribution (%) of the cow in the box during phase 1 of the adoption test and comparison with hazard repartition (test toward compared hazard repartition) (n=9).

Legend: ContactP1 = Percentage of SCANs where the cow is seen in contact with the barrier during the 1st phase of the adoption test ; NearP1 = Percentage of SCANs where the cow is seen between 50cm from the fence and the first half of the stall ; FarP1 = Percentage of SCANs where the cow is seen in the second half of the pen (away from the fence). When P<0.05 there is a "" and when P<0.01 there is a "***".*

Results

6. Adoption Test

The following four indicators were retained since they showed variability in results between cows: observation directed to the calf in phase 1, contact time at the barrier during phase 1, sum of threats and kicks directed at the calf in phase 3, and number of days required for successful adoption

Cows were observed in contact with the barrier more often (***Fig.5***) than would be expected by chance during the experiment ($t = 6.25$, $df = 8$, $P < 0.001$). In contrast, cows were observed less often than chance in the area away from the barrier ($t = 4.50$, $df = 8$, $P < 0.001$). These criteria were not correlated ($P>0.1$) with the frequency of threat/kick in phase 2 of adoption as well as the number of days to successful adoption (***Table 2***).

Variables retained in the adoption test to qualify the relationship between the calf and the nurse:

- Calf_obs_P1 = observation directed at the calf in phase 1
- Contact_P1 = contact at the barrier during phase 1
- Threat_Kick_P3 = threat and kick directed towards the calf in phase 3
- Day_required_successful_adoption = number of days required for a successful adoption

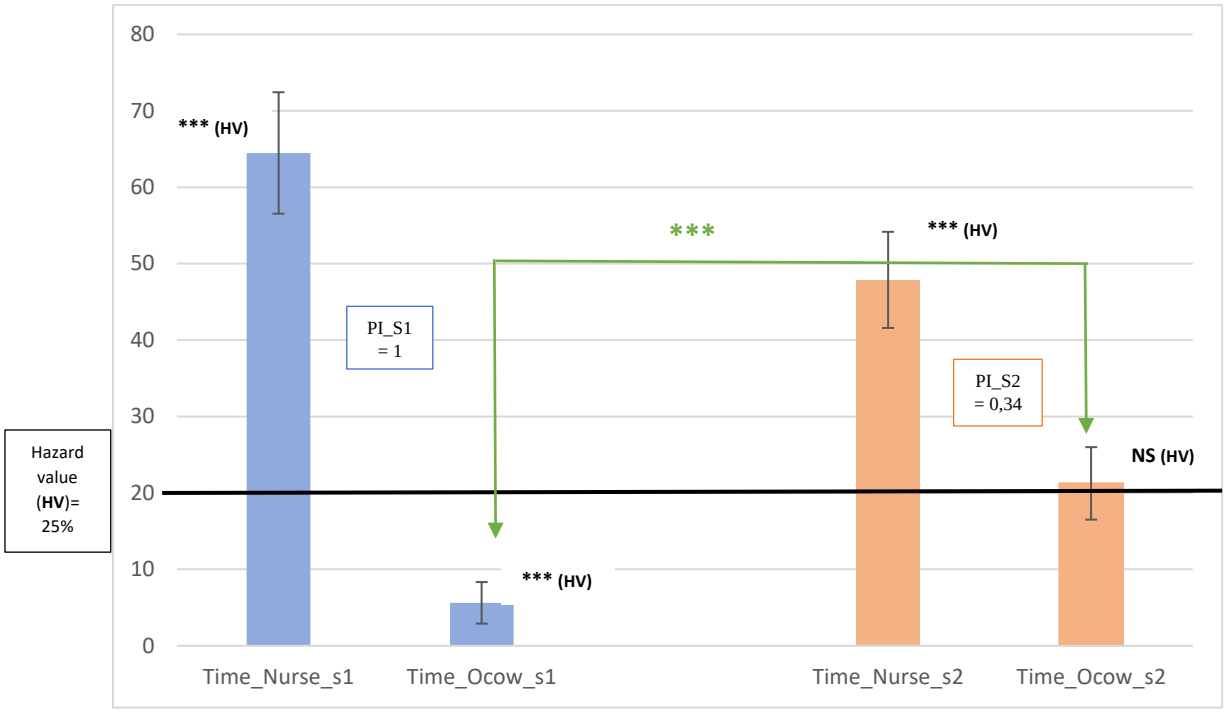


Figure 6: Distribution of the time (%) spent by the calf in each area of the box during the choice test in session 1 and session 2 (n=16)

*Legend: Time_Nurse_S1 = Percentage of time spent by the calf in the area close to its nurse session 1 (S1) ; Time_Ocow_S1 = Percentage of time spent by the calf in the area close to the other cow in session 1 (S1) ; Time_Nurse_S2 = Percentage of time spent by the calf in the area close to the nurse in session 2 (S2) ; Time_Ocow_S2 = Percentage of time spent by the calf in the area close to the other cow in session21 (S2). PI_S1: Preference Index in session 1and PI_S2: Preference Index in session 2. When P>0.05 there is non-significant differences "**NS**"; P<0.05 there is a "*" and when P<0.01 there is a "***".*

