PhD thesis

Maternal behaviour and use of maternity pens in parturient dairy cows

by

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NERD

[nurd] noun.

An intellectual badass.

To my grand-mother,

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Preface

The present PhD thesis entitled "Maternal behaviour and use of maternity pens in parturient dairy cows" was submitted to the Graduate School of Science and Technology (GSST), Aarhus University, as part of the requirements in the Ministerial Order for the degree of Doctor of Philosophy at the Department of Animal Science at Aarhus University, Denmark. I declare that I have composed the present PhD thesis. The work presented is my own and all assistance has been duly acknowledged. None of the work described has been submitted for any other degree or professional qualification.

The experiments presented in this thesis were conducted in the period September 2014 until January 2018 at the Department of Animal Science, AU Foulum, Aarhus University.

The PhD project was part of the research project called 'The self-guided cow' and funded by the Green Development and Demonstration Programme (GUDP) of the Danish Ministry of Environment and Food, Denmark, and GSST, Aarhus University. The research project was a cooperation between Department of Animal Science, AU Foulum and the private company Jyden Bur A/S, Vemb, Denmark.

The main objective of this PhD project was to obtain knowledge on the maternal behaviour of parturient dairy cows in relation to their use of maternity pens, through experimental work combined with state-of-the-art literature synthesis. This new knowledge may ultimately contribute to the improvement of animal welfare through optimised housing systems and management routines for parturient cows.

The thesis contains five original research papers based on five separate studies. The introduction to the thesis provides a brief overview of the topic and lists the incentives for conducting the PhD project. The background section explains current practice as well as details and challenges of the environment of parturient dairy cows. More in-depth knowledge on the background for each specific study is provided within each paper (Chapter 5 "Studies 1-5"). During the PhD project, all new discoveries and practical experiences obtained from the studies were subsequently incorporated when a new study was conceived, thereby facilitating a coherent and well-functioning line of experiments. The last paper of the thesis constitutes a comprehensive literature review highlighting new aspects of, and evolutionary reflections on, the causation of pre-partum maternal behaviour of cows, and is linked to the overall interpretation of the results from the experimental studies. The discussion critically reviews the results across all five studies in relation to the current literature while reflecting on new aspects in a broader perspective.

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I was privileged to be working together with the company Jyden Bur A/S when conducting two of the experiments. I would like to thank everybody involved, but especially thanks to director Jens Agergaard and product developer Ole Andersen.

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I could never have completed this PhD without the love and support from my family and friends. Louice H. Bang and Lonni Bisgaard always inspired by to do what I loved the most and supported me all the way. Thanks to the countless questions on what this PhD 'actually was about', my parents and brother, my grandparents and my parents in law, have helped me stay in focus and communicate my passion in a broader perspective.

Lastly, I acknowledge and thank my partner, Frederik Pachler, for being there every day, for discussing nerdy science, and for being a consistently smiling face. You definitely have cow-whispering skills and you make me happier than you will ever know.

Maria Vilain Rørvang, Aarhus, January 2018

Contents

List of included papers and manuscripts

I. **Rørvang, M. V.,** Herskin, M. S., Jensen, M. B., 2017. Cows with prolonged calving seek additional isolation. Journal of Dairy Science 100: 2967-2975. DOI: 10.3168/jds.2016-11989.

II. **Rørvang, M. V.,** Nielsen, B. L., Herskin, M. S., Jensen, M. B., 2017. Short communication: Calving site selection of multiparous, group-housed dairy cows is influenced by site of a previous calving. Journal of Dairy Science 100: 1467-1471. DOI: 10.3168/jds.2016-11681.

III. **Rørvang, M. V.,** Jensen, M. B., Nielsen, B. L., 2017. Development of test for determining olfactory investigation of complex odours in cattle. Applied Animal Behaviour Science. 196: 84-90. DOI: 10.1016/j.applanim.2017.07.008.

IV. **Rørvang, M. V.,** Herskin, M. S., Jensen, M. B., 2018. The motivation-based calving facility: Social and cognitive factors influence isolation seeking behaviour of Holstein dairy cows at calving. PLOS One 13 (1). E0191128. DOI: 10.1371/journal.pone.0191128.

V. **Rørvang, M. V.,** Herskin, M. S., Nielsen, B. L., Jensen, M. B., Understanding pre-partum behaviour of a domesticated ungulate: Cattle revisited as wild. Frontiers in Veterinary Sciences 5: article 45. DOI: 10.3389/fvets.2018.00045.

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Summary

A successful reproduction is important for a sustainable dairy production. The pre-partum maternal behaviour of dairy cows is important in the design of calving facilities and when aiming to ensure a successful calving. The causation of the behaviour is, however, currently not fully understood, and therefore this PhD thesis aimed to obtain new knowledge about the behaviour of parturient cows and the use of maternity pens. Five specific research questions were formed based on areas of interest within this scope; a) Do dairy cows prefer a certain type or degree of isolation when calving, and does the choice of isolation influence pre-partum maternal behaviour? b) Is calving site selection influenced by the site where another cow had previously been calving? c) Are parturient cows and heifers able to detect and distinguish between complex odours and may some odours evoke more attention than others? d) Does insertion of a gate in an individual maternity pen increase the proportion of cows calving in such pens, and might social factors influence this? and e) What is the causation of pre-partum maternal behaviour of cattle?

Five consecutive experiments were conducted and the results revealed new aspects of maternal behaviour of parturient cows. Parturient dairy cows showed no preference for a specific level of physical cover in the individual maternity pen. However, a higher level of physical cover was chosen by cows with prolonged calving duration. Insertion of a gate at the entrance of an individual maternity pen did not increase the proportion of cows calving in the pens, due to social factors. High social dominance increased the probability of a cow calving in the pens, whereas presence of alien calves decreased the probability of a cow calving in the pens. Calving site of group-housed dairy cows was influenced by the site where another cow had previously been calving, potentially due to attracting effects of birth fluids in the bedding. Parturient cows and heifers were able to detect and distinguish between complex odours, with some odours evoking more attention than others.

Based on these results and a literature review, the causation of pre-partum maternal behaviour of cattle is suggested to be the motivation to locate an appropriate calving site, by means of isolation achieved through a combination of distance and physical cover. Isolation can be achieved through a continuum of physical cover and distance and the motivation for level of isolation may increase with increasing level of disturbance (e.g. social dominance and presence of alien calves and/or birth fluids). The collective results from this thesis may contribute to the future development of calving facilities and thereby assist in safeguarding the welfare of parturient cows. Furthermore, the results highlight unexploited opportunities for using odours in management of dairy cows and design of housing systems.

Sammendrag

Succesfuld reproduktion er centralt for en bæredygtig mælkeproduktion. Koens maternelle adfærd op til kælvning er vigtig for forståelsen af, hvordan opstaldningsfaciliteter til drægtige køer bedst designes for at opnå en problemfri kælvning. Man kender endnu ikke den fulde underliggende forklaring på koens adfærd op til kælvning og derfor var formålet med denne Ph.d.-afhandling at opnå ny viden om drægtige køers adfærd og brug af kælvningsbokse. Fem specifikke forskningsspørgsmål blev formuleret baseret på den eksisterende viden inden for emnet. Disse havde til formål at afdække; a) om drægtige køer har en specifik præference for fysisk dække i kælvningsboksen, b) om en låge indsat i indgangen til kælvningsboksen har en gunstig effekt på køernes brug af kælvningsboksen, c) om valget af kælvningssted er påvirket af andre køers tidligere kælvningssted, d) om køer og kvier kan kende forskel på forskellige lugte og om nogle lugte er mere interessante end andre, samt e) hvad den underliggende forklaring er på den adfærden som ses hos drægtige køer op til kælvning.

Fem eksperimentelle forsøg blev gennemført, og alle afdækkede nye aspekter af køers maternelle adfærd. Køerne viste ingen præference for et specifikt design af fysisk dække i kælvningsboksen, dog havde køer med langvarige kælvninger en præference for mere isolation/mere fysisk dække. En låge indsat i kælvningsboksen øgede ikke andelen af køer, som kælvede i boksen da pga. social faktorer. Høj social dominans øgede chancen for at køerne kælvede i boksen og tilstedeværelse af kalve reducerede chancen for at køerne kælvede I boksen. Køernes valg af kælvningssted var påvirket af hvor andre køer tidligere havde kælvet. Dette kan skyldes at fostervæsker i strøelsen har en tiltrækkende effekt på drægtige køer. Både køerne og kvierne kunne kende forskel på forskellige lugte og udviste samtidig mere interesse for nogle lugte end for andre.

Baseret på resultaterne af de fire forsøg, samt litteratur reviewet, forstås den maternelle adfærd som et udtryk for koens motivation for at finde et passende kælvningssted, hvilket hun opnår ved at isolere sig fra forskellige forstyrrelser og/eller trusler. Koen kan isolere sig ved hjælp af en kombination af fysisk dække og afstand til de faktorer som forstyrrer hende, og graden af forstyrrelse (f.eks. social dominans eller rovdyr) kan resultere i en højere motivation for isolation. De samlede resultater kan bidrage til det fremtidige arbejde med at optimere kælvningsfaciliteter og derved på sigt være med til at sikre velfærd for drægtige køer.

Resultaterne belyser også helt nye muligheder for at bruge køers lugtesans i management af køer samt i arbejdet med at designe staldsystemer.

1. Introduction

Dairy production relies on the cows´ ability to reproduce. A successful reproduction is, therefore, crucial to achieve and sustain a viable milk production enterprise. The period around calving is typically termed the transition period, defined as three weeks before and three weeks after calving (Grummer, 1995; Drackley, 1999). The term 'transition' refers to the comprehensive physiological changes from being a dry to a lactating cow with calving marking the point of transformation. Transitioning from dry to lactating is associated with a high risk of disease (Atkinson, 2016) as nearly 75% of all disease cases in dairy cows occur within the first month after calving (reviewed in Ingvartsen et al., 2003; Ingvartsen, 2006; Heikkilä et al., 2012). In addition, the process of giving birth places a physiological demand on the cow as the body undergoes profound changes, many of which are associated with pain (Mainau and Manteca, 2011). Hence, cows are vulnerable during this period, and need special attention and care if animal welfare and production are to be safeguarded.

Based on a range of studies of health and behaviour (e.g. paratuberculosis: Donat et al., 2016; Pithua et al., 2013; endometritis: Cheong et al., 2011; sickness and calving behaviour: Proudfoot et al., 2014a) of cows in the transition period, guidelines often recommend farmers to move parturient cows to individual maternity pens when calving is imminent. Calving facilities are recommended to comprise group pens for cows that are close in time to calving, connected to individual maternity pens to which cows are moved when calving becomes imminent (by recommendation of The Canadian Dairy Code of Practice (NFACC, 2009)). For cows housed indoors, some countries even prescribe calving in individual maternity pens by law (In Denmark by Ministry of Environment and Food (Anonymous, 2014)). In this way, monitoring the calving progress is facilitated, which is important, as approximately 50% of calvings in commercial dairy production are assisted (Mee, 2004; 2008; Lombard et al., 2007). In theory, calving in a secluded individual maternity pen would enhance the welfare of the cow by allowing her a quiet and clean calving site adapted for her assumed motivation to be physically isolated (e.g. Proudfoot et al. 2014a), whilst at the same time allowing the farmer easier surveillance of her. However, the practicality of moving cows to individual maternity pens has proven a challenge. First of all, farmers may have access to a limited number of individual maternity pens. Therefore, determining the optimal time of moving a cow has received much interest in order to minimise the time each cow spends in a pen and thus the number of pens (and investments) required per the farm. It has previously been suggested that a parturient cow should be moved before the 2nd stage of labour (def: from initiation of contractions until the calf is born, Noakes et al., 2001), as moving cows during this stage may be disturbing and consequently prolong the calving process (Proudfoot et al., 2013). A prolonged calving increases the risk of complications (e.g. higher risk of stillbirth: Gundelach et al., 2009; Barrier et al. 2013a) and subsequent diseases (e.g. Schuenemann et al., 2011) and maternal behaviour (Barrier et al. 2012a). Therefore, the appropriate time for moving cows is at the latest during the 1st stage of labour (def: from initiation of pelvic ligaments relaxation, suddenly enlarged and tense udder and tail raises until visible abdominal contractions) (Ball and Peters, 2004; Saint-Dizier and Chastant-Maillard, 2015). Although farmers extensively monitor parturient cows (e.g. on average once every 4 h on Canadian farms: Vasseur et al., 2010), they might, however have trouble determining the onset of the 1st stage of labour. This may result in late detection of imminent calving. Hence, parturient cows may end up being moved too late and potentially being recurrently disturbed by frequent visits from husbandry personnel. The current international trend towards increased herd size (Barkema et al., 2015) indicates that future farmers will have a higher number of cows to supervise, and thus face even greater challenges in terms of calving surveillance and moving of parturient cows.

A possible solution consists of providing farmers with tools for more reliable and precise detection of the onset of labour. Studies to develop such tools have been carried out with the aims of detecting and monitoring behavioural and physiological changes occurring during calving. Changes reported prior to calving include reduced rumination (Schirmann et al., 2013; Ouellet et al., 2016), increased number of lying bouts (Miedema et al., 2011a; Schuenemann et al., 2011; Jensen, 2012; Ouellet et al., 2016) and reduced vaginal temperature (Burfeind et al., 2011; Streyl et al., 2011; Ouellet et al., 2016). Sensors to detect these parameters have been developed (e.g. vaginal temperature measures by Vel'Phone, developed by: Medria, P.A. de la Gaultiére, 35220 Châteaubourg, France). However, there is large individual differences in behavioural indicators (Ouellet et al., 2016) and indicators differ with respect to the timing of reliable changes before calving. For instance, rumination and lying bouts may change markedly within the last 6 h prior to calving according to Ouellet et al. (2016), and similarly for steps, lying bouts and standing time during the last 6 h before calving (Titler et al. (2015). Borscher et al. (2017) combined activity, rumination and lying bouts in a neural network machine-learning method and succeeded to predict calving on a daily and 8 h basis. Hence, calving indicators may change during the beginning of the 1st stage of labour but as the farmer cannot receive the information until after a change has occurred, moving cows to individual maternity pens may already be too late. Therefore, there is a need for practical solutions facilitating cows to be moved at an appropriate time before calving. One possible solution is to develop a motivation-based calving facility, taking advantage of the assumed natural motivation of the pre-parturient cow to seek isolation.

If animal welfare is to be safeguarded in the future, while also taking into consideration the farmers´ need for more effective calving management routines, more basic knowledge of the controlling mechanisms of maternal behaviour in parturient dairy cows is needed. Knowledge of the preferences and underlying motivations of parturient cows, may allow for improvement of the housing systems and management routines, all of which may contribute to improve animal welfare and production.

2. Background

2.1. Phases of ungulate maternal behaviour

Maternal behaviour functions to promote survival of the offspring (Lent, 1974) and is largely under hormonal control in mammals. The definition of maternal behaviour used in this thesis is inspired by Clutton-Brock's (1991) definition of parental care: behaviours displayed by the female that appears likely to support the development and growth of her offspring. To the extent that maternal behaviour is sensitive to external cues, it may provide an opportunity of adapting the offspring to the expected environment (Beery and Francis, 2011). The Clutton-Brock (1991) definition is not limited to specific periods (as opposed to e.g. Crump, 1995: after birth). In this specific case, maternal behaviour is, however, restricted to after fertilization, as according Blumer (1979) and more specifically focussed around the period where the female becomes increasingly responsive towards maternal cues (e.g. alien/own offspring, olfactory cues) in late gestation (Dwyer, 2008). This definition was chosen as the PhD project focussed on mechanisms causing the behavioural changes seen in parturient cows as calving becomes imminent and at calving (see Chapter 2.2. below). Maternal behaviour is thus initiated before the event of parturition itself (Nowark et al., 2000) resulting in three phases of the behaviour: pre-partum, parturition and post-partum maternal behaviour. The pre-partum period refers to the period leading to the event of parturition including behaviour preparing the female for parturition e.g. separation behaviour in sheep (reviewed in: Dwyer and Lawrence, 2005) and nest building in pigs (Algers and Uvnäs-Moberg, 2007). Parturition involves the expulsion of the foetus and post-partum maternal behaviour relates to caring and protection of the offspring after parturition (reviewed for mammals in Bridges (2015)). In mammals, the onset of pre-partum maternal behaviour is governed by endocrine responses whereas post-partum maternal behaviour is mainly controlled by sensory stimulation (Krasnegor and Bridges, 1989, sheep: Poindron and Le Neindre, 1980; Keverne and Kendrick, 1994, rats: Rosenblatt et al., 1988, pigs: Algers and Uvnäs-Moberg, 2007). The regulation of maternal behaviour, thus, undergoes a transition marked by parturition. This thesis focuses on aspects related to the behaviour of dairy cows during the pre-partum period and at calving. The post-partum period is considered only to the extent to which it is important for the causation of the pre-partum behaviour.