Table 3: Results of the choice test for session 1, session 2 and calculation of p-value between sessions and compared to hazard

Legend: Time_Nurse (%) = Percentage of time spent by the calf in the area close to its nurse; Time_Z0 (%) = Percentage of time spent by the calf in the neutral area; Time_Ocow (%) = Percentage of time spent by the calf in the area close to the other cow (Ocow)

	Session 1		Session 2		P-value	
Variable	**Mean**	Standard error	**Mean**	Standard error	Session effect	Hazard effect
1st choice_Nurse (%)	**93,80**	0,06	**93,80**	0,06	W = 1,5, P = 1	S1 : W = 120, P<0,005 S2 : W = 120, P<0,005
Time_Nurse (%)	**64,50**	7,94	**47,90**	6,29	t = 1.698, df = 15, P = 0.110	S1 : W = 4, P<0,005 S2 : t = -0.789, df = 15, P = 0.442
Time_Z0 (%)	**27,81**	7,38	**29,63**	4,70	W = 57.5, P = 0.605	S1 : W = 20, P = 0.013 S2 : t = -4.328, df = 15, P < 0,005
Time_Ocow (%)	**5,63**	2,73	**21,25**	4,75	W = 9, P = 0.006	S1 : W = 4, P<0,005 S2 : t = -0.789, df = 15, P = 0.442
Latency exploration (sec)	**102,31**	27,79	**33,81**	6,22	W = 109.5, P<0,05	
Frequency exploration	**3,13**	0,61	**9,44**	0,65	t = -7.468, df = 15, P<0,005	
Frequency of zone change	**2,75**	0,62	**5,94**	0,59	W = 11, P=0,005	
Latency Vocalizations (sec)	**248,44**	27,79	**49,31**	21,78	W = 129, P = 0.001	
Frequency Vocalizations	**0,38**	0,22	**2,19**	0,88	W = 4.5, P = 0.037	
Preference Index (-1 ---> 1)	**0,82**	0,07	**0,34**	0,14	W = 94, P = 0.010	S1 : W = 134,P < 0.005 S2 : t = 2.3561, df = 15, P = 0.032

7. Choice Test

The results of the choice test are presented in ***Table 3***.

Fifteen of the 16 calves in the experiment had their nurse as their "first choice" in session 1 (P=0.015) and in session 2, three months later (P=0.015). The only calf that did not choose its nurse was different between the two sessions. The preference index confirms a very clear preference of the calves for the nurse in session 1 (W = 134, dF= 15, P < 0.001) with a mean value of PI_S1 = + 0.82 (+/-0.07) (***Fig.6***). This preference is still significant (t = 2.356, df = 15, p<0.05) in session 2: PI_S2 = +0.34 (+/-0.14).

However, we observe a significant decrease (W = 94, df =15, P = 0.01) of this preference index between session 1 and session 2: -0.47 (q25= -0.007; q75= 0.827). There was no correlation for the preference indexes of each individual between session 1 and session 2 (r_s=0.02, df=15, P>0.1) nor in relation to the age of the calf (session 1: r_s=-0.03, df= 15, P>0.1; sessions 2: r_s=0 .48, df=15, P=0.06).

There was no significant difference in the time spent in the nurse area between session 1 and session 2 (t = 1.698, df = 15, P>0.05) (***Fig.6***), nor was there any correlation between the two sessions (r_s=-0.04, df=15 P>0.05). The correlation with age was not significant for session 1 (session 1: r_s=-0.22, df=15, P>0.1) but it was for session 2 (r_s=0.51, df=15, P<0.5).

In contrast, calves in session 2 spent significantly more time on the other cow's side than in session 1 (W = 9, df = 15, P<0.01) (***Fig.6***). Exploratory behavior also changed from session 1 to session 2: the frequency of exploration increased significantly (t = -7.468, df = 15, P>0.05) between session 1 and session 2 and the exploration latency decreased significantly (W=109, df=15, P<0.05) between session 1 and session 2. In addition, calves in session 2 changed significantly more zones than calves in session 1 (W=11, df=15, P<0.005). These results indicate that calves do not explore only in their nurse's area. Finally, calves in session 2 vocalized more than in session 1 (W = 4.5, df =15, P >0.05).

In summary, the results of the choice test show that calves spend more time in the nurse's area, but this time decreases with age. The preference index (PI) is close to 1 at session 1 but decreases afterwards. On the other hand, calves spend relatively little time in the other cow area but this time increases in session 2, as does the exploratory behavior and the frequency of area change.

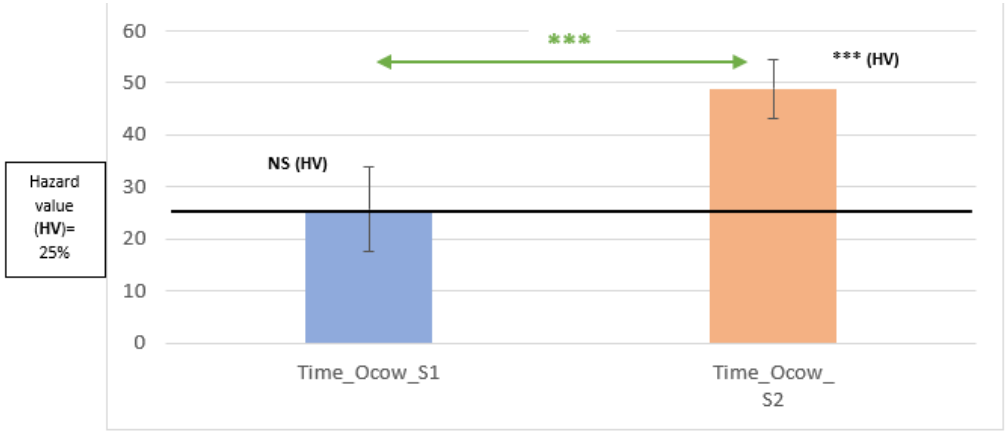


Figure 7: Evolution, between session 1 and session 2, of the time (%) spent in the other cows' area during the separation test and the comparison with a hazard distribution (n=16)

*Legend: Time_Ocow_S1 = Percentage of time spent by the calf in the area close to the other cow (Ocow) in session 1 ; Time_Ocow_S2 = Percentage of time spent by the calf in the area close to the other cow (Ocow) in session 2. When P>0.05 there is non-significant differences "**NS**" ; when P<0.01 there is a "*******".*

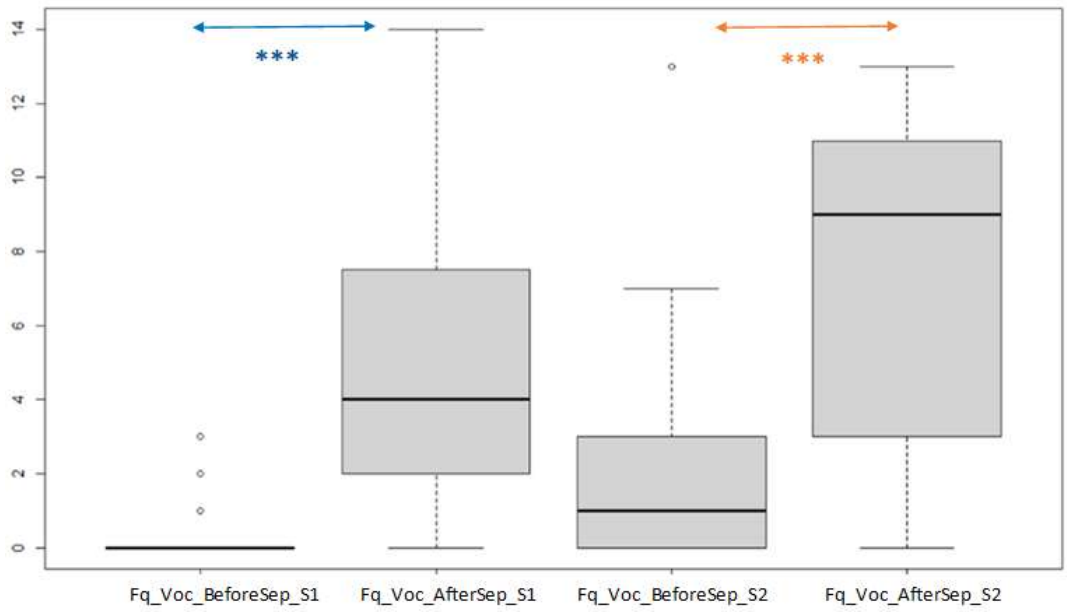


Figure 8: Evolution of vocalization frequency before and after the separation of the nurse in session 1 (S1) and session 2 (S2) (n=16)

*Legend: Fq_Voc_BeforeSep_S1 = Frequency of vocalizations emitted by the calf during the choice test (nurse present) in session 1 ; Fq_Voc_BeforeSep_S2 = Frequency of vocalizations emitted by the calf during the choice test (nurse present) in session 2 ; Fq_Voc_AfterSep_S1 = Frequency of vocalizations emitted by the calf during the separation test (nurse absent) in session 1 ; Fq_Voc_AfterSep_S2 = Frequency of vocalizations emitted by the calf during the separation test (nurse absent) in session 2. Time_Ocow_S1 = Percentage of time spent by the calf in the area close to the other cow (Ocow) in session 1 ; Time_Ocow_S2 = Percentage of time spent by the calf in the area close to the other cow (Ocow) in session 2. When P>0.05 there is non-significant differences "**NS**" ; when P<0.01 there is a "*******".*

8. Separation Test

The results of the separation test are presented in ***Table 4***.