2.2. The natural pre-partum maternal behaviour of cows

A cow typically synchronizes her behaviour with conspecifics while staying in close proximity of the herd (Miller and Wood-Gush, 1991). However, during late gestation, this pattern changes. There are, to date, only very few studies on the parturient behaviour of wild or semi-wild cattle (Maremma cattle (Vitale et al., 1986), Chillingham cattle (Hall, 1989), Masai cattle (Reinhardt et al., 1977) and Camargue cattle (Schloeth, 1958)) and these were all carried out decades ago. Unfortunately, these few studies only provide limited insight into the behaviour of calving cows. The studies agree, however, that the pre-partum behaviour of cows changes as calving approaches and that the cow (to some extent) separates herself, or hides her calf away from the herd. This may thus be the reason for the very few observations of calving cows in feral conditions – as they seek away and hide and become more difficult to observe. Reinhardt et al. (1977) and Schloeth (1958) reported cows of Masai and Camargue herds to leave the herd before calving. Hall (1979) observed Chillingham calves hiding after birth and Vitale et al. (1986) reported that Maremma calves expressed both hiding and following behaviour in the early post-partum weeks depending on the availability of cover (further description and discussion of this behaviour can be found in Study 5, Chapter 5.5.).

2.3. Implementation of behaviour in housing and management recommendations

Optimal housing and management of parturient cows is important in order to facilitate a smooth calving. Calving difficulties, or prolonged calving, are generally associated with negative effects on health, behaviour and productivity, which relate to the overall welfare of dairy cows (Schuenemann et al., 2011; Barrier et al., 2012a, 2013a; b). Prolonged calving, resulting from calving difficulties, may mean that the cow needs to be assisted (extraction of the calf) and has been shown to increase the risk of stillbirth (Mee, 2004; Lombard et al., 2007), calf mortality (Lombard et al., 2007; Mee, 2008; Barrier et al., 2013b), trauma for the dam, uterine diseases (Sheldon et al. 2009) and lower milk yield (Dematawewa and Berger, 1997). In addition, calves born from difficult calvings may suffer long-term risk of mortality and reduced milk production (Eaglen et al., 2011; Heinrichs and Heinrichs, 2011; Henderson et al., 2011).

Newer studies on commercially kept, indoor-housed cattle, have confirmed that the behaviour of cows change during late gestation (as in feral cattle herds), especially as calving becomes imminent. Cows become restless (Miedema et al., 2011a and b; Jensen, 2012; Barrier et al., 2012b) and some studies reported spatial isolation behaviour when cows were kept on pasture (Lidfors et al., 1994) and hiding-like behaviour when housed indoors (Proudfoot et al., 2014a and b). Moreover, Proudfoot et al. (2014a) showed that 80 % of the parturient cows preferred isolation behind a barrier, while sick and newly calved cows (from the same study) spent more time behind the barrier the first 3 days post-partum. A range of studies have highlighted the advantage of calving in individual maternity pens in relation to biosecurity and hygiene: For cows, calving in individual maternity pens, it reduces the risk of paratuberculosis (e.g. Donat et al., 2016; Pithua et al., 2013), and endometritis (Cheong et al., 2011) and in heifers, *Salmonella* infections are reduced (Losinger et al., 1995). Additionally, diarrhoea (Frank and Kaneene, 1993) and respiratory diseases (Svensson et al., 2003) are reduced in calves born in individual maternity pens. It is such knowledge that, combined with industry-accepted standards, have contributed to the recommendations for managing parturient cows in several countries. The United States Department of Agriculture (UDSA) National Animal Health Monitoring System, Dairy 2014, provided recommendations alongside current practices based on 77% of all dairy operations in the US (USDA, 2014). In the report, it is recommended that the calving area is kept quiet, clean, dry and spacious in order to allow cows to separate from each other. The corresponding Canadian Dairy Code of Practice for the Care and Handling of Dairy Cattle (NFACC, 2009) also requires that the calving area provides warmth, comfort, insulation, dryness, traction and adequate space if the calving area is a group pen. In the UK, Department for Environment Food and Rural Affairs also has specific recommendations (Code of Recommendations for the Welfare of Livestock: Cattle; DEFRA, 2003) stating that parturient cows must be kept in a well-drained and bedded lying area separate from other animals (other than parturient cows) when calving indoors. The UK, US, and Canadian recommendations all allow indoor group housing of parturient cows, which is different from the Danish recommendations and legislation (Anonymous, 2014). In Denmark, parturient cows must be housed in individual calving pens when calving indoors. All recommendations, however, incorporate an aspect of adequate space or opportunity to separate (by housing in individual pens), indicating an adaptation of the scientific knowledge on visual hiding or separation behaviour of the parturient cow. Individual maternity pens may offer farmers an easier way of monitoring the calving progress of each individual cow and allow intervention if needed. It may also be easier to clean an individual maternity pen as compared to a group calving area, thereby potentially offering cows a cleaner calving site in terms of biosecurity (e.g. Pithua et al., 2013). This may also be part of the rationale behind the recommendations of moving parturient cows to individual maternity pens (NFACC, 2009; Anonymous, 2014; USDA, 2014). In Denmark, the recommendations further add that cows are moved to individual maternity pens as close to calving as possible (as according to the US recommendation) (Holm 2010).

2.4. Calving environment of indoor-housed cows in commercial production

In the US, USDA has reported that 59% of all dairy operations have cows calving in group-calving areas (examples in Figure 1A and B), whereas 29% have individual maternity pens (USDA, 2014). In the survey, the remaining 12% did not specify the calving area. Furthermore, 69% of all operations have a 'usual calving area' defined as a place where to a cow is moved prior to calving. It is currently recommended that cows are moved to the 'usual calving area' as close to calving as possible, which is also what happens in most cases, as 42% of all operations move cows 1 day or less prior to calving. In Canada, a recent study on 236 dairy operations from three different provinces showed that 30% of the participating operations used individual maternity pens, and 35% used group calving areas (Villettaz Robichaud et al., 2016). The remaining 25% consisted of farms using tie stalls or different combinations of individual maternity pens and group calving areas. The Canadian study showed that dairy farmers predominantly move cows to the calving site 3 weeks prior to calving when using group or tie stall solutions, whereas appearance of the first signs of imminent calving was used to move cows to individual maternity pens without further definition of these signs. For many countries, including UK and Denmark, data on the current practice are not available in the same detail. In Denmark, the only production data currently available is based on current practices from 2002-2004. This data state that 43% of cows in loose housing calved in a group pen (example in Figure 1C), whereas 38% calved in an individual maternity pen (example in Figure 1D). Additionally, 18% of the cows were not separated at all and thus calved within the normal herd.

Figure 1. Pictures A) and B) illustrate typical group calving conditions on US farms. Pictures are kindly provided by Dr. Kathy Proudfoot, The Ohio State University. Picture C) illustrates conditions from a typical Danish group calving area, and picture D) represents a typical individual maternity pen in Denmark.

Based on the current international practice emphasized above, only few cows will have access to an individual maternity pen. Individual maternity pens are labour intensive as determining the right time to move parturient cows is difficult (Chapter 1 and Cook, 2011). Individual maternity pens also take up space and are expensive to install (Cook, 2011). This may be reasons why farmers use group calving areas more frequently than individual maternity pens (Cook, 2011; Durst, 2011). Additionally, the advantages of using individual maternity pens raised above (Chapter 2.3.) may not have been clearly communicated to farmers, which might add to why farmers mainly use group calving areas. Irrespectively of the underlying reasons, cows in modern commercial production environments are often surrounded by herd mates when calving. In addition, cows may be influenced by the physical constraints within the environment. Parturient cows may, therefore, be exposed to various influential factors when housed in a commercial indoor calving environment (Figure 2). These are reviewed in the following Chapters.

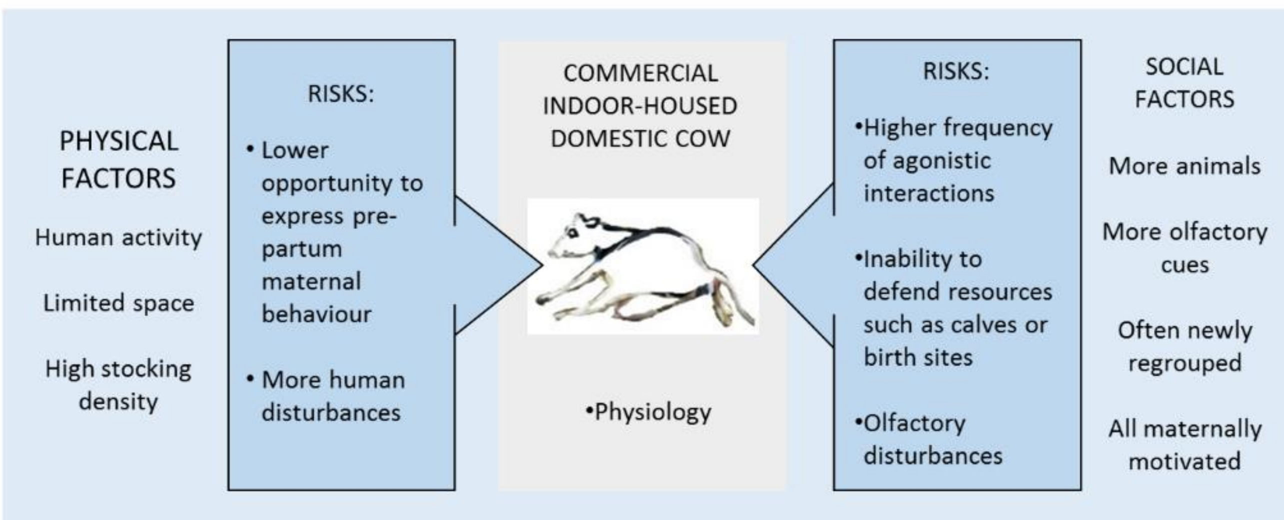

Figure 2. An overview of social, environmental and cow-level (individual) factors, which may influence the behaviour of parturient cows housed in a commercial indoor environment.

2.4.1. Cow-level factors

The initiation of pre-partum maternal behaviour is characterized by marked physiological changes. During late gestation, plasma progesterone levels drop whereas prolactin, oestrogen and placental-derived oestradiol levels rise (Dwyer, 2014; Kendrick and Keverne, 1991). These hormonal changes accompanied by the vaginocervical stimulation from the passage of the foetus, leads to a central release of oxytocin and an increase in expression of oxytocin receptors in several areas of the brain, thereby preparing the ungulate female for parturition (Kendrick et al., 1997). Although rarely studied in ungulates, there seem to be a profound attraction towards olfactory cues related to parturition, the initiation of which may arise from these physiological changes. The most studied species in this respect is sheep, where several studies have shown female attraction towards amniotic fluid. In sheep, this can be induced by a steroid treatment, followed by vaginocervical stimulation resembling the expulsion of the lamb (Poindron and Levy, 1990). Ewes are attracted to the amniotic fluid of their own species during a short time window after lambing (Lévy et al., 1983). Furthermore, ewes respond to ovine amniotic fluid regardless of origin and to amniotic fluid originating from goats but not cattle (Arnould et al., 1991). In cattle, attraction towards amniotic fluid has also been shown. Pinheiro Machando et al. (1997) reported that cows' attraction towards amniotic fluid started as early as 12 hours prior to calving, whereas no attraction towards the placenta appeared in this period. Post-partum, however, the cows showed attraction towards both the amniotic fluid and the placenta persisting up to 24 hours after calving (which was the duration of the study period). Additionally, cows also ingested donor placentas and amniotic fluid. Similar findings have been reported in ewes (Lévy et al., 1983; Basiouni and Gonyou, 1988), rats and mice (Kristal, 1991). George and Barger (1974) noted that dairy cows remained in the same area where their amniotic fluid was discharged, until calving was completed. Cows close to calving may thus be affected by olfactory cues originating from own or other conspecific birth fluids.

Olfaction in general plays a crucial role in relation to mammalian reproduction. In cattle, bulls are capable of detecting specific compounds in the urine of cows in oestrus (Archunan and Rameshkumar, 2012), female puberty can be boosted via a bull pheromone (Rekwot et al., 2001), and the responsiveness of cows towards their offspring has been shown to be directed by neonatal pheromones (Griffith and Williams, 1996). However, until now, it has not been known whether such olfactory influence affect the motivations underlying pre-partum maternal behaviour in cows. The described change in olfactory responsiveness towards birth fluids indicates that cows develop a different preference to odours as calving approaches as it has also been suggested for humans (reviewed in Cameron, 2014).

Despite olfaction having a huge impact on the expression and development of behaviour, studies linking olfaction and behaviour are rare (Campbell-Palmer and Rosell, 2011; Nielsen et al., 2015). The sense of smell is very likely to influence cattle in a wide range of management and housing aspects and thus there may be a potential of exploiting odours and olfaction in the management of cattle (reviewed in: Archunan et al., 2014). Currently, not much is known about the olfactory capacities of cattle in general. The cow is genetically capable of smelling fatty, sour, floral, woody, lemony, green, lily of the valley, vanilla, spearmint, caraway, sweet, hay-like, lemon, rancid and spicy (Lee et al., 2013). Other studies have shown olfactory preferences for mineral oil and propylene glycol with or without rum-ether over an odour-less sample (Corley et al., 1999). In a more applied setting, Madsen et al. (2010) found that visits to the milking robot increased when cows were fed concentrates of a specific type and additionally, Herskin et al. (2003) noted that sniffing duration increased when presenting cows with novel foods in baskets rather than in the usual feed trough. This may indicate that it is possible to manipulate the cows' attention to some extent even though it is not clear whether increased visits or sniffing reflected a degree of novelty or preference for a specific taste or smell. From these results it is, however clear, that olfaction plays an important role in feed preferences of cattle (Engen, 1982; Maruniak, 1988) and that the behaviour of the cow potentially can be manipulated by odours.

2.4.2. Physical factors

At first glance, commercial indoor housing offers dairy cows an unchanging environment as compared to more natural environments such as large pastures. Indoor calving areas are often bedded with sand or deep straw (Cook and Nordlund, 2004), and have feed and water available. In natural environments, climate varies, feed availability vary, predators may be present, and other animals may interfere. The commercial indoor environment may thus be more stable in terms of climate, feed and water. Disturbances may, however, still occur within an indoor commercial environment. The presence of humans and machinery may act as disturbances potentially affecting the expression of pre-partum maternal behaviour and the process of calving. Proudfoot et al. (2014b) found that more cows calved in a shelter when calving during daytime, whereas during night time, equally many cows calved inside and outside the shelter. Other studies have accordingly shown that the majority of cows calve during quiet periods in the barn when housed indoors (Arthur et al,. 1961; Edwards, 1979; von Keyserlingk and Weary, 2007). Disturbance (in terms of manual moving by farm staff) during the late 1st stage of labour has been found to prolong the 2nd stage of labour (Proudfoot et al. 2013). Being in an environment with higher risk of disturbance could thus, pose a risk to parturient cows even when not manually moved between pens. Additionally, space allowance per animal is typically lower in commercial indoor housing facilities as compared to cattle on pasture or in feral environments. These arrangements may allow less opportunity for the animals to express behaviour for which they are motivated. In a study of feral cattle by Hall (1979), 55 Chillingham cattle had access to 1.3 km^2 and in a study by Vitale et al (1986) feral Maremma cattle in Italy had access to 12.5 km^2 of non-maintained marshes and maquis. These studies observed parturient cows separating from the herd before calving, and Vitale et al. (1986) reported that cows displayed hiding-like behaviour when the habitat offered an opportunity for physical cover such as woods and maquis. Isolation may thus appear to vary with the characteristics of the environment. From studies of domestic cattle on pasture, observations of separation or hiding-like behaviour is scarce (e.g. Aitken et al., 1982; Lidfors et al., 1994), but Lidsfors et al. (1994) also noted that at least for cows calving in the forest area, cows chose to calve in isolation behind bushes, scrubs or besides trees. Interpretation of the observations of pre-partum isolation from indoor-housed dairy cows are not conclusive. Dufty (1971) and later Proudfoot et al. (2014a and b) observed cows separating from the group, whereas Edwards and Broom (1982) noted that only some cows displayed this behaviour. These conflicting results could be due to the different environments. A thorough review of this topic comparing cattle studies to other related ungulate species in relation to pre-partum isolation and effects of the physical environment can be found in Study 5 (Chapter 5.5.).