At the time of separation from the nurse, the calves will distribute their time in each area more evenly. In session 1, the time spent near the other cow was not different from chance (25%; +/-8.13) (W = 71, P = 0.897) (***Fig.7***). In session 2, the calf spent an average of 48% (+/-5.56) of its time in the other cow's area, this difference is significant compared to random allocation (t = 4.796, df = 15, p-value <0.001). So, there is a significant increase in time spent near the other cow between session 1 and session 2 (***Fig.7***). There was no correlation between the time spent in the nurse's area before separation and the time spent in the other cow's area after separation in either session 1 (r_s=0.284, df=15, P>0.1) or session 2 (r_s=0.088, df=15, P>0.1). Calves vocalized more after separation from the nurse than before separation whether in session 1 (W = 0, df=15, P <0.001) or session 2 (W = 7.5, df=15, P <0. 001) (***Fig.8***) but did not change area any more regardless of session (session 1: W =15, df=15, P>0.1, session 2: W =51.5, df=15, P>0.1,) nor did they explore differently (session 1: W = 78, df=15, P>0.1, session 2: W = 134.5, df=15, P>0.1,).

There was a significant difference in the frequency of zone change during this separation phase (t = -4.130, df = 16, p-value <0.001) between session 1 and session 2. Calves in session 2 changed zones more times than in session 1. Exploration frequency also increased significantly (Wilcoxon Test; W = 15, p-value = 0.006) between calves in session 1 and session 2.

In summary, the results of the separation test show an increase in the time spent in the area of the other cow when the nurse is removed from the test. This increase was even more pronounced in session 2. Vocalizations, frequency of zone change and exploratory behavior were also found to increase after separation from the nurse and more markedly in session 2.

Table 4: Results of the separation test for the session 1, session 2 and calculation of p-value between sessions and compared to hazard

Legend: Time_Nurse (%) = Percentage of time spent by the calf in the area close to its nurse; Time_Z0 (%) = Percentage of time spent by the calf in the neutral area; Time_Ocow (%) = Percentage of time spent by the calf in the area close to the other cow (Ocow)

	Session 1		Session 2		P-value	
Variable	**Mean**	Standard error	**Mean**	Standard error	Session effect	Hazard effect
Time_Nurse (%)	**38,22**	7,78	**22,97**	4,77	W= 86, P = 0.147	S1 : t = 1.459, df = 15, P = 0.165 S2 : W = 46, P = 0.442
Time_Z0 (%)	**36,97**	6,85	**29,30**	3,68	t = 1.018, df = 15, P = 0.324	S1 : t = -1.738, df = 15, P = 0.102 S2 : t = -5.378, df = 15, P <0,001
Time_Ocow (%)	**25,91**	6,00	**49,41**	5,26	W = 28, P = 0.041	S1 : W= 28, P = 0.041 S2 : W = 59.5, P = 0.678
Latency exploration (sec)	**57,85**	10,33	**35,68**	6,72	t = 0.387, df = 6, P = 0.711	
Frequency exploration	**0,70**	0,21	**2,31**	0,37	W = 14, P = 0.008	
Frequency of zone change	**2,81**	0,59	**5,68**	0,77	W = 9, P = 0.003	
Latency Vocalizations (sec)	**35,42**	8,01	**15,31**	4,73	W = 113.5, P= 0.019	
Frequency Vocalizations	**4,88**	0,86	**9,00**	1,00	t = -2.744, df = 15, P = 0.015	

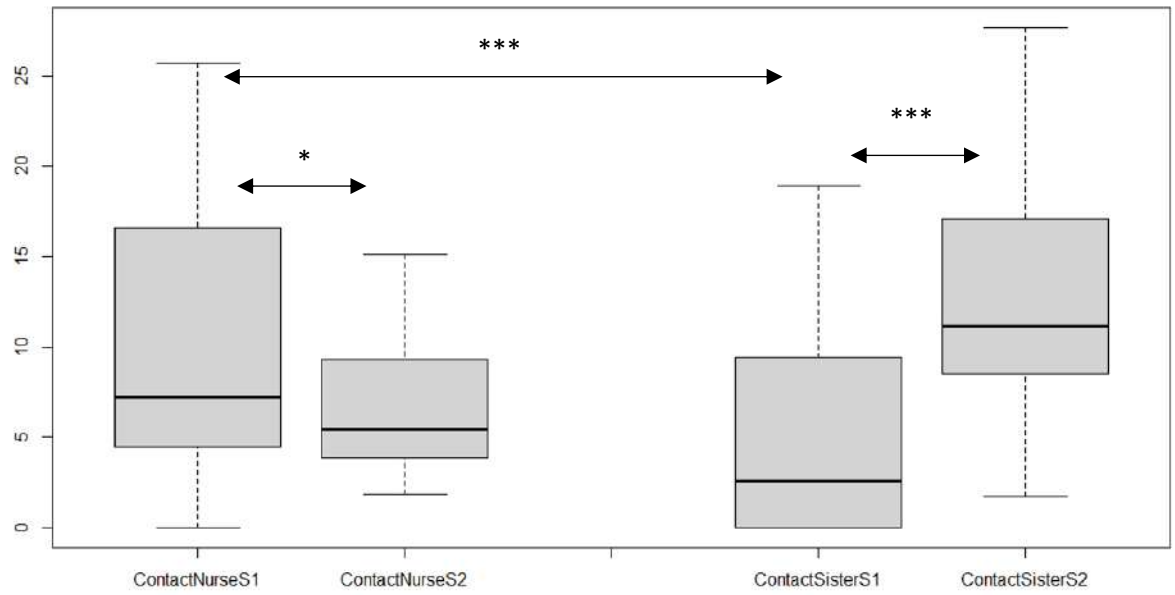


Figure 9: Evolution of the percentage of "contact" SCANs for calf/nurse and calf/sister pairs between the session 1 and session 2 (n=22)