2.4.3. Social factors

In addition to the implications from the physical constraints, parturient cows are exposed to a number of social factors. Cows close to calving are often kept in small and newly established groups. Regrouping has been shown to result in higher frequencies of agonistic interactions (von Keyserlingk, 2008), and maternally motivated ungulate females are known to express defensive and aggressive behaviour to protect their offspring after parturition (Buddenberg, 1986; Turner and Lawrence, 2007; Dwyer, 2008; Arey, 1992). Hence, pre-parturient groups with short inter-individual distances risk increased aggression. Likewise, differences in social status may also result in uneven abilities to gain access to resources e.g. the calf or a preferred calving site, as dominance determines the outcome of cow-cow discrepancies (Val-Laillet, 2008). This is for example seen in Lidfors et al. (1994) who found that parturient cows separated from the herd and noted that one cow was disturbed by the group during calving and subsequently isolated in the forest area. Accordingly, Proudfoot et al. (2014b) found that pair-housed parturient cows increased the separation distance to their partner upon calving. Another example is cases of mismatch between calves and their biological dams reported in terms of cows licking and grooming alien calves (Edwards, 1983; Edwards and Broom, 1982; Illman and Spinka, 1993). In such cases, disturbances arising from being housed in a social group may have compromised the establishment of the mother-offspring bond leading to mis-mothering (i.e. cows licking and nursing calves that are not their own offspring, reviewed in Study 5, Chapter 5.5.). Hence, if particular calving sites are also perceived as resources, the chance of gaining access to such a site may differ with social dominance. Thus, access to calving sites in a group-housing situation may depend on social dominance. If this is the case, it would inevitably affect group-housed parturient cows and the functionality of future calving facilities based on the natural motivation of parturient cows.

2.4.4. Calving in individual maternity pens

Compared to group housing, keeping cows in individual maternity pens theoretically removes the risk of agonistic encounters, social disturbances and mis-mothering. Nevertheless, if the cow is separated from the herd before she is motivated to move away from conspecifics, she may experience this as aversive (Boissy and LeNeindre 1997). The timing of when to move the cow is, thus, critical in order to limit potential negative effects of social isolation. If moved at the optimal time, an individual maternity pen may offer the cow a calm place to calve alongside an opportunity to rest and nurse her calf. A calm calving may contribute to ensure bonding between cow and calf (Alexander and Shillito 1977; Espmark 1971) and facilitate timely provision of colostrum (discussed further in Study 5, Chapter 5.5.). The optimal time of moving parturient cows would coincide with the time of motivational change, i.e. the shift from preferring to stay within close proximity of the herd members to be motivated to separate from them. This specific motivational shift constitutes the basic idea underlying a motivation-based calving facility.

At present, the pre-partum maternal motivation of dairy cows is, however, not fully understood, and thus it is not known how such a motivation-based calving facility should be designed in order to stimulate the parturient cow to use individual maternity pens. Several factors in the environment may affect the motivations and thus the behaviour of parturient cows inevitably affecting the functionality of a motivation-based calving facility. Hence, studies are needed to elucidate what specific factors affect the expression of the pre-partum maternal behaviour of cows.

3. Aim of the thesis and research questions

The main aim of this thesis was to obtain new knowledge about the behaviour of parturient cows and factors affecting this behaviour and the use of maternity pens. The main emphasis was on the preferences and motivations of dairy cows around the time of calving, in particular the period leading up to calving and the calving itself.

Research questions addressed by the work underlying this thesis:

a. Do dairy cows prefer a certain type or degree of isolation when calving, and does choice of isolation influence pre-partum maternal behaviour? (Study 1 and paper 1)
b. Is calving site selection influenced by the site of a previous calving? (Study 2 and paper 2)
c. Are parturient cows and heifers able to detect and distinguish between complex odours and may some odours evoke more attention than others? (Study 3 and paper 3)
d. Does insertion of a gate in an individual maternity pen increase the proportion of cows calving in such pens, and do social factors influence this? (Study 4 and paper 4)
e. What is the causation of pre-partum maternal behaviour of cattle? (Study 5 and paper 5)

4. Overview of the study line and pre-experimental considerations

This thesis comprises five studies, all linked to explore the main aim and research questions of the PhD project. Knowledge obtained from each study was used in the design of following studies (illustrated in Figure 3). Collectively, the included studies were designed to meet the main aim of the thesis and seek to provide knowledge to answer the research questions. This section includes a summary of materials, methods and main results underlying the pre-experimental rationale applied in the studies illustrating the experimental flow of the PhD project. Details of each study can be found in the corresponding paper (Chapter 5). Chapter 6 includes a critical joint discussion of the collective results in relation to state-of-the-art literature and corresponding post-experimental considerations across all the studies.

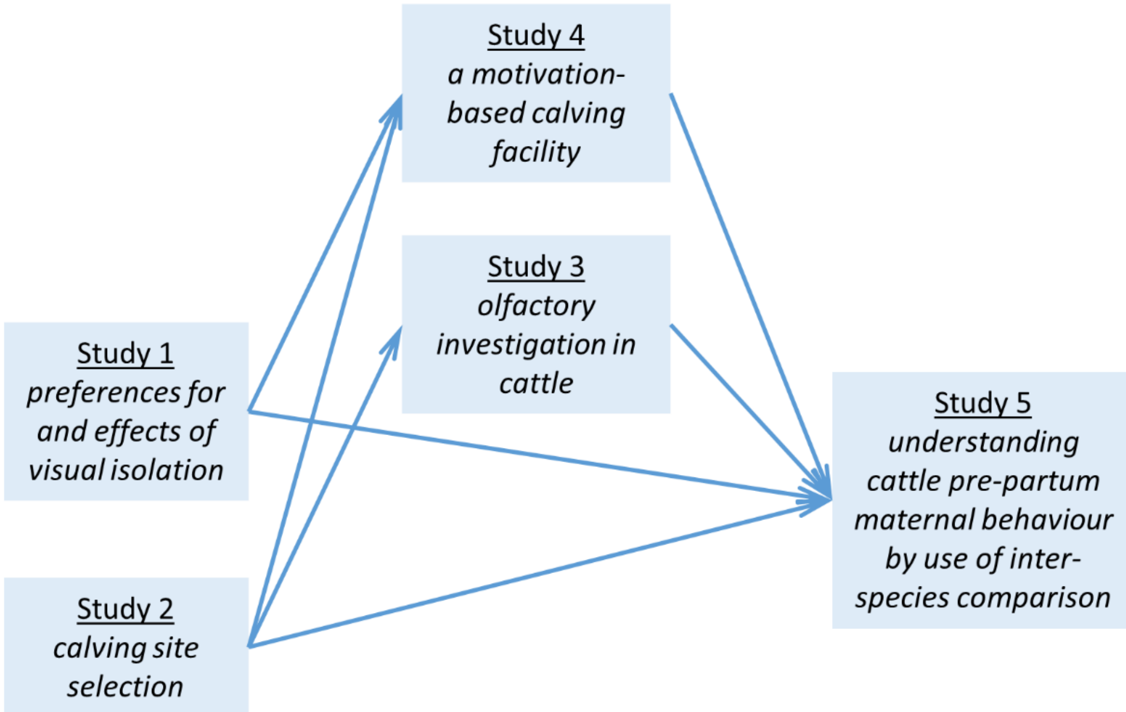

Figure 3. Overview of the studies included in the thesis and inter-study development. Results from Study 1 affected design of Studies 4 and 5. Study 2 was intended as a pilot study for Study 4, but showed important stand-alone results, providing the rationale for Study 3, allowing inclusion of olfaction as an aspect of the work. Study 5 was motivated by the results from Studies 1-4 and, as the only one of the five studies, used a more theoretical approach in order to explore the causation of pre-partum maternal behaviour of cattle.

4.1. Study 1 – preferences for, and effects of visual isolation at calving

This study involved 37 parturient Holstein dairy cows housed in groups of six in an experimental section of the resident barn at Dept. Anim. Sci., Aarhus University (Figure 4). The experimental section consisted of a group pen and two separate maternity units. Each maternity unit was divided into three differently designed, individual maternity pens. A cow from each group would be moved manually from the group pen to either of the two maternity units three days prior to expected calving. In the maternity unit, the cow could choose between the three individual maternity pens, which were accessible via a rubber mat aisle at the feed manger (Figure 4). No other cows could enter the maternity unit (hence the manual moving) allowing the cow free entrance (and no competition) to all individual maternity pens. The three-day period before calving allowed the cow to familiarise with the maternity unit (i.e. all three individual maternity pens) before calving.

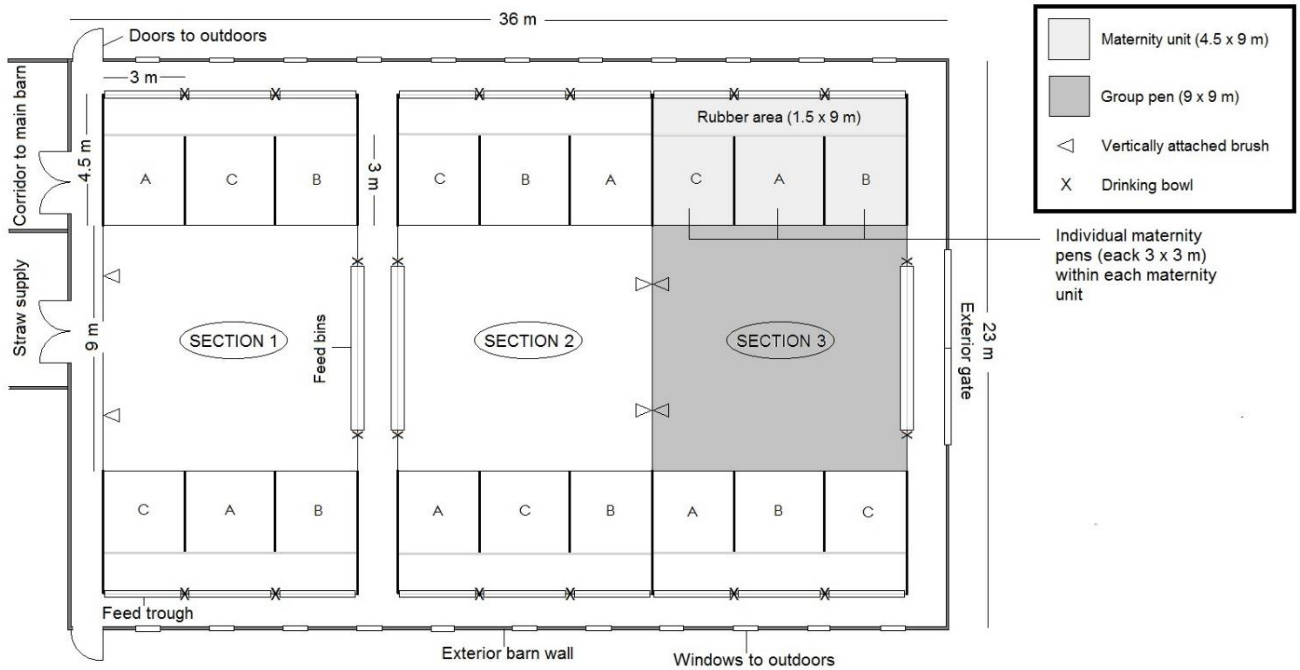

Figure 4. Overview of the experimental barn including top view of the three experimental sections, water bowls, brushes and feed bins. Covered sides within and between the maternity units are illustrated by thick black lines. The rubber mat aisle connecting the three individual calving pens is shown by the grey line indicating the transition from rubber to straw. Letters A, B, and C represent design of the barrier in the specific pen and the balancing of these designs within each maternity unit; A = tall and narrow (1.8 x 1.5 m), B = low and wide (1.0 x 2.5 m) and C = tall and wide (1.8 x 2.5 m). The specific barrier with design A, B, or C is represented by the thin black line separating group pen and individual maternity pens (Figure from Rørvang et al., 2017, Chapter 5.1.).

The individual maternity pens all had deep barley straw bedding and a water bowl. They were shielded from the other individual maternity pens within the maternity unit by use of grey plastic barriers. The third side of each individual maternity pen, facing the group pen, was also shielded, but the grey plastic barrier only covered part of the side, being either: A) tall and narrow, 1.8 m x 1.5 m, B) low and wide, 1.0 m x 2.5 m, or C) tall and wide, 1.8 m x 2.5 m. The remaining part of the barrier was fitted with metal bars, allowing the cow visual and limited tactile contact with the members of the group (Figure 5). Hence, all cows were able to choose freely between three differently designed individual maternity pens (A, B or C, Figure 4 and 5) without any social competition or disturbance while still being able to have visual and limited tactile contact with the rest of the group.

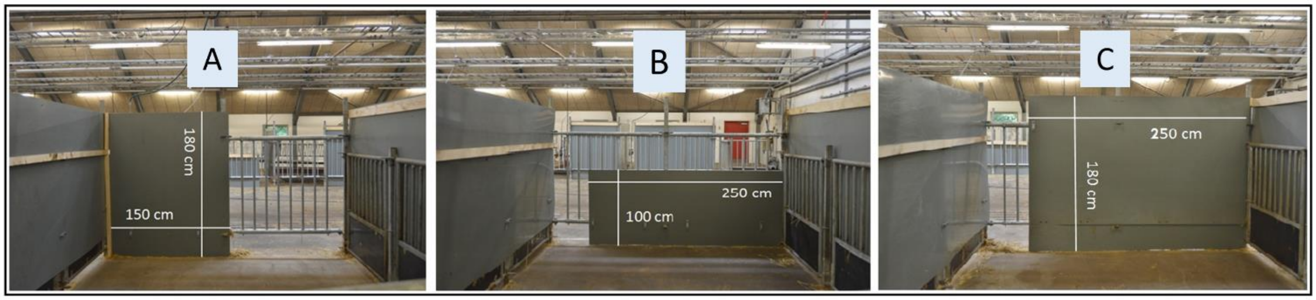

Figure 5. Overview of the three individual maternity pens within each maternity unit (seen from inside the maternity unit). The placing of each individual maternity pen design (A, B or C) was randomly assigned to the maternity units to balance effect of placement. Design A; being the tall and narrow, 1.8 x 1.5 m, B; being low and wide, 1.0 x 2.5 m and C; being tall and wide, 1.8 x 2.5 m (Figure from Rørvang et al., 2017, Chapter 5.1.).

Contrary to the hypothesis, the cows did not show significant preference for any of the three individual maternity pen designs (number of cows calving in individual maternity pens A, B and C respectively: 13, 9 and 15), and cows calving in pens A, B and C did not differ significantly in their pre-partum maternal behaviour. However, a post hoc analysis comparing duration of 2nd stage labour for cows choosing pens A or B (corresponding to 50% isolation) to cows choosing pen C (corresponding to 75% isolation) showed that cows choosing the highest level of isolation had significantly longer 2nd stage labour. Additionally, 12 cows changed (their choice of) pen after the onset of abdominal contractions (onset of 2nd stage of labour), and these cows had significantly longer 2nd stage labour, as well as more frequent postural changes and more contractions.

Although it was not possible to separate cause and effect, these results suggest that the parturient dairy cows did not prefer specific features of isolation in the individual maternity pen unless having a prolonged and potentially difficult calving. Based on this finding, we decided to proceed using the tall and narrow (A) individual maternity pen design in subsequent studies to allow a mixture of isolation and visual contact to the group.

4.2. Study 2 – calving site selection

Initially, the second study was planned as a pilot study to a larger experiment (Study 4), and therefore involved a relatively small sample size. Unexpectedly, as the pilot study progressed, observations indicated important aspects of calving site selection when housing parturient cows in groups. Results were subsequently published as a stand-alone paper in addition to serving as a pilot for Study 4.