Legend: ContactNurseS1 = Percentage of SCANs where the calf is seen in contact with his nurse during the session 1; ContactNurseS2 = Percentage of SCANs where the calf is seen in contact with his nurse during the session 2; ContactSisterS1 = Percentage of SCANs where the calf is seen in contact with his sister during the session 1; ContactSisterS2 = Percentage of SCANs where the calf is seen in contact with his sister during the session 2. When P<0.05 there is a "" and when P<0.01 there is a "***".*

9. Pasture Observations

9.1. SCAN Results

9.1.1. Session 1

Based on the percentages of SCANs taken in Session 1, calves had significantly more contact with their nurse than with their sister(s) on pasture (W=98, df=21, P<0.005) (***Fig.9***). Nevertheless, the percentages of "nurse contact" and "sister contact" scans are significantly correlated (r_s=0.465, df=21, P<0.05). Finally, there was no correlation between the time spent near the nurse during the choice test and the observed contacts between calves and their nurse (r_s=0.107, df=15, P>0.1).

9.1.2. Session 2

The analysis of the percentages of SCANs shows that calves had significantly more contact with their sister than with their nurse (W=25, df=21, P<0.005) (***Fig.9***). The proportion of SCANs "sister contact" averaged 12.75 (+/-2.01) versus 5.08 (+/-0.96) for "nurse contact". There was no correlation between "nurse contact" and "sister contact" in session 2 (r_s = 0.08, Df=21, P>0.1).

9.1.3. Sessions 1 and 2

There is a significant decrease in the percentage of scans where the calf is seen in contact with its nurse between session 1 and session 2 (W = 191, df=21, P<0.05) (***Fig.9***). Conversely, there is a significant increase in the percentage of contact with the sisters between session 1 and session 2 (W = 47, df=21, P = 0.010) (***Fig. 9***). On the other hand, there is a positive correlation of "nurse contact" between session 1 and session 2 (r_s = 0.49, Df=15, P<0.05). For both sessions, there is no correlation between the age of the calves and the proximity between individuals (r_s=0.17, df=21, P>0.1).

9.2. FOCUS results

Thanks to the results obtained with the focus groups, it is possible to characterize the interactions between the individuals, as well as their evolution.

The type of partner has an influence on the quantity of interactions observed at pasture. There are significantly (W = 66, df=21, P<0.001) more calves interacting with their nurse (median=1; q25=1; q75=1) than with another cow (median=0; q25=0; q75=1). However, in session 2, there was no significant difference between the number of interactions between the calf and his nurse and the calf and another cow (W=10.5, P>0.05).

On the other hand, there is a significant difference in the frequency of suckling breakage generated by the calf with its nurse between session 1 and session 2. The calves in session 2 generated more feed breaks than in session 1 (W = 54, p-value = 0.007). Thus, the calf is the cause of the rupture of the feedings in session 2.

There is a significant difference in the duration of the "calf head" pose between the session 1 and the session 2. Calves in the session 2 rested their head on another calf more times than the session 1 (W = 20, p-value = 0.008).

Table 5: Results of the human avoidance test on nurses and calves in session 1 and session 2.

Legend: ID Cow: cow identification number; ID Calf: nurse identification number

Session	ID COW	Avoidance Distance (cm)	Session	ID COW	Avoidance Distance (cm)
S1	3004	0	S2	3004	5
S1	2352	100	S2	2352	70
S1	803	30	S2	803	135
S1	2367	30	S2	2367	15
S1	175	50	S2	175	5
S1	5087	100	S2	5087	85
S1	5078	50	S2	5078	225
S1	3002	30	S2	3002	25
			S2	2316	0
Session	ID CALF	Avoidance Distance (cm)	Session	ID CALF	Avoidance Distance (cm)
S1	1001	200,0	S2	1001	50
S1	1002	290,0	S2	1002	160
S1	1003	580,0	S2	1003	80
S1	1007	430,0	S2	1007	190
S1	1009	250,0	S2	1009	15
S1	1010	230,0	S2	1010	75
S1	1018	330,0	S2	1018	125
S1	1019	200,0	S2	1019	85
S1	1020	360,0	S2	1020	110
S1	1023	380,0	S2	1023	155
S1	1028	150,0	S2	1028	0
S1	1034	350,0	S2	1034	150
S1	1035	250,0	S2	1035	90
S1	1037	390,0	S2	1037	135
S1	1040	290,0	S2	1040	125
S1	1041	220,0	S2	1041	120
S1	1043	380,0	S2	1043	135
S1	1045	310,0	S2	1045	160
S1	1046	330,0	S2	1046	275
S1	1047	190,0	S2	1047	165
S1	1049	430,0	S2	1049	85
S1	1050	290,0	S2	1050	205
			S2	1052	285
			S2	1053	120
			S2	1055	190

In summary, it appears that in the first session, calves prefer to be in contact with their nurse rather than their sisters. Thus, there is more interaction between the calves and their nurse than with another cow. The calves that are most in contact with their nurse in session 1 are also in contact with their nurse in session 2. The bond created between the two individuals is therefore durable. Moreover, we can see that the nurse is a "gathering" element for the calves.

However, over time, the calves are less in contact with their nurse in session 2 and get closer to their sisters. In general, they even interact more with the other calves.

10. Human Test

The results of the human test did not show a correlation between the calves' avoidance distance from humans and the nurses' avoidance distance from humans in session 1 (r_s = 0.166, dF=, P>0.1) and in session 2 (r_s = 0.014, dF=, P>0.1).

Calves in session 2, had a lower avoidance distance (mean=122cm, +/-13) than in session 1 (mean=310cm, +/-21). (t = 8.656, df = 21, p-value < 0.001) (***Table 5***).

In session 2 their avoidance distance correlated with the age of the calves (r_s= 0.44, dF=21, P <0.05), which was not the case in session 1 (r_s=0.037 dF=21, P>0.1).

Table 6 - Correlation test between the different variables of the adoption test and those of the grazing observations and choice test in session 1 (n=12).

Legend: Calf_obs_P1 = observation directed at the calf in phase 1 of adoption; Contact_P1 = contact at the fence during phase 1 of adoption; Threat_Kick_P2 = threat and kick directed towards the calf in phase 2 of adoption; Day_required_successful_adoption = number of days required for a successful adoption; ContactNurseS1 = proportion of SCAN where the calf is seen in contact with its nurse in session 1 during the pasture observation; Time_Nurse_S1 = Percentage of time spent by the calf in the area close to its nurse in session 1 during the choice test

	Calf_obs_P1	Contact_P1	Threat_Kick_P3	Day_required_successful_adoption
ContactNurseS1	**r_s = 0.461** **p = 0.05**	r_s = 0.257 p = 0.30	**r_s = - 0.461** **p = 0.05**	**r_s = - 0.520** **p = 0.01**
Time_Nurse_s1	r_s = 0.431 p = 0.16	r_s = - 0.107 p = 0.74	r_s = - 0.431 p = -0.431	r_s = 0.023 p = 0.93

Table 7 - Correlation test between the different variables of the adoption test and those of the grazing observations and choice test in session 2 (n=12).

Legend: Calf_obs_P1 = observation directed at the calf in phase 1 of adoption; Contact_P1 = contact at the fence during phase 1 of adoption; Threat_Kick_P2 = threat and kick directed towards the calf in phase 2 of adoption; Day_required_successful_adoption = number of days required for a successful adoption; ContactNurseS2 = proportion of SCAN where the calf is seen in contact with its nurse in session 2 during the pasture observation; Time_Nurse_S2 = Percentage of time spent by the calf in the area close to its nurse in session 2 during the choice test

	Calf_obs_P1	Contact_P1	Threat_Kick_P3	Day_required_successful_adoption
ContactNurseS2	r_s = 0.119 p = 0.63	r_s = - 0.015 p = 0.95	r_s = - 0.119 p = 0.63	r_s = - 0.239 p = 0.28
Time_Nurse_s2	r_s = - 0.194 p = 0.54	r_s = 0.367 p = 0.24	r_s = 0.194 p = 0.55	r_s = 0.019 p = 0.94

11. Study of the correlations between the results of the adoption test and the following tests (observation on pasture and choice test)

11.1 Session 1

Observation directed at the calf during the adoption test was positively correlated with the percentage of SCANs where the calf was seen in contact with its nurse at pasture (***Table 6***).

Conversely, the frequency of "threatening/kicking the calf" behaviors as well as the days required for successful adoption were negatively correlated with the proportion of SCANs where the calf was seen in contact with its nurse at pasture (***Table 6***).

11.2 Session 2

In session 2, a very different picture emerges regarding the bonding of the animals. Here, there is no correlation between the criteria related to the quality of the adoption and the bonds created afterwards observed at the pasture and at the choice test (***Table 7***).

These last results, although they cannot predict the quality of the relationship between the calf and its nurse, highlight a coherence of the results between the different tests of the experiment: a cow concentrated on the calf in the adoption phase, is closer to the calf in session 1.

To conclude this exploratory study, we wanted to investigate certain criteria specific to the cows in the experiment (such as the age of the cow, the date of the last calving, the quantity of milk produced and the parity of the cows) that could facilitate adoption. These results are only suggestions for future work.

At first glance, it appears that the amount of milk produced by the cow before adoption tends to increase the motivation of the cow at the time of adoption (Contact_P1 and QuantityMilk: r_s= 0.639; p = 0.06).