Ten cows were housed in two separate group calving facilities (5 cows in each), each consisting of a group pen and six freely accessible adjacent individual pens (each 3 m x 4.5 m). All areas had sand bedding topped with 15 cm barley straw and self-filling water cups. Feed was only available in the group pen. The cows were allowed to choose freely where to calve in the group calving facility, and barn staff removed soiled straw after each calving. Each cow and calf pair was removed from the group calving facility 5-12 hours after calving.

In the first group, all cows calved in close proximity to each other (Figure 6a). The first cow calved in the group pen at the spot where her amniotic sac broke (grey circle, Figure 6a). All four subsequent calvings, and ruptures of amniotic sacs, happened within a radius of one cow-length from where the first cow calved (black cows, Figure 6a). In the second group, the first cow also calved where her amniotic sac had broken in the group pen (grey cow, Figure 6b), but after a thorough cleaning of the calving site (removal of all soiled straw and sand), the next cow calved inside an individual pen where her amniotic sac had broken (grey circle, Figure 6b). The following three cows calved within one cow-length from the second calving in the individual pen, where their amniotic sacs also broke (black cows, Figure 6b). Overall, seven out of 10 cows calved where a previous cow had calved and did, therefore, not appear to select calving sites randomly. The influence from a previous calving may potentially be explained by attracting components from birth fluids in the bedding.

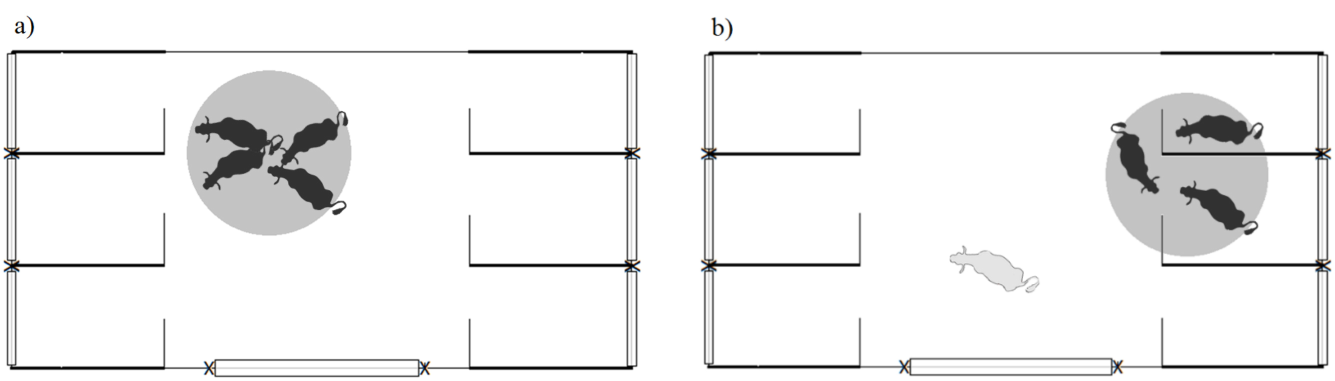

Figure 6. Overview of calvings in the two groups of 5 cows, representing a) the first group; and b) the second group. The light grey cow was the first to calve in the second group (see text for details), (Figure from Rørvang et al., 2017, Chapter 5.2.).

The finding of a potential attracting effect of birth fluids led to a reconstruction of the experimental procedures for Study 4, as the cleaning procedures needed adjustment to ensure that potential effects of birth fluid attraction were limited. The results also provided the rationale for developing a test to explore the olfactory capacities of cattle (Study 3).

4.3. Study 3 – olfactory investigation in cattle

Based on the unexpected but important finding of Study 2, olfactory capacities of cattle were given more attention than originally planned for the work underlying this thesis. The third study was, therefore, developed to explore olfaction in cattle – a test aiming to determine olfactory investigation of cattle. The development of the experimental design of Study 3 relied on test designs originally developed for rodents as none such tests had been done on larger mammals. Based on the theory behind the rodent tests, a test situation for cattle was adapted in order to elucidate which odours cattle are capable to detect. The original Habituation/Dishabituation Test, relies on the animals´ motivation to investigate new odours (Yang and Crawley, 2009). In the present case, the Habituation/Dishabituation Test was used in combination with an olfactory preference testing procedure (Witt et al., 2009) to determine which odours the cows could detect but also which odours evoked the most interest (Saraiva et al., 2016). This approach of combining tests is unusual, but a pilot study indicated that cows habituated rapidly to the odours presented and to the test situation. Therefore, the Habituation/Dishabituation Test was chosen to determine what complex odours cows were able to detect, and subsequently first odour presentations from the Habituation/Dishabituation Test were used to compare the level of interest. Interest was quantified as sniffing duration (def. the cows' muzzle less than the length of a cows' muzzle away from the odour sample), which was also the response variable used in the rodent studies (e.g. Corona-Samano et al., 2016).

Twenty-three parturient cows and heifers participated in the study. The test situation was adapted to the environment of cattle by presenting the odour in a test bucket in the home environment (Figure 7) instead of placing the animal with the odours in a confined space, as is usually done in the rodent tests. Tests were conducted using three different compounds being coffee and orange juice representing complex odours and tap water representing a presumably neutral odour (Witt et al., 2009). All were chosen based on accessibility, price, possibility for standardization, and because they were presumably unknown to the animals in the study. Each cow or heifer was tested in her home environment with each odour being presented twice in a row for 2 minutes with inter-trial and inter-odour pauses of 2 minutes. A balanced odour order presentation scheme was applied to ensure that all odour order combinations were represented. Each cow or heifer was randomly assigned to a specific presentation order of the three odours.

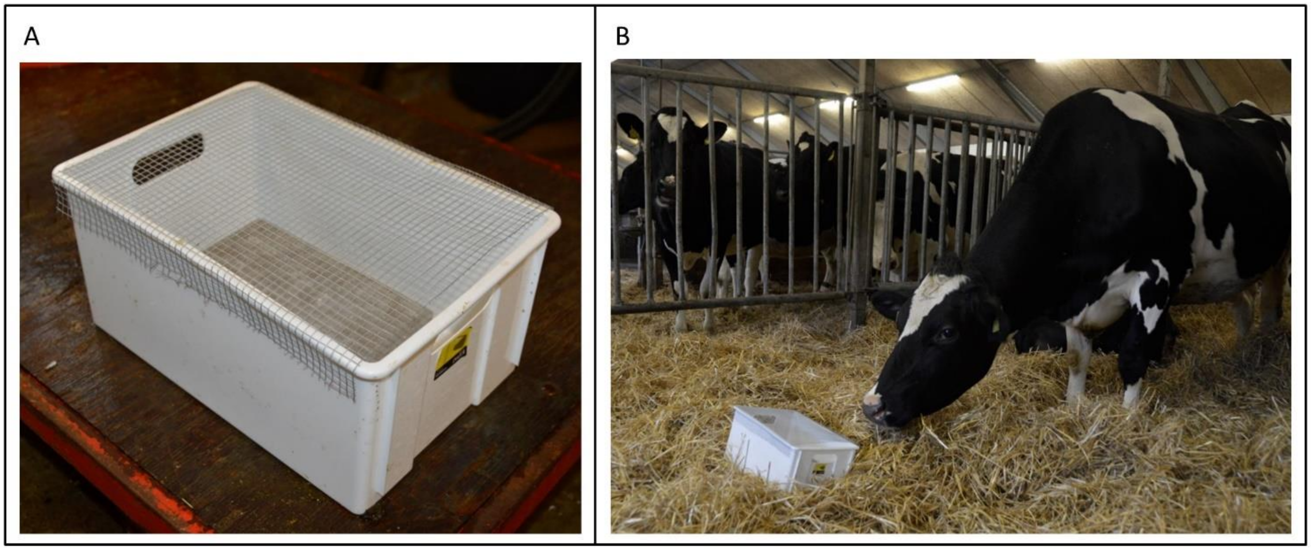

Figure 7. Test bucket (A) and test situation (B) from the Habituation/Dishabituation Test adapted for cattle (pictures adapted from Rørvang et al., 2017, Chapter 5.3.).

All cows and heifers sniffed an odour significantly less when presented the second time implying habituation to the odour. All cows and heifers also sniffed a new odour significantly more implying dishabituation. Cows and heifers sniffed coffee and orange juice significantly more than tap water, and coffee samples were sniffed significantly more than orange juice samples. Cows and heifers did not differ in this behaviour.

The results showed that cows and heifers were able to detect and distinguish between different complex odours and showed elevated interest for one specific odour. These findings emphasized the importance of taking olfactory capacities of cattle into consideration as olfaction may both facilitate and impede motivations of parturient cows. Hence, olfaction was given more focus in the remaining studies.

4.4. Study 4 – a motivation-based calving facility

The collective results from Studies 1-3 suggested that many factors might affect the behaviour of parturient cows, when kept in a group calving environment. Primarily, Study 4 aimed to test a motivation-based calving facility designed to facilitate the movement of parturient cows into individual maternity pens based on pre-partum motivations. Additionally, Study 4, investigated if social factors (dominance relations and presence of newborn calves) influenced pre-partum separation behaviour of dairy cows when kept in a motivation-based group calving facility.

For this study, special individual maternity pens were designed to allow parturient cows visual and spatial separation from the group by means of a specially designed gate (Figure 8). The gate was designed to allow a cow to move away from the rest of the group into an individual maternity pen – when motivated to do so – by providing her with a confined and shielded maternity pen which only one cow could enter at a time (thereby the name: *the motivation-based calving facility*). Groups of parturient dairy cows were housed in group calving facilities allowing access to individual maternity pens with the gates installed, permitting either free cow traffic in and out the pens (the gates to the pens were kept permanently open, Figure 8A) or access for only one cow at a time (functional/closed gate, Figure 8B).

Figure 8. Treatments in Study 4: A) The gate to the individual maternity pens was kept permanently open; and B) functional gate allowing only one cow access to the individual maternity pen at a time (seen from inside the individual maternity pens)(pictures adapted from Rørvang et al., 2018, Chapter 5.4.).

In total, 13 groups of six cows were housed in these facilities with either open gates or functional/closed gates. Cows were trained prior to calving to use either of the two treatments (i.e. permanently open gates or functional/closed gates, depending on which treatment they were assigned to) and all cows complied with a specific learning criterion. Social dominance was assessed during the last 12 h prior to calving. Sixty-six dairy cows were included in the study and 34 of these calved in an individual maternity pen regardless of treatment. Contrary to the hypothesis, the functional gates designed to aid separation, did not facilitate the use of the individual maternity pens. Although not significant, a logistic regression model showed that having a functional gate tended to affect the odds of calving inside an individual maternity pen negatively. The model also showed that social dominance within the group at the time of calving had a significant positive effect on the odds of calving in an individual maternity pen. Contrarily, the presence of an alien calf in the group pen within 8 h from calving lowered these odds. Hence, factors arising from being housed in a social group influenced the behaviour of the cows around the time of calving. These results collectively emphasized that many factors are at play in the control and expression of pre-partum maternal behaviour of dairy cows and that a deeper understanding of the biology underlying these factors would be advantageous.

4.5. Study 5 – understanding pre-partum maternal behaviour of cattle by use of inter-species comparison

The four preceding experimental studies yielded new, and to some extent unexpected results, jointly highlighting the need for a deeper and broader understanding of the causation of pre-partum maternal behaviour of cattle. A review of the very limited body of available literature on feral cattle was conducted, and the findings were compared to pasture-kept and indoor-housed cattle, as well as other ungulate species, in order to elucidate similarities and dissimilarities.

One hundred and twenty-eight papers, including more than 40 cattle studies, were included and the main findings were:

Maternal pre-partum behaviour varies among species, but the final proximate goal of ungulate mothers appears to be the same: locate an appropriate birth site to ensure and safeguard a calm parturition and optimal surroundings for post-partum maternal behaviour by lowering the risk of predators, disturbances, and mistaken identity of offspring

Features of birth sites vary among species, and depend largely on the environment. Ungulate females display a considerable ability to adapt to their surroundings; hence, the previous strict dichotomy of classifying a species as either 'hider' or 'follower' may be overly simplistic.

For commercial indoor-housed dairy cows, confinement and high stocking density offer limited possibility for birth-site selection behaviour. This poses a risk of agonistic behaviour, disturbances, and mis-mothering, as well as exposure to olfactory cues influencing pre- as well as post-partum maternal behaviour. Additionally, pre-partum dairy cows seem particularly affected by olfactory cues (as compared to e.g. sheep) as they are attracted to birth fluids already before calving. Dairy cows are exposed to several factors (i.e. olfactory cues, social dominance, presence of newborn calves and limited access to cover or isolation opportunities), which may thwart their maternal motivation and influence their behaviour and welfare. Providing an environment that allow dairy cows to perform the pre-partum maternal behaviour for which they are motivated may ensure an efficient calving without complications and safeguard productivity and animal welfare.

5. Studies 1-5

The results obtained from the five studies are presented in this chapter as five published papers. Studies correspond to papers in the follow order:

Study 1:

Published paper: Rørvang, M. V., Herskin, M. S., Jensen, M. B., 2017. Cows with prolonged calving seek additional isolation. Journal of Dairy Science 100: 2967-2975. DOI: 10.3168/jds.2016-11989.

Study 2:

Published paper: Rørvang, M. V., Nielsen, B. L., Herskin, M. S., Jensen, M. B., 2017. Short communication: Calving site selection of multiparous, group-housed dairy cows is influenced by site of a previous calving. Journal of Dairy Science 100: 1467-1471. DOI: 10.3168/jds.2016-11681.

Study 3:

Published paper: Rørvang, M. V., Jensen, M. B., Nielsen, B. L., 2017. Development of test for determining olfactory investigation of complex odours in cattle. Applied Animal Behaviour Science 196: 84-90. DOI: 10.1016/j.applanim.2017.07.008.

Study 4:

Published paper: Rørvang, M. V., Herskin, M. S., Jensen, M. B., 2018. The motivation-based calving facility: Social and cognitive factors influence isolation seeking behaviour of Holstein dairy cows at calving. PLOS One 13 (1): E0191128. DOI: 10.1371/journal.pone.0191128.

Study 5:

Published paper: Rørvang, M. V., Herskin, M. S., Nielsen, B. L., Jensen, M. B., 2018. Pre-partum maternal behavior of domesticated cattle: *a comparison with managed, feral, and wild ungulates*. Frontiers in Veterinary Sciences 5: article 45. DOI: 10.3389/fvets.2018.00045
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5.1. Study 1

Paper 1:

Cows with prolonged calving seek additional isolation.

Rørvang, M. V., Herskin, M. S. and Jensen, M. B.

Published in Journal of Dairy Science 2017 100: 2967-2975.
DOI: 10.3168/jds.2016-11989.

J. Dairy Sci. 100:2967–2975
https://doi.org/10.3168/jds.2016-11989

Dairy cows with prolonged calving seek additional isolation

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ABSTRACT

In modern calving facilities, dairy cows either calve in a group pen or are moved to a separate individual pen when calving is imminent. In practice, cows are often moved too close to calving, which poses a health risk to cow and calf. Thus, a need exists for new calving facility designs and management practices that better align with the motivations of the cow. This study examined dairy cow preferences for individual calving pens by offering 3 different levels of isolation (tall and narrow, low and wide, and tall and wide) by analyzing the association between precalving behavior, choice of degree of isolation, and the progress of calving. The hypotheses were that cows would prefer the highest level of isolation when giving birth, and that calving in a high level of isolation would be associated with less restlessness and a shorter calving duration. Contrary to these hypotheses, no specific preference between degrees of isolation or difference in calving behavior in the different calving pens was found. However, cows experiencing a longer calving duration chose to calve in the most secluded calving pen (tall and wide). These results cannot determine cause and effect, but may suggest that interactions between motivation for isolation seeking and calving behavior exist.
Key words: isolation seeking, maternal behavior, calving site selection, cow preferences

INTRODUCTION

The transition from dry to lactating is a high-risk period for dairy cows. Calving itself places high demands physically but is also painful (Maineau and Manteca, 2011), and the transition to lactation is associated with a high risk of disease (Atkinson, 2016). In recent years, farm size has increased (Barkema et al., 2015) and the high number of calvings require surveillance, which can be challenging in modern dairy production, where it is recommended that cows calve in separate calving facilities that they are introduced into when calving is imminent (Marcussen and Laursen, 2007; Rushen et al., 2008). The use of individual calving pens is recommended based on the known preference of the preparturient cow to seek isolation as calving approaches (Proudfoot et al., 2014a,b); in some countries, calving in individual pens has been adopted by law (Ministry of Environment and Food of Denmark, 2014) to ensure an undisturbed parturition in a clean pen. Furthermore, allowing the cow to separate herself from the group when motivated to do so before calving may also contribute to good animal welfare. In practice, however, the management of preparturient cows is not simple. If cows are moved based on predicted calving date they may occupy the individual calving pen for several days, but when cows are moved late in the first stage of calving this may prolong the second stage of calving (Proudfoot et al., 2013), which may lead to uterine infections, calving complications, and other production related diseases. Hence, it is a management challenge to introduce cows to a clean, secluded, and undisturbed calving pen in time, and this requires intensive surveillance of the cows. Farmers could potentially benefit from on-line monitoring (e.g., accelerometers or rumination collars) of behavioral and physical changes occurring before calving. However, reduced rumination (Schirmann et al., 2013), increased number of lying bouts (Miedema et al., 2011; Jensen, 2012), and reduced vaginal temperature (Ouellet et al., 2016) did not occur until the last few hours before calving, and moving of the cow based on these signs may be too late in practical farming. An alternative option is the development of new cow motivation-based systems, where cows voluntarily move into individual calving pens when motivated to separate from the group. To develop such a system, knowledge of the cow's preferences for calving pen design is a prerequisite to stimulate isolation from the herd before calving. Campler et al. (2014) showed that cows avoid calving on a rubber surface as compared with sand, and Proudfoot et al. (2014b) found that cows prefer to be secluded at calving. Thus, a certain degree of isolation and provision of a soft and non-slip surface in individual calving pens may increase the motivation of dairy cows to enter.

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However, at present, the preferred degree of isolation is not known. In addition, potential effects of being able to isolate before giving birth on the calving behavior of dairy cows have not been examined.

By providing cows access to individual calving pens with soft bedding and offering different levels of isolation, we investigated dairy cows' preference regarding degree of isolation at calving. Additionally, we investigated precalving behavior, use of the opportunity to isolate, and the progress of calving in relation to the final choice of degree of isolation. We hypothesized that cows would prefer the highest degree of isolation when giving birth, as this isolation opportunity would seclude the cow the most while still offering minimal opportunity for visual and tactile contact to cows in the group pen. Moreover, we hypothesized that calving in the most secluded pen would be associated with less restlessness and a shorter duration of calving than when the calves were born in pens with lower degrees of isolation.

MATERIALS AND METHODS

The experiment took place at the Danish Cattle Research facility at Aarhus University (Foulum, Denmark) between September 2014 and March 2015.

Animals, Housing, and Management

Initially, the study included 49 multiparous Danish Holstein cows, which was the number of cows calving in the resident barn during the study period. Prior to calving, these cows were allocated to 9 groups of 5 to 6 cows according to expected calving date [insemination date + 280 d (Danish mean number of gestation days in Holstein cows; Marcussen and Laursen, 2007)] to reach approximately the same number of days between successive calvings within groups. All cows were of the resident herd at Aarhus University, AU-Foulum, Tjele, Denmark, and were group housed in a cubicle barn before the experimental start, and thus familiar with other cows in their group. A block consisted of 3 groups, each moved to the group pen of their experimental section (Figure 1) approximately 2 wk before the first expected calving in that group. Cows were thus blocked according to expected calving date. All group pens had deep straw bedding, and 6 individual feed bins (bin width = 75 cm, Jyden, Vemb, Denmark) and 2 automatic self-filling water cups (model 2177–4010, Jyden).

Each group pen was connected to 2 maternity units (Figure 1 and 2), each of which was subdivided into 3 interconnected individual calving pens between which a cow could move freely via a rubber-floored alley [Kura Flex, Kraiburg, Tittmoning, Germany; a 19-mm-thick, pebbled-surface rubber mat with 5-mm studs on the lower side (24 mm including 5-mm studs)]. All individual calving pens had 1.3-m high sides made from tubular metal bars on 3 sides and a feed trough (model 1318–8210, Jyden) on the fourth (facing the outer wall). A 1.8-m-high light gray, plastic barrier (low-density polyethylene compound, 10 mm thick) covered 2 pen sides. The third pen side, facing the group pen, was partly covered by either 50% (height × width) a tall and narrow, 1.8 × 1.5 m barrier (A); a low and wide, 1.0 × 2.5 m barrier (B); or covered 75% by a tall and wide, 1.8 × 2.5 m barrier (C). This resulted in 3 different individual calving pens in each maternity unit (Figure 2). These dimensions were chosen to compare effects of height or width or both. The uncovered part of the third side of each individual calving pen allowed some visual and tactile contact with animals in the group pen. The allocation of barriers A, B, or C to each individual calving pen was balanced to account for possible side bias of the cows (allocation indicated in Figure 1). In all individual calving pens, 30 cm of deep barley straw bedding covered the floor facing the group pen (3 × 3 m), and a rubber mat covered the alley part facing the outer walls (1.5 × 9 m; Figure 1). This arrangement ensured that cows did not lie in the alley between the 3 individual calving pens. In each maternity unit, water was available for ad libitum intake from 2 self-filling water cups (identical to the ones in the group pen).

Clean straw was added daily to the group pens by the barn staff. In the maternity unit, fresh straw was added daily after removal of manure and any soiled straw, and the rubber alley was cleaned daily. Before introduction of a new cow, the maternity unit was cleaned out (i.e., all straw and manure removed) and fresh straw added. Bedding quality of each individual calving pen was evaluated daily before manure removal and scored according to a 5-point scale developed prior to the experiment: (0) dry, no feces or urine; (1) moist, less than 3 spots with feces; (2) slightly wet, some dry straw and more than 2 spots with feces; (3) wet, mainly wet straw and feces; (4) very wet, only wet straw, feces and urine spread over the whole pen (median: 1, range: 0–4). Barn staff used this measure as a reference to minimize differences in bedding quality between individual calving pens and maternity units.

Within each experimental section, cows were moved individually to one of the 2 maternity units at least 3 d before expected calving (mean ± SD: 5.8 ± 2.7 d) or if signs of imminent calving (i.e., enlarged udder, soft ligaments, attentive toward abdomen or udder, licking the udder) appeared before this time. After calving, each cow and her calf remained in the maternity unit for 72 to 96 h.

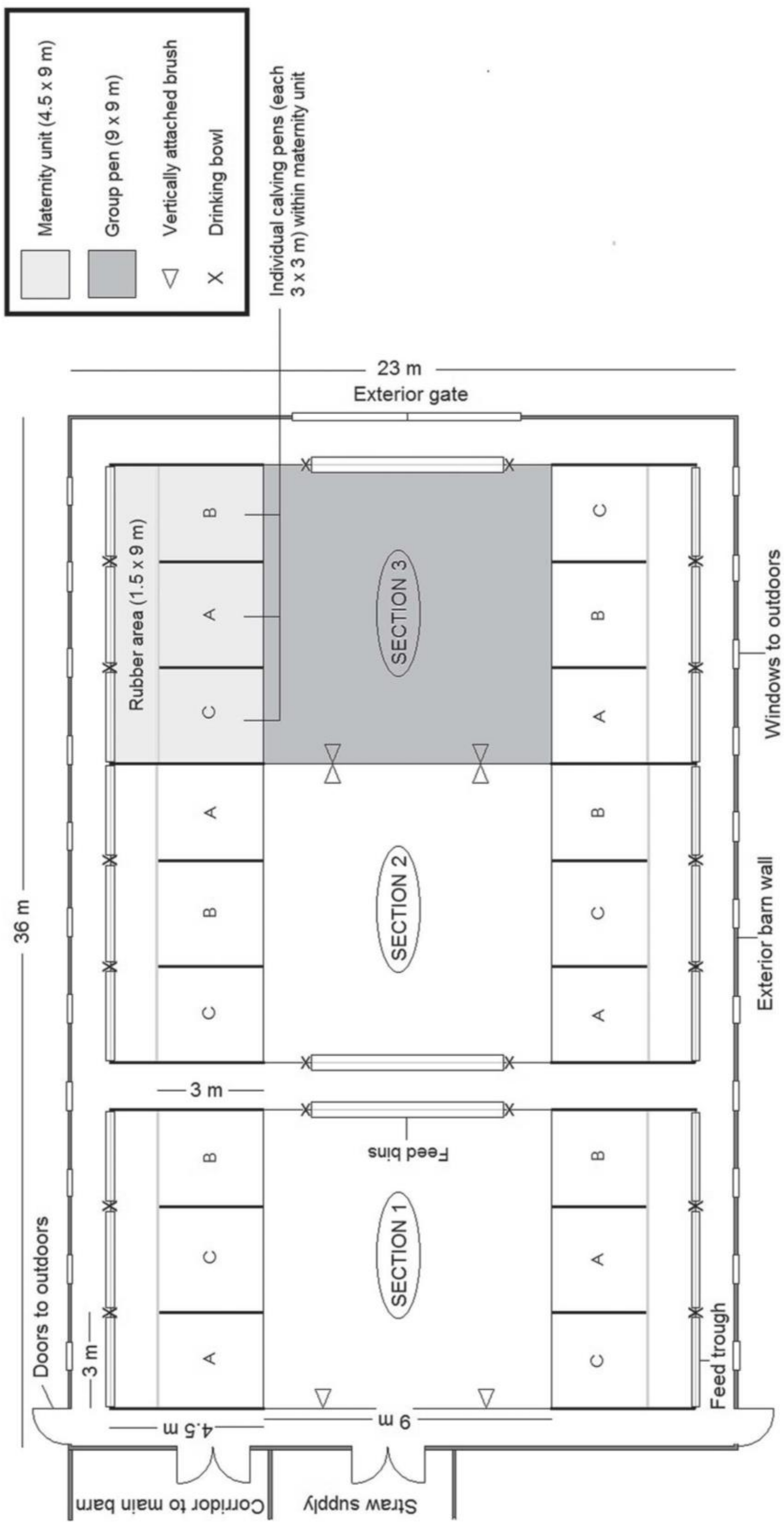

Figure 1. Floor plan of the experimental barn, with top view of the 3 experimental sections; position of vertically attached brushes, drinking bowls, and feed bins are shown. Thick lines around maternity units and between individual calving pens represent covered sides. The maternity unit consists of the rubber area connecting the 3 individual calving pens, allowing the cow to move freely between all areas. Gray lines separating rubber area from the individual calving pens represents the transition from rubber flooring to straw flooring. A, B, and C indicate the type of seclusion offered by a barrier placed on the pen side toward the group pen; A = tall and narrow (1.8 × 1.5 m); B = low and wide (1.0 × 2.5 m); and C = tall and wide (1.8 × 2.5 m). These barrier sides are represented by the thin black line dividing individual calving pens and group pen.

Figure 2. The 3 types of isolation offered by barriers A, B, and C separately (seen from inside the maternity unit from the rubber area) placed in the individual calving pens facing the group pen. The uncovered part of the pen side offered the cows inside the maternity pen an opportunity to have visual and tactile contact to the group members in the group pen. Color version available online.

Delivery of a calf was assisted if the calf was not born within 4 h after the appearance of the amniotic sac. Calvings were supervised using video surveillance cameras (described below), enabling the barn staff to observe the appearance of the amniotic sac or the legs of a calf without disturbing the cows.

Cows were excluded from the experiment if they calved in the group pen (n = 3), if they spent less than 3 d in the maternity unit before calving (n = 1), if they had to be moved between barn sections due to lack of space (n = 2), if they were diagnosed by the herd veterinarian with milk fever, mastitis, or retained placenta within 24 h of calving (n = 4), or if their calving was assisted (n = 2), as all of these situations could have influenced the choice of the cow. Thus, 37 cows were included in the analysis; 21 cows entering their second parity, 14 entering their third parity, and 2 older cows.

Behavioral Observations and Variables

Behavior was monitored via black-and-white digital video cameras (model TVCCD-624, Monacor, Bremen, Germany) mounted 3.5 m above each individual calving pen. An experienced technician monitored and stored the video recordings and an experienced observer collected all behavioral data. To avoid bias, the observer collecting the data was unable to distinguish between the different individual calving pens (i.e., A, B, or C) from the video recordings. During the 24 h before birth of the calf, cow behavior was recorded continuously according to an ethogram (Table 1). To evaluate the cow's choice of calving pen at the moment of calving, the location and orientation of the cow inside the calving pen was recorded when the hips of the calf were fully expelled. This measure adds information about the chosen level of isolation, as well as to what extent the visual or tactile contact with the group was favored or not (Table 1). If half of a cow's body was in one half of a calving pen (as defined in Table 1), the placement of the head of the cow determined the outcome. In addition, the location, posture, and orientation of the cow were determined (Table 1). The individual calving pen, where the calf was born, was scored as the final choice of calving site. As contractions are among the early signs of second stage labor (Noakes et al., 2001), the second stage of labor was, per definition, initiated at the first visible rhythmical abdominal contraction bout (while the cow was lying) and finalized when the hips of the calf were fully expelled (Proudfoot et al., 2013; Table 1). The frequency of lying bouts, number of contractions, and number of times the cow moved between individual calving pens were summarized.

Cows were gait-scored when entering and exiting the experimental sections. The scoring was done by 2 experienced observers according to a 5-point scoring system (Thomsen et al., 2008). No cows were scored as obviously lame (above score 3) and the median score was 1 (range = 1–3). All cows were weighed by use of an automatic scale (Danvægt, Hinnerup, Denmark). The cows weighed on average 673 (SD = 59) kg when entering the experiment and 646 (SD = 53) kg when leaving.

Statistical Analysis

Based on Shapiro-Wilk normality test and visual assessment of histograms, normality of the recorded variables could not be assumed as data were skewed. All variables were thus analyzed by nonparametric tests using the base system of the statistical software R (R version 3.1.2.; R Core Team, 2014) and results evaluated using a significance level of 5%.

Behavior 24 Hours Before Calving

Preference for calving site was analyzed by Chi-squared test. When analyzing whether the duration of the second stage of labor or time spent inside the final choice of calving pen were affected by the final choice of individual calving pen, a Kruskal-Wallis test was used (Siegel and Castellan, 1988). A post hoc analysis was conducted using a Wilcoxon rank sum test to compare cows choosing either individual calving pen A or B (the lowest level of isolation, 50%) to cows choosing individual calving pen C (the highest level of isolation, 75%). To analyze if the choice of individual calving pen affected the orientation of the cow at calving, the association of these categorical variables was analyzed by a Chi-squared test. When analyzing if cow orientation at calving affected the duration of the second stage of labor, a Wilcoxon rank sum test was used.

Table 1. Ethogram of recorded location, posture, and behavior of the dairy cows during 24 h before calving and at the time of calving

Item	Description
Prior to calving (24 h)	
Location[1]	
In left individual calving pen	>50% of the body placed in the left individual calving pen
In middle individual calving pen	>50% of the body placed in the middle individual calving pen
In right individual calving pen	>50% of the body placed in the right individual calving pen
Posture	
Lying brisket[2]	Lying on sternum, head may be rested or raised
Lying flat[2]	Lying flat on side (no rest on sternum), head may be rested or raised
Upright[3]	Body supported by 4 legs, standing or walking
Calving event	
Abdominal contractions[2]	Uterus and abdominal muscles contract and release repeatedly. This is visible as rhythmical movements of the abdomen, or abdomen and hind part of the cow. Each contraction bout consists of at least 3 successive contractions.
At calving	
Calving event	
Calving[4]	The hips of the calf are fully expelled from the dam
Location[1]	
In left individual calving pen	>50% of the body placed in the left individual calving pen
In middle individual calving pen	>50% of the body placed in the middle individual calving pen
In right individual calving pen	>50% of the body placed in the right individual calving pen
Orientation (within pen)	
Toward group	The front of the body is directed toward the group pen
Away from group	The front of the body is directed away from the group pen
Position (within pen)[5]	
Shielded	The majority of the body of the cow is positioned in the side of the calving pen where the barrier is placed on the pen side toward the group pen
Not shielded	The majority of the body of the cow is positioned in the side of the calving pen where a part of the pen's side toward the group pen is uncovered
Posture	
Lying brisket[2]	Lying on sternum, head may be rested or raised
Lying flat[2]	Lying flat on side (no rest on sternum), head may be rested or raised
Upright[3]	Body supported by four legs, standing or walking

[1]Each of the 3 individual calving areas had a different level of seclusion in a different order. "Left," "middle" and "right" refer to left, middle and right from the video recordings to blind the observer from the treatment (A, B or C).