There was a positive correlation between the date of last calving and the number of days needed to consider adoption successful (r_s = 0.760; dF=11, p= 0.01). This means that the longer it has been since the cow calved, the less time she will take to adopt the calves presented to her.

Discussion

The results of the present experiment suggest a coherence between the nurse behavior at the adoption and those observed for the calves that followed afterwards (on pasture and choice test). Indeed, the results highlighted the strong preferential bond developed by the calves for their nurse even nurses are not their biological mother. All calves, except one, chose their nurse as their first choice, even some months later. They also vocalized more after a provocated separation. First observations around two months after adoption and at pasture also highlighted the strong proximity that the calf had to its nurse. However, three month later, calves seem much more attracted by the "sister" calves even if the calves that are the closer to their nurse at the first session remain the same at the second session. Moreover, the avoidance distance of calves from the human decreases with time. More interesting in this exploratory study, the individual variability in the "nurse" behaviors observed during the adoption test seem allow to assume some criteria that could predict the success of a system of rearing calves under nurse. All future nurses showed strong attention toward the calves before adoption even if they were out of their calving time. However, some cows accepted the calves much more easily compared to some others that rejected it at first. Our study suggests that individual variation is related to the later calf-nurse relationship. Easier is performed the adoption by the nurse, and stronger will be the proximity between them at least during the next three months. Afterwards, this relationship was no longer apparent in our data.

To my knowledge, it is the first scientific study that shows the bond created between a nurse and a calf outside of the calving period and the relationships changes with the other calves before weaning. The literature was focused on the bond created between the mother or a nurse and its calf since calving [10, 8, 56]. The calf's search for contact with the nurse in the choice test and during pasture observations and the increase in vocalizations by the calves (when the nurse is removed from the calf's field of vision) are similar criteria found in studies assessing the bond between the young and its mother [57, 58]. So, this similarity suggests that the nurse could have common properties than the biological cow for the calf. The nurse is a source of food as the mother is suckling the calf which reinforce the bond in mammals [58]. Moreover, vocalizations during the separation test can be interpreted as distress response [57]. These vocalizations could be explained by a possible search of sucking as the calf was separated for his nurse since one hour. However, this duration is rather limited and they would have also vocalized during the choice test if they wanted to suck. So, we believe that the more likely explanation relies in the distress separation of the nurse or social isolation from the herd. However, in this last case, calf should have searched for the other cow proximity. But it was not the case at the first test session as calves did not spent more time close to her than by chance. So we believe that it was really induced by the nurse removal.

Our results show that nurse is not the only partner of the adopted calf. At the first session, the calves are more often observed close to the nurse than to the sisters. A correlation concerning the proximity of the calves to their sisters as well as the proximity to their nurse was also put forward. This result suggests a local attraction of the two sisters for the nurse presence. Ligout et al. point out that ewes can directly (through suckling) or indirectly promote proximal contact and thus mutual familiarization between individuals of the same litter [36]. This mutual long-lasting familiarization seems confirm by the observations of the second session. Calves are observed much more in contact with their sisters than the nurse and less close to her.

The correlation between the proximity of the nurse and the proximity of the sisters is not observed anymore. In accordance to the literature with mothered reared calves, this seems indicate that growing calves become more and more independent of their nurse and attracted together [56]. It is important to notify that calves of the experimentation are gather with their nurse at a maximum of 14 days of age so they spent the majority of their first month together. According to Raussi, this period before 3,5 month of age appears important for calves to develop long lasting preferential relationship [59].

Considering the relationship with humans, we didn't observe any correlation between the avoidance distances of the calves from the human and those of the nurse from the human. These results are not in agreement that few studies in the literature [60]. Cows of the study are selected by the technicians for their docility that could explain a short avoidance distance. The relatively small variability of these results makes more difficult to reveal a significant correlation between the avoidance distances of the cow from the human and those of the calves from the human. The results of the human avoidance test showed that there was a significant decrease of the avoidance distance between the session 1 and the session 2. This is probably due to a habituation of the animals to the experimenter [60].

Finally, one of these major perspectives is related to the fact that we observe a great variability of certain behaviors of the nurse toward the calf during the adoption process. Successful adoption is defined here as acceptance of suckling with the manifestation of maternal care directed toward the calf such as licking [50]. During and around calving, the mother's behavior is directed towards her calf, she licks it and lets him suckle [61]. In this study, the first adoption phase highlights a directed observation toward the calf. These directed observation toward the calf is also correlated with the nurse contact in the pasture for the first three months. To the contrary, when there are rejection behaviors, they observed some kicks toward the calf [61]. The study showed that when there are some kicks toward the calf, the proximity at the pasture is lesser. In anyway, it is particularly interesting to point out that the literature highlights the "sensitive period" around and after calving, based notably on an amniotic fluid attraction and consumption [62]. It is synonymous with significant exchanges between the calf and its mother (neonate calve sniffing and licking that contributes to its vigor). Such period is considered as a key time for establishing a bond between the two individuals [22]. This "sensitive period" is respectively at the time of calving for the adult and birth for the young [25]. However, in our study, the animals are all far from this sensitive period (cows as well as calves) and there are not amniotic fluids and we still observe a relationship approaching a maternal model. It becomes obvious to ask why some cows keep a strong motivation outside the sensitive period. We didn't find any studies in literature suggesting potential mechanism. Preliminary results concerning criteria such as the amount of milk produced before adoption to facilitate adoption remain speculative. However, they suggest interesting work on the subject.