[2]Modified from Jensen, 2012.

[3]From Jensen, 2011.

[4]Modified from Proudfoot et al., 2013.

[5]The calving pen was considered as 2 equally sized halves (each 1.5 m × 3 m).

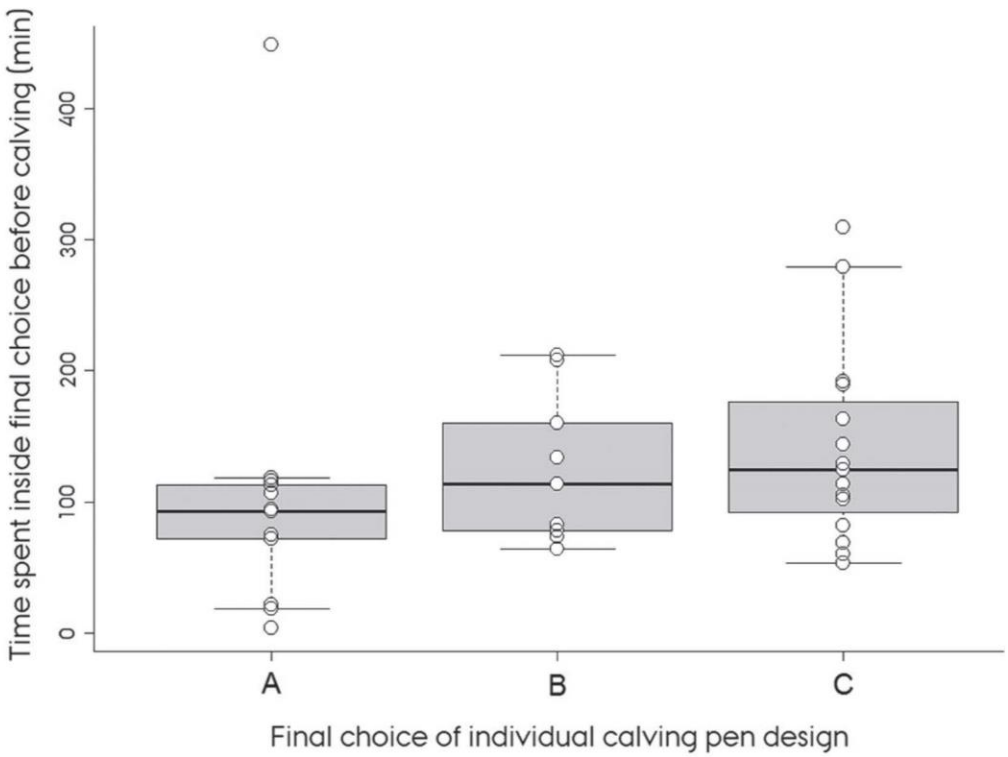

Figure 3. Box plot of the duration of time (min) spent in the individual calving pen finally chosen (from entering an individual calving pen the last time until calving) for the 3 individual calving pens A (tall and narrow), B (low and wide), and C (tall and wide) during the final 24 h before calving. Whiskers indicate maximum and minimum, box represents the 25 and 75% quartiles, and the thick black line indicates the median.

Effects of Changing Calving Area During the Second Stage of Labor

Twelve cows changed individual calving pen after the first visible abdominal contractions (definition in Table 1). These cows were compared with the 25 cows not changing individual calving pen after the first abdominal contractions in a post hoc analysis. A Wilcoxon rank sum test (two-sided) was used to compare the 2 groups with respect to the variables: duration of second stage of labor, number of lying bouts, number of contractions, and number of individual calving pen changes.

RESULTS

Behavior During 24 Hours Before Calving

The cows showed no significant preference for a specific individual calving pen (number of cows giving birth in individual calving pen A, B, and C were 13, 9, and 15, respectively; $\chi^2 = 1.51$; df = 2; $P = 0.38$). No difference between the 3 individual calving pens was found for the duration of time spent in the individual calving pen where the calf was born ($\chi^2 = 3.50$; df = 2; $P = 0.17$; Figure 3). We found no effect of final choice on the duration of the second stage of labor [($\chi^2 = 0.53$; df = 2; $P = 0.77$; 108.44 (SD = 66) min].

We ran a post hoc analysis comparing cows choosing 1 of the 2 individual calving pens with the lowest level of isolation (A or B) with cows choosing the individual calving pen with the highest level of isolation (C). Results of the analysis showed that the cows choosing the higher level of isolation had a significantly longer duration of second stage labor (w = 44; $P = 0.047$; Figure 4). Twenty out of the 37 cows faced away from the group when calving (cows facing away/all cows: A = 7/11, B = 5/11, C = 8/15). For these cows, the particular individual calving pen (A, B or C) did not affect their orientation at calving ($\chi^2 = 2.20$; df = 2; $P = 0.33$). Furthermore, cow orientation did not affect the duration of the second stage of labor (w = 190; $P = 0.56$).

Effects of Changing Individual Calving Pen After Initiation of Contractions

Twelve cows were observed having contractions before entering the individual calving pen where the

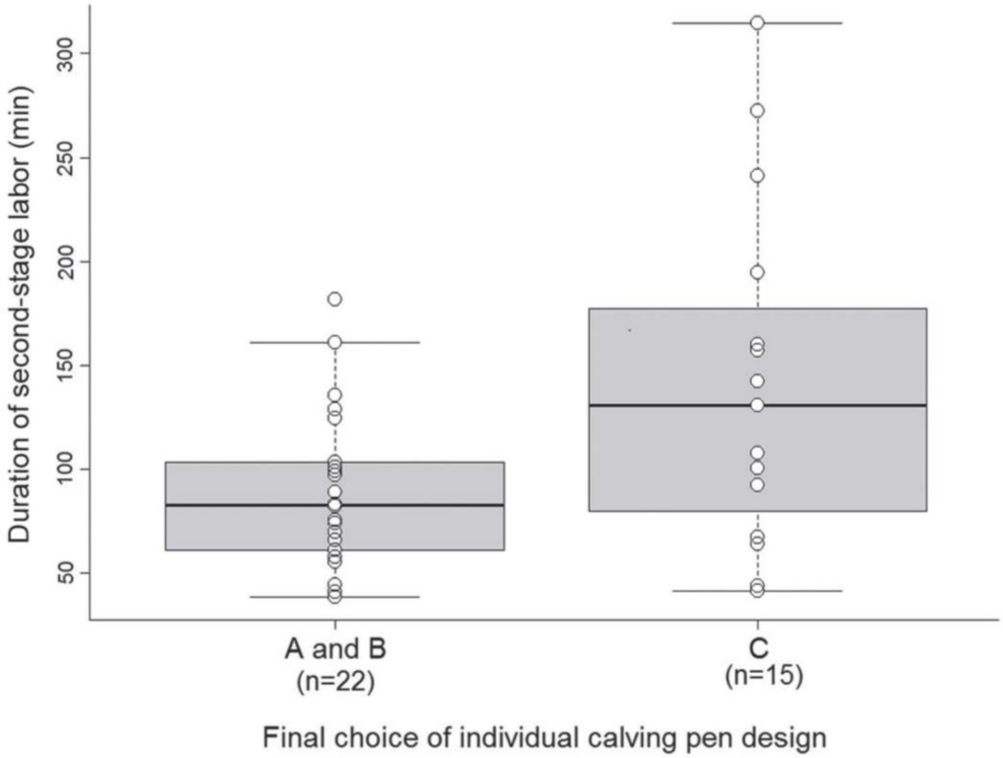

Figure 4. Box plot illustrating the post hoc analysis comparing the duration of the second stage of labor of cows choosing individual calving pen A or B (50% isolation) to cows choosing individual calving pen C (75% isolation). Whiskers indicate maximum and minimum, box represents the 25 and 75% quartiles, and the thick black line indicates the median.

calf was born (final choice), and these cows had significantly longer second stage labor compared with cows not changing individual calving pen after initiation of contractions (Table 2). These 12 cows did not change individual calving pen more often when calculated for the 24 h before calving, but had a significantly higher number of lying bouts and contractions (Table 2) when compared with cows not changing their preference during the second stage of labor. Moreover, 7 out of these 12 cows changed from individual calving pen A or B to C, whereas only 2 changed from A or C to B and 3 from B or C to A.

DISCUSSION

This study examined aspects of calving site selection in dairy cows by allowing preparturient cows to choose between individual calving pens offering different types (in term of height, width or both) and levels of isolation (in term of percentage coverage), and by examin-

Table 2. Calving duration (min), number of changes cows made between individual calving pens, number of lying bouts, and number of contractions during the last 24 h before calving[1]

	Cows changed individual calving pen during the second stage of labor [median (25–75% interquartile range)]			
Variable	Yes (n = 12)	No (n = 25)	w	P-value
Duration of second stage labor (min)	159.2 (102.9–206.1)	75.6 (61.2–100.6)	45.0	<0.001
Frequency of individual calving pen changes (no./24 h)	72.5 (58.0–83.5)	55.0 (33.0–82.0)	115.5	NS
Frequency of lying bouts (no./24 h)	42.5 (40.3–64.0)	30.0 (26.0–35.0)	50.5	<0.01
Frequency of contractions (no./24 h)	49.5 (32.3–62.3)	27.0 (21.0–35.0)	65.0	<0.01

[1]Data are shown for cows changing individual calving pen during the second stage of labor and cows not changing individual calving pen during the second stage of labor, respectively. Statistics from the Wilcoxon rank sum test are shown in column w with corresponding P-value. In the tests of these variables, smaller values of w are more significant.

ing possible consequences of these choices in terms of calving behavior. Based on data from 37 calvings, no preference for a specific individual calving pen design or for a specific isolation level (50 vs. 75%) could be found. Furthermore, no difference between calving behavior in the different pens was found. However, cows experiencing a longer calving duration (longer second stage labor) gave birth in the most isolated individual calving pen, and cows that changed individual calving pen after the initiation of the first rhythmical abdominal contractions (onset of second stage labor) had a longer calving duration, more contractions, and a higher number of lying bouts as compared with cows not changing pen after the onset of second stage labor. These results provide new information specifically useful for future design of calving pens for dairy cows and for the understanding of possible effects of being able to isolate before giving birth.

We found no preferences for a specific calving pen design (A, B, or C), and no preference for a specific level of isolation (A and B, or C) in the preparturient dairy cows. We hypothesized that most cows would calve in the individual calving pen with the highest level of isolation or the most secluded design, but the results did not confirm this. It is, however, possible that dairy cows show a preference for a high degree of isolation, but that the present design of the 3 different individual calving pen barriers were not perceived as being different by the cows. For instance, the relatively low variation in the degree of isolation (from 50 to 75%) or the short distance between the cows in the group pen and the individual calving pens may have caused this. Comparing the 3 different types of isolation, we did not find differences in periparturient behavior, nor did the 3 different types of isolation affect the orientation of the cows according to the group when calving, which may suggest that the cows did not perceive the individual calving pens as different and thus behaved alike in all cases. Under natural or seminatural conditions, cows move away from the group when calving is imminent (Lidfors et al., 1994), possibly implying that distance might play role in how cows perceive isolation. Sows have been shown to walk considerable distances to select a suitable place to give birth (Jensen, 1988, 1989), and free-ranging and wild sheep have also been shown to move away from the herd when lambing (free-ranging, Alexander at al., 1990; wild, reviewed in Dwyer and Lawrence, 2005). Another interpretation, however, could be that the seclusion provided by the 3 different calving pen designs all fulfilled the motivation for isolation. Future studies, manipulating other aspects of social isolation (e.g., the distance to neighboring cows, or the opportunity for olfactory or auditory isolation) are needed to clarify these points.

Unexpectedly, the present results did not support the hypothesis that calving in the individual calving pen with the highest level of isolation was associated with less restlessness and a shorter duration of calving. In fact, all cows changed calving pen often, which may imply restlessness within the last 24 h before calving. This may also reflect that cows (due to the interconnected calving pens and their common feed table) could be moving alongside the feed table while feeding and, as a consequence, change calving pens without fully entering the straw area. On the other hand, giving birth in a pen offering the highest level of isolation was associated with a longer calving duration, indicating that, even though no clear preference for birth site was shown, the calving pens were not perceived as being similar. Cows having the longest calving durations chose the highest level of isolation, possibly explained by an increased level of restlessness and increased discomfort during calving. It is difficult to determine when calving becomes difficult and when it becomes more painful for the cow; however, we speculate that a prolonged calving and exhaustion adds to this. Previously, a longer calving duration has been associated with more frequent uterine contractions (Barrier et al., 2012), implying that dystonic cows are exposed to a higher level of pain from the increased number of contractions during calving (reviewed in Rushen et al., 2008), potentially leading to higher levels of restless behavior (Maineau and Manteca, 2011). Recently, a tendency of compromised cows to choose a secluded area after calving has been shown for sick cows (Proudfoot et al., 2014a), which may have been motivated by pain or discomfort. However, this possibility warrants further study involving behavioral as well as physiological and metabolic measures.

The present findings of interactions between periparturient behavior of dairy cows and their birth site selection need further investigation to clarify cause and effect. However, from a practical point of view, the present knowledge that indoor-housed preparturient cows did not show a clear choice between the 3 different types and levels of isolation, as well as the described interaction between long calving duration and the choice of the high degree of isolation, might suggest that providing dairy cows with calving pens providing 75% of isolation might be the best of the present options. What still remains uncertain, and needs further clarification before motivation-based calving facilities can be designed, is whether isolating behind a barrier is sufficiently attractive in order for a preparturient cow to move away from the group and isolate. As suggested, further studies are needed to understand what mechanisms drive isolation-seeking behavior in preparturient dairy cows.

CONCLUSIONS

Contrary to the hypotheses, we found no preference for specific types or levels of isolation and no difference in calving behavior between dairy cows giving birth in the individual calving pens with different seclusion designs. Cows experiencing a longer calving duration gave birth in the most secluded individual calving pen (75% isolation), and cows that changed individual calving pen after initiation of the first rhythmical abdominal contractions had a longer calving duration, more contractions, and more lying bouts. These results cannot determine cause and effect, but suggest that interactions between motivation for isolation seeking and calving behavior exist. Before new management solutions for calving cows based on isolation-seeking behavior of the dam can be developed, further studies of the mechanisms underlying motivational changes in the hours before giving birth are needed.

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5.2. Study 2

Paper 2:

Short communication: Calving site selection of multiparous, group-housed dairy cows is influenced by site of a previous calving.

Rørvang, M. V., Nielsen, B. L., Herskin, M. S., Jensen, M. B.