Limitation of the study and perspectives

My exploratory work had some limitations regarding methodology and representativeness of results. First of all, this exploratory study done on a limited number of nurses and calves. Thus, it is more difficult to find some correlations between the different variables of the experience and the results could be less robust when inferring them to the larger population. It will be important to repeat this experimentation to confirm these results.

Some other methodological aspect needs to be discussed.

Even if the results of the choice test allowed us to determine a clear preference of the calf towards its nurse, it was not possible to relate them with the results of the pasture observations. Indeed, the lack of variability in the results of the choice test does not allow to evaluate an intensity of bonding between the calf and its nurse but only to give a qualitative value. Moreover, a randomly selected cow was chosen to face the nurse without considering the affinity of the calf toward this cow. It could be interesting to investigate further if the variation of preferences of the calf could depend on the social relationship of this cow with the calf. It would have been relevant to also test the withdrawal of this cow and compare it to the withdrawal of the nurse.

Considering the time available for the observers (COVID-19 period) to perform SCAN and FOCUS on pasture, we did not obtain a large amount of data (Sessions 1 + 2: 112 SCANS and 40 minutes of FOCUS). The instantaneous nature of the data collection does not allow us to obtain a representative image of the periods and therefore does not allow us to characterize the quality of the relationships. Even if we were able to find significant variation and correlation among the different period of test and observations, it would be interesting to increase the time to observation in pasture, covering the whole daylight period, to have a more robust conclusion. Finally, the observation protocol was essentially aimed at observing the proximity with the nurse, so it was not possible to see the interest that a calf could have towards another cow and thus answer the question of the construction of the relational network of the dairy calf.

The human test should have been performed by a technician unknown to the animals. The animals in the experiment were aware of the experimenter as she was also a regular observer of the herd, which may represent a bias for this experiment. However again, for a time constraint it was not possible and it should be further one in the future.

Finally, the results, which assume a close relationship with a maternal model, may suggest a possible notion of attachment between the calf and its nurse. To answer this question, it would be necessary to evaluate the nurse as a "secure base" for the calf. This avenue should be explored in the future. It would be particularly interesting to compare calves reared by their nurse to calves reared by their biological dams or calves reared artificially. The experiment was also performed with many genetical types. Breed can be an important factor determining the cow-calf relationship [63]. Genetic factor will need a further exploration with a very large sampling probably on farm.

Conclusion

In conclusion, this preliminary and exploratory study shows that the adoption practices of dairy calves by a nurse cow lead mostly to a long-lasting and preferential bond between the calf and its nurse. However, the most difficult adoptions, even if successful, could lead to a weaker bond between the calf and its nurse. The intensity of the nurse's observation of the calf during the adoption phase and the lower frequency of rejection behaviors by the nurse towards the calf seem to be early indicators of the quality of the subsequent bond. As in the biological mother-calf relationship, an evolution of interactions is observed over time, such as the calf's closeness to its sisters and to the other calves. The practice of adoption had already shown its value for the farmer's work, the reuse of unproductive animals and the health of the dairy calf. The results of this study, if confirmed, seem to show that this adoption practice could be beneficial to the construction of the relational network of the dairy calf by having the calf under the mother as a reference.

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ABSTRACT

In a context where societal demand is influencing rearing conditions by demanding better consideration of animal welfare, alternatives are being developed. The rearing of calves under nurse is one of these relatively new practices that is becoming more widespread. However, few papers refer to the relationship between the calf and the nurse (as a mother figure), which are not biologically linked and where the adoption is distant from calving. Therefore, the purpose of this exploratory study was to investigate the social interactions before the weaning in a pasture-based cow-feeder system and to see how they evolve between two sessions (May and August 2021). The experimental design had 9 nurses and their 25 calves. To evaluate this link, choice/separation and human avoidance tests were carried out as well as pasture observations during the two sessions.

Despite a time lag between the last calving date of the nurse cow and the adoption date (up to 18 months), during the adoption test, a certain attention of the cows towards the calf was clearly shown, characterized in part by the observation of the cow directed towards the calf and the few rejection behavior. Afterwards, a bond seems to be created between the calf and its nurse, characterized by the proximity between the individuals and a preference put forward during the choice test. Later, observations show a decrease in contact between the calf and his nurse, but an increase in contact with his sisters and interactions with other calves. Finally, it does not exist a link between the avoidance distance human/cow and between human/calf. This construction of the relational network of the dairy calf evocate that observed in a model of veal under the mother.

Keywords: Social behavior, Preferential relationship, Calf-nurse bond, Changing relationships, Animal welfare