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Short communication: Calving site selection of multiparous, group-housed dairy cows is influenced by site of a previous calving

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ABSTRACT

A calving cow and her newborn calf appear to have an attracting effect on periparturient cows, which may potentially influence the functionality of future motivation-based calving pen designs. In this pilot study we examined whether calving site selection of group-housed Holstein dairy cows was affected by the site of a previous calving. Ten multiparous cows moved to 1 of 2 group pens 11 (range = 4–27) d before calving were included. Each pen consisted of an open area (9 × 9 m) connected to 6 secluded areas (4.5 × 3 m each), where cows could move freely between all areas. Time of calving, location of the breaking of the amniotic sac, as well as the place of birth were recorded. In all but 1 case cows calved within a distance of 1 cow length from where the previous calving took place, suggesting that the cows did not select calving site at random. These preliminary observations indicate that choice of calving site may be affected by the site of a previous calving, potentially explained by the presence of amniotic fluids.
Key words: parturition, maternal behavior, cow preference, calving site selection

Short Communication

The use of motivation-based calving facilities may potentially increase cow welfare in modern dairy production. Based on the preferences of calving cows to seek isolation from the group (Proudfoot et al., 2014) and the finding that moving a calving cow to an individual pen late in the birth process may lead to prolonged calving (Proudfoot et al., 2013), a motivation-based calving facility may consist of a group pen joined to single calving pens into which cows can withdraw when calving is imminent (Rørvang and Jensen, 2016). However, calving site selection is likely to be influenced by factors other than motivation for isolation, knowledge of which may be needed to ensure the functionality of motivation-based calving pen designs. Campler et al. (2014) indicated that cows prefer to calve on sand over rubber, most likely because sand better supports the frequent changes in posture (Bak et al., 2016) seen during the final 6 h before calving (Miedema et al., 2011; Jensen, 2012). In lactating dairy cows various factors, such as depth of straw bedding (Tucker et al., 2009), dryness of bedding (Reich et al., 2010), and design of the lying area (Abade et al., 2015), influence where and for how long the cows prefer to lie down. At present, similar knowledge is not available for cows approaching calving. Edwards (1983) showed that calving cows, as well as newborn calves, seem to have an attracting effect on cows in late pregnancy, which may be mediated by the presence of amniotic fluid, known to contain olfactory cues and to facilitate maternal behavior in many mammalian species [e.g., rodents (Kristal, 1991), sheep (Lévy et al., 1983), dogs (Dunbar et al., 1981), cats, horses, pigs, and goats (Fabre-Nys et al., 1993)]. In addition to the presence of calving cows and calves, periparturient cows have been shown to be attracted to amniotic fluids (Pinheiro Machado et al., 1997). However, irrespective of the underlying mechanisms, knowledge about factors influencing calving site selection is important for the successful development of motivation-based calving facilities. Thus, the objective of the present study was to investigate whether calving site selection in group-housed dairy cows was influenced by the site of a previous calving.

The study took place at the Danish Cattle Research facility at AU Foulum (Aarhus University, Tjele, Denmark) in September 2015 and was originally planned as a pilot study before a larger experiment on calving behavior of dairy cows. Twelve multiparous Danish Holstein dairy cows (mean parity ± SD: 2.2 ± 0.4) participated in the study. Prior to calving, the cows were allocated to 1 of 2 groups (I and II) with approximately equal dispersion of expected calving dates (average of 2 d between each calving). Each group was moved to a calving area in the experimental barn (calving areas 1 and 2 in Figure 1) approximately 1 wk before the first expected calving date in the group. Each calving area

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included an open area (9 × 9 m) with 6 individual feed bins (each 75 cm wide; Jyden, Vemb, Denmark) and 6 adjoining secluded areas (4.5 × 3 m each). Each secluded area was surrounded by 3 solid 1.8-m walls, with one wall having the entrance (1.5 m wide) from the open area. The fourth side was a metal gate with a feed bunk (model: 1318–8210; Jyden). The freely accessible but secluded areas offered the cows an opportunity to calve more isolated than when within the group pen area. The whole calving area was bedded with 15 cm of sand (Kosand brand; Dansand, Brædstrup, Denmark; mean grain size = 0.322 mm) topped with approximately 15 cm of barley straw bedding. Two water bowls (model: 2177–4010; Jyden) were located in the open area and one in each of the secluded areas.

All cows had ad libitum access to a TMR with a forage-to-concentrate ratio of 80:20 (DM basis). Feed was provided twice daily, between 0930 and 1200 h and between 1730 and 1800 h. After calving, the cow and calf were removed from the barn within 12 h after calving.

All areas had feces removed and straw added on a daily basis (between 0930 and 1200 h) to minimize differences in bedding quality between the areas. After the cow-calf pair had been removed, a light cleaning of the calving area was carried out, which included removal of blood, wet straw, and remains of the amniotic sac. In addition, a thorough cleaning was carried out after the first calving in group II, where all soiled sand and straw in a radius of 1 m of where the amniotic sac broke was removed and replaced with clean sand and straw. This was done to test possible effects on the calving site selection of the subsequent cow.

Within group, all 6 cows were introduced to the calving area at the same time and allowed 24 h to acclimatize. Two cows calved within this period and were therefore excluded from the experiment, leading to data obtained from 10 cows (i.e., 5 cows in each group). Each calving area was cleaned thoroughly after these 2 calvings, removing all blood, wet straw, and remains of the amniotic sac, and the subsequent calvings occurred 46 and 39 h after this procedure, respectively.

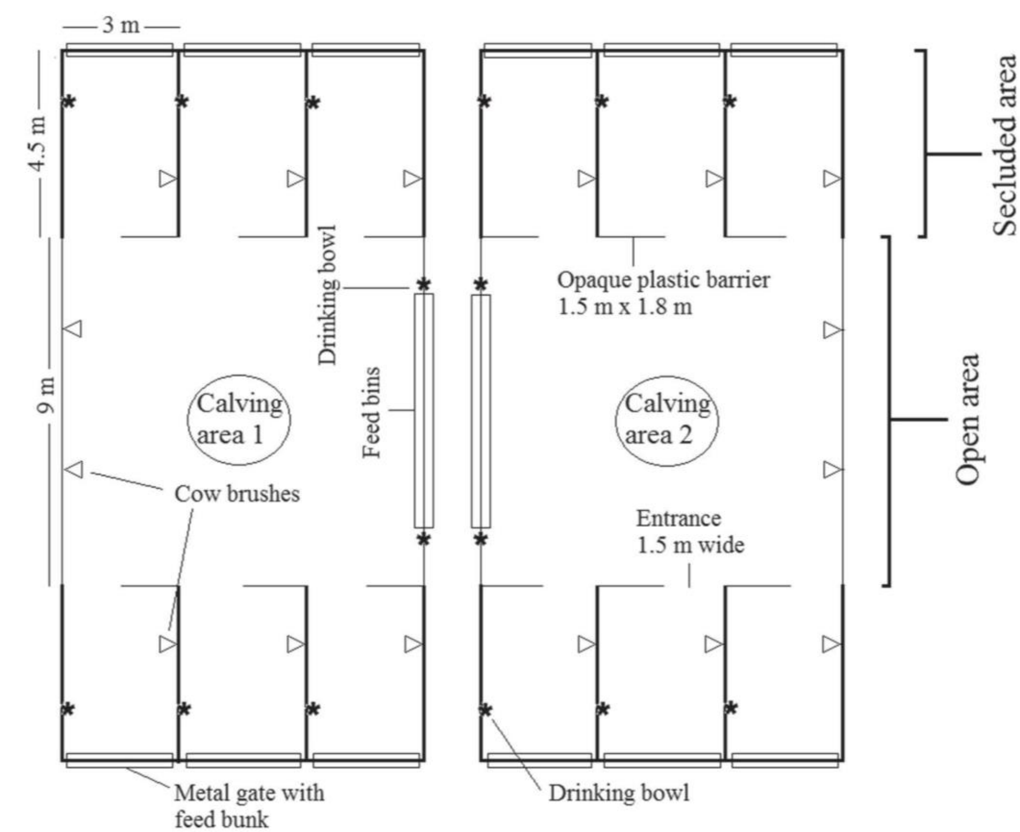

Figure 1. Floor plan of the 2 calving areas in the experimental barn. Thick lines around secluded areas (each 4.5 × 3 m) represent covered sides made from gray opaque plastic. Position of vertically attached brushes, drinking bowls, and feed bins are shown.

Table 1. Time difference and physical distance between calvings in groups I and II, respectively

	Group I		Group II	
Order of calvings	Time (h)	Distance (m)	Time (h)	Distance (m)
First and second	16	<2.5	120[1]	>8[1]
Second and third	27	<2.5	43	<2.5
Third and fourth	61	<2.5	31	<2.5
Fourth and fifth	20	<2.5	120	<2.5

[1]A thorough cleaning procedure was done after the first calving.

The groups were monitored by digital video cameras (model: TVCCD-624, Monacor, Bremen, Germany) mounted above the calving areas. One camera was mounted above the open area and one above each secluded area; 14 cameras in total. Videos were stored continuously throughout the experimental period, and the behavior of each cow was observed for 24 h before calving by an experienced observer using these videos. Position, posture, and behavioral elements were recorded using 5-min instantaneous sampling (Martin and Bateson, 2007) to keep track of the calving process. The exact time of calving (when the hips of the calf were fully expelled from the dam) was recorded, and the precise location of the birth was noted as well as the location of the breaking of the amniotic sac. The breaking of the amniotic sac was visible from the video as either the sac breaking outside the cow or as a sudden fluid bursting out of the cow when the sac ruptured inside the cow. To analyze whether the cows calved at random locations, a 1-sample proportion test with continuity correction was used (Teetor, 2011). The statistical analysis was performed using the R software, version 3.1.2 (R Core Team 2014).

In group I, all 5 cows calved in close proximity to each other (Figure 2a and Table 1). The first cow calved in the open area, where her amniotic sac broke. The 4 subsequent cows calved and their amniotic sacs broke within a radius of approximately 1 cow length of where the first cow calved and where her amniotic sac broke (dark gray circle in Figure 2a).

In group II, the first cow calved in the open area (Figure 2b and Table 1), where her amniotic sac also broke. After this calving, all bedding in a radius of 1 m of where the calving took place was removed and replaced. The second cow calved inside a secluded area (approximately 8 m away from the first calving, Table 1), where her amniotic sac broke. For the 3 subsequent cows, their amniotic sacs broke, and they also calved, within a radius of approximately 1 cow length of the second calving.

In all cases (7 out of 10) where no thorough cleaning was carried out, cows calved within a distance of 1 cow length (maximum 2.5 m) from where the previous calving had taken place. Statistical testing indicated that the cows did not select calving site by random, but chose to calve in very close proximity to where the previous calving took place ($\chi^2 = 5.14$, df = 1, $P = 0.023$). Although our study was too small to yield conclusive results, these preliminary observations indicate that choice of calving site may be affected by the site of a previous calving, potentially explained by the presence of amniotic fluid from other cows. The possibility that visual or physical cues affected calving site selection cannot be excluded, but the thorough cleaning after the first calving in group II resulted in a novel site chosen, suggesting that olfaction might outweigh the effects of other cues. The third cow in group II chose to calve on the other side of the barrier from where the previous calving took place (Figure 2b); however, she appeared to do so because no space was left for her in the pen of the previous calving (because this was occupied by 2 other cows when she was due to calve), and she calved in the closest possible proximity to the previous calving site by choosing the nearest corner in the neighboring pen. One might speculate whether all cows in the group were attracted to the calving site and not just cows close to calving. However, a cow's attraction toward amniotic fluid occurs 12 h before calving and not before that time (Pinheiro Machado et al., 1997); because the other cows in the group calved more than 12 h after the second calving (Table 1), it is unlikely that these may have been affected by olfactory cues from the previous calving.

If the present findings are confirmed by a larger study, this may have implications for management procedures in the modern dairy industry, especially for the development of motivation-based calving facilities.

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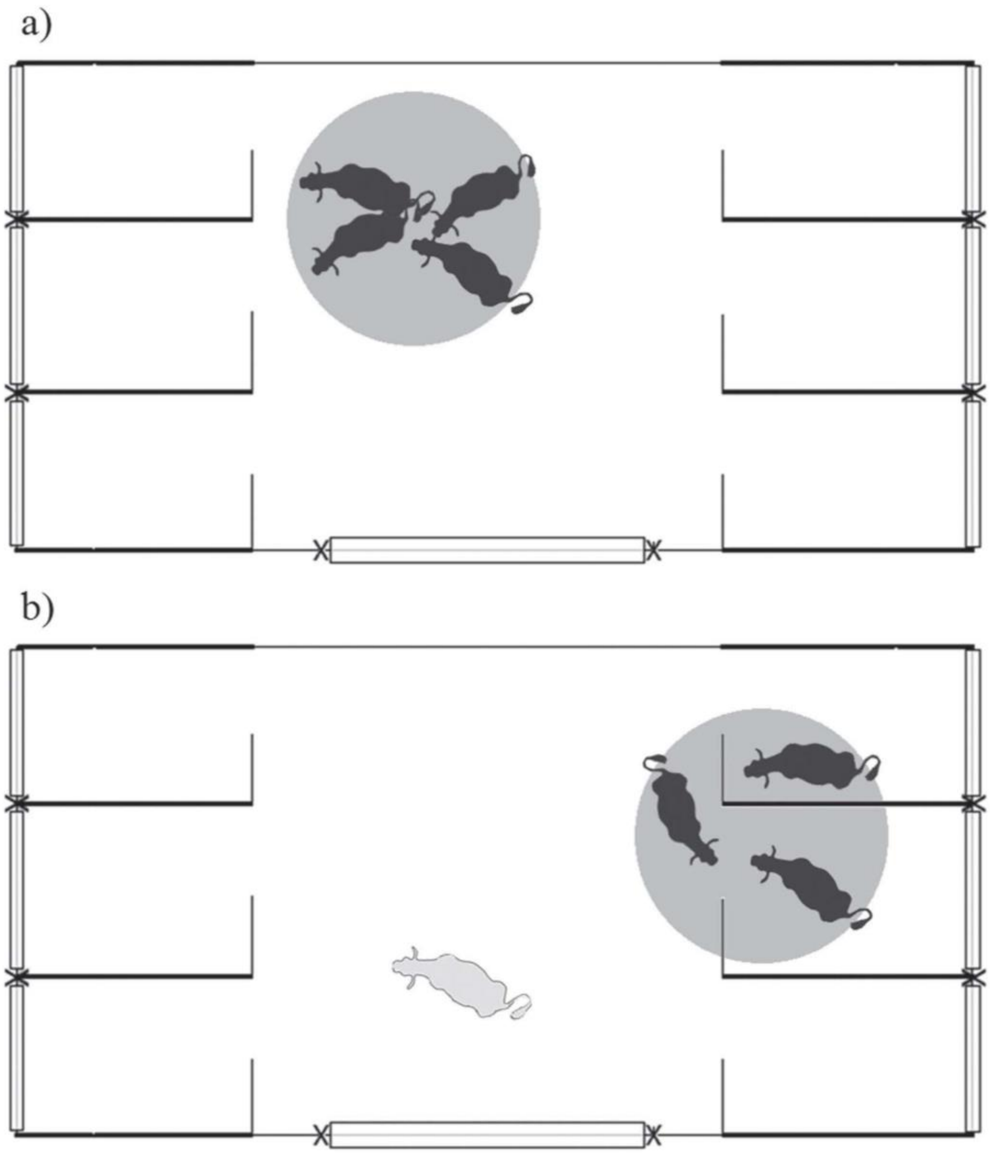

Figure 2. Calving sites in group I (a) and II (b). The center of the large gray circle indicates the calving place of the first cow and the periphery of this circle indicates 1 cow length from where the calving took place (diameter of approximately 2.5 m). The black cows illustrate the position of each subsequent calving. In (b) the light gray cow illustrates the position of the first calving cow, after which a thorough cleaning and replacement of sand and straw was carried out.

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5.3. Study 3

Paper 3:

Development of test for determining olfactory investigation of complex odours in cattle.

Rørvang, M. V., Jensen, M. B., Nielsen, B. L.

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Development of test for determining olfactory investigation of complex odours in cattle

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ABSTRACT

The sense of smell is likely to influence the behaviour of domestic and captive animals in a wide range of management and housing situations. In domestic cattle, there may be unexploited potential for using odours and olfaction in the management; however, published studies on bovine olfactory capacity are scarce. By applying an olfactory Habituation/Dishabituation test developed for rodents, this study aimed to assess olfactory ability in cattle. Twenty-three cows (n = 10) and heifers (n = 13) were tested with three different odours (orange juice, liquid coffee and tap water as an odourless control) presented in a test bucket. The test was conducted on individual animals in their home pen and consisted of each odour being presented twice in a row for 2 min each with an inter-trial pause of 2 min. Following another 2-min pause without odour the animal was presented with a new odour, with order of odour presentation balanced among animals. Duration of sniffing (muzzle in proximity or contact with) the test bucket as well as the occurrence of licking or biting the test bucket were recorded by direct observation. All animals sniffed an odour (i.e. the test bucket) less when presented for the second time (habituation; all 1st vs. 2nd presentations: $P < 0.001$). Sniffing duration increased at the subsequent presentation of a new odour (dishabituation; all 2nd vs. 1 st presentations: $P < 0.001$). All animals sniffed coffee and orange longer than water (water vs. coffee: $P < 0.001$ and water vs. orange: $P < 0.001$), but they also sniffed coffee longer than orange ($P = 0.021$). Licking or biting behaviour occurred only when presented with coffee or orange (13 out of 23 animals for both samples). The test showed that cows and heifers were able to distinguish between different complex odours (coffee and orange juice), and that the animals showed increased interest for one of the odours (coffee). This is the first example of a Habituation/Dishabituation test being adapted for and applied to cattle. The test may require further development, but represents the first steps towards exploring the possibility of using odours when adapting or enriching the environment in which we keep cattle.

1. Introduction

Olfaction is the main sensory modality in the majority of mammalian species and plays a key role in their interactions with the environment as well as in sexual and social behaviour (Brown and Macdonald, 1985; Wyatt, 2003). Despite having a huge impact on the expression and development of behaviour, the link between chemical signals and animal behaviour has often been ignored, especially with respect to large mammalian species (Campbell-Palmer and Rosell, 2011). This may be due to volatile chemical compounds and odours being hard to control and measure (Nielsen et al., 2015). Nevertheless, the sense of smell is likely to influence domestic and captive animals in a wide range of management and housing situations, whether these are laboratory, companion, zoo or farm animals. There may thus be unexploited potential for using odours and olfaction in the management of animals.

One of the areas where olfaction may play a crucial role is in cattle management (reviewed in: Mucignat-Caretta et al., 2012). There are relatively few published studies on the olfactory capacity of domestic cattle (one of the first examples; Baldwin, 1977), although olfaction likely plays a central role in diet selection of this species. However, the olfactory subgenome has been sequenced, and we do therefore know that cattle should be capable of sensing the following: fatty, sour, floral, woody, green, lily of the valley, vanilla, spearmint, caraway, sweet, hay-like, lemon, rancid and spicy (Lee et al., 2013). Corley et al. (1999) noted that cows preferred mineral oil, propylene glycol and rum ether in propylene over an odourless sample and lime oil when testing preferences related to feed intake. Also, Herskin et al. (2003) found an

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increased sniffing time, but a decreased feed intake, when adding a low dose of fish oil to the feed, whereas providing cows with a novel feed induced a higher sniffing duration compared to normal feed treated with a dose of eucalyptus oil. Furthermore, Herskin et al. (2003) noted that sniffing behaviour increased when cows were presented with a known feed in a basket rather than in the cowś usual feed trough indicating that the novelty of the presentation also influenced investigation time. In a study by Madsen et al. (2010) it was found that cows visited an automatic milking system more frequently when offered concentrates of a specific type inside the automatic milker. Whether the above results reflect a preference for novelty or preference for a specific taste or smell is not clear, but it is very likely that olfaction plays an important role in feed preferences of cattle (Engen, 1982; Maruniak, 1988) and that the feeding motivation of cattle potentially can be manipulated by odours.

Olfaction also plays an important role in the reproductive behaviour of cattle. A bull can detect if a cow is in oestrus, or not, by a specific compound in her urine (Archunan and Rameshkumar, 2012). Furthermore, a bull pheromone has been found to induce puberty in heifers (Rekwot et al., 2001) and the responsiveness of a cow towards her offspring has been shown to be governed by signature odours (Griffith and Williams, 1996). Anosmic cows can compensate for the inability to smell using visual cues, but blind and anosmic cows do not form a maternal bond and will prevent their own calf from suckling (Griffith and Williams, 1996; Williams et al., 1996). The studies on olfactory aspects of reproduction have in general focused mainly on prompting successful contact between males and females and on maternal care of the new-born, i.e. offspring recognition, care and protection. However, an aspect of maternal behaviour during the period prior to parturition may have been overlooked in ruminants. It is suggested that the dam develops a high state of responsiveness towards odours associated with neonates during the period just before parturition (e.g. sheep: Lévy et al., 1983, cattle: Pinheiro Machado et al., 1997). The change in olfactory responsiveness could mean that the cow's preference or perception of odours changes as calving approaches which has also been suggested for humans (reviewed in Cameron 2014). Therefore, knowing the olfactory preferences and capacities could be beneficial in the management of pregnant cows, for instance to attract the cow to a certain place prior to calving. Furthermore, certain odours may calm the pre-parturient cow and reduce the stress that the cow experience when being handled and moved which often occurs during late gestation (Mee, 2004; Proudfoot et al., 2013).

Knowing more about the olfactory capacities and preferences of cattle may enable us to adapt the odorant environment to the preferences of the animals. This study used methods developed by neuroscientists for testing olfactory capacity and preferences in rodents and adapted these to investigate olfaction in cattle. The main aims were to investigate if an olfactory test for rodents could be used to test olfaction in cattle, and to investigate if pregnant cows and heifers could detect and distinguish between two complex odours. The secondary aims were to test if the animals investigated one of the two odours more (implying more interest), and if cows and heifers differed with respect to odour investigation.

2. Materials and method

2.1. Developing the test situation

In order to investigate the olfactory capacities of cattle we relied on a test mainly developed for and used in rodents. To investigate what odours an animal is capable of detecting, a Habituation/Dishabituation test can be applied (Yang and Crawley, 2009). This test relies on the animal's interest in novel odours and a reduced willingness of the animal to sniff odour samples when presented repeatedly. The willingness of domestic animals to explore novel items has been used for various purposes e.g. when studying the episodic-like memory in rats and pigs (Kouwenberg et al., 2009) which was our basis for arguing that this test could be adapted for cows. In this study, the Habituation/Dishabituation test was used in combination with the olfactory preference testing procedure (Witt et al., 2009) to ascertain which odours the cows could detect and distinguish between, but also if cows found some odours more interesting than others. The olfactory preference test proposed by Witt et al. (2009) uses water as an odourless neutral reference and thus determines whether the tested odours are investigated more or less than water. A similar approach was used in mice by Saraiva et al. (2016) when testing attractiveness and aversiveness of odours. Habituation/Dishabituation and preference testing are not usually combined; however, a pilot experiment indicated that cows rapidly habituated to the odours and lost interest in the test situation after a few trials. To prevent the cows from losing interest in the odours we utilized the Habituation/Dishabituation test to investigate both whether cows could detect and discriminate the odours. Subsequently we used the first introduction of each odour to determine which of the odours the cows showed the most interest in. Interest was measured as time spent sniffing the odour as in the rodent version of this test; e.g. Coronas-Samano et al. (2016), but we also included direct contact with the test bucket (see Section 2.5.).

2.2. Test buckets

The test buckets (Fig. 1A) were five white plastic boxes (height x length x width: 16 cm x 34 cm x 23.5 cm, 13 L; model 9950, Harald Nyborg, Odense, Denmark), which were covered with a wire mesh (galvanized wire (0.9 mm Ø) mesh (width: 6 mm) model 6321, Rancho®, Odense, Denmark)). The wire mesh lid made the test buckets permeable to air, and hence odours, but prevented the cows from licking and touching the odour samples. The test buckets each had a ballast weight in the shape of a concrete block (model SF-Klostersten® Camfered, Type 2, pure and clean concrete, height x width x thickness: 14 cm x 21 cm x 5.5 cm, weight: 3.53 kg; IBF, Ikast, Denmark) in order to prevent cows from tilting the test buckets. All test buckets were placed in the testing area of the barn (Fig. 1C) two weeks prior to the habituation procedures started to ensure that any odour associated with the test buckets had disappeared, and so that the test buckets did not smell any different from the cows' home environment. During this period the cows were able to see, but not investigate, the test buckets.

2.3. Animals, housing and management

Pregnant (mean days of pregnancy: 281, range: 278–282) Danish Holstein dairy cows (n = 10; 6 cows entering their second parity and 4 cows entering their third parity) and heifers (n = 13) were housed in a straw-bedded group pen positioned in one end of a large barn at Aarhus University's cattle facilities in Foulum, Tjele, Denmark. The group pen measured 8 m × 12 m, the long pen sides had headlocks and the shorter sides were made of vertical tubular metal bars. A 9 m long barrier, also made of tubular metal bars, divided one end of the pen into two equally wide halves (Fig. 1C). Experimental animals were fed a total mixed ration (TMR) with a forage-to-concentrate ratio of 80:20 (%, DM basis) for ad libitum intake. Feed was allocated twice daily (at 0800 and 1400 h) along one of the pen sides with headlocks such that all cows could access the feed at the same time. Fresh straw as bedding was provided daily between 1100 and 1300 h. Group composition and size (group size median: 4; range: 2–6) varied over time as cows and heifers were moved into the group pen 1–2 weeks prior to the expected calving date and removed from the group pen when calving was imminent. Before being tested, all experimental animals had at least 2 (range: 2–10) days to become familiar with the group pen and the animals in it. The cows were tested in this pen and testing the cows in their home environment meant that none of the cows were socially isolated during the tests.

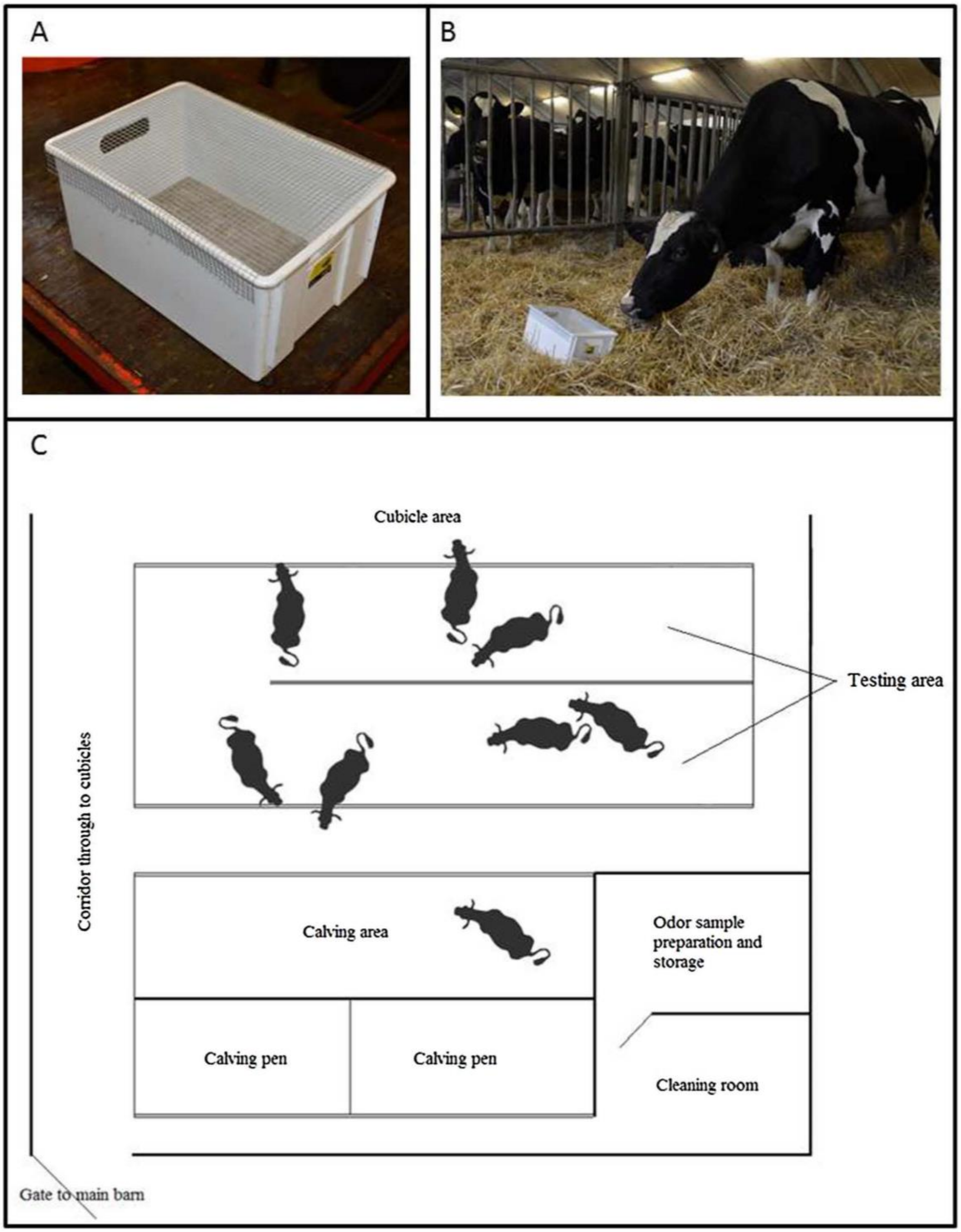

Fig. 1. A) An example of one of the 4 test buckets. B) An example of a cow in the test situation. The rest of the group is separated from the test area by fixtures made of tubular metal bars dividing the pen. The cow being tested is sniffing the test bucket i.e. the cow's muzzle is within the distance of one muzzle length from the test bucket. C) Overview of the barn area where the experiment was conducted. The black thick lines indicate solid concrete walls, the grey thick lines indicate fixtures with vertical tubular metal bars, and double lines indicate the pen side having headlocks. The whole pen area had deep straw bedding. The odour preparation and storage room was separate from the rest of the barn. The cleaning room was not used during this experiment.

2.3.1. Acclimatization procedure

Once a week, an experienced trainer started an acclimatization procedure. The experienced trainer was always the same person and was known to all cows from prior handling in another behavioural study, i.e. all cows were used to being handled. The acclimatization procedure was carried out on those animals which had less than one week until expected calving (mean ± s.d.: 5 ± 4 days before expected calving). In this way no cows or heifers were trained on the day they were moved into the group or on the day they were expected to calve. It also ensured that tested animals were removed before the next group of animals were acclimatized.

The acclimatization procedure consisted of placing a test bucket (i.e. a test bucket, which never had any odour added) in front of the cow, 1.5 m away from the head of the cow, for 3 min. The cow was able to investigate the acclimatization test bucket (Fig. 1B) by sniffing, touching, licking, biting and pushing it during this period whilst other cows in the group were kept away by the trainer. The acclimatization criterion was that the cow should approach the bucket at least once within the 3 min period and investigate it for more than 10 s but no more than 2 min and without displaying any behaviour indicative of fear (such as immobilization or freezing behaviour, flight responses, backwards movements or vigilant behaviour (i.e. head raised above shoulder height while ears pointing forwards). Each experimental animal had up to three 3-min trials distributed over 2 days (maximum 2 trials per day) in order to comply with the acclimatization criterion and no animals failed to comply with this criterion. When complying with the criterion the cow or heifer was considered to be ready for testing and was tested for the first time in the afternoon of the same day or on the next day regardless of the progress of the other animals in the group. This resulted in a total of 10 cows and 13 heifers proceeding to the test.

2.4. Odours and odour sample preparation

Substances chosen as odours for the test were orange (orange juice, batch 661101, Rynkeby Foods A/S, Ringe, Denmark) and coffee (instant coffee, 35 g diluted in 500 mL of boiling tap water, subsequently cooled to room temperature; LOT: L-0463, BKI Foods, Højbjerg, Denmark), and tap water was used as an odourless control. These represent complex odours (i.e. each composed of many different odorants) and were chosen because we wanted to test natural, non-toxic odours, which were both cheap and accessible, novel to the cows as well as easy to standardize. Cows were not familiar with orange or coffee odours as staff was never allowed any food or drinks when working in the cow barn.

Fresh odour samples were prepared before each new testing trial (approximately once a week). Odour samples were made by placing one filter paper (unbleached (light brown) filter paper model 7607, Harald Nyborg, Viborg, Denmark,) in the test bucket, and then pouring on to it 5 mL odour sample at room temperature. The filter paper absorbed the odour fluid and, due to its colour, made all odour samples look alike, thus limiting any form of visual difference between the samples. One distinct test bucket was used for each different odour in order to prevent any cross-contamination of odours. At the end of a test day all test buckets had the filter papers removed, and all equipment used (test bucket, wire mesh and ballast weight) were cleaned with warm water. When cleaned, the materials were left to dry for at least 2 days before the next test was initiated. Odour preparations and cleaning of test buckets were done in a room separate from the testing area (Fig. 1C) and the person handling odour samples wore latex gloves for all preparation procedures.

2.5. The Habituation/Dishabituation test

On each test day a maximum of four animals were tested to limit potential odour contamination of the testing area. The trainer separated the particular cow or heifer from the rest of the group by a distance of 4 to 10 m. A balanced odour order was determined beforehand and each experimental animal had a distinct odour order assigned randomly before the testing commenced (Table 1).

In the test situation the particular test bucket was moved from the preparation room to the testing area and placed in front of the cow in the same manner as in the habituation procedure. The same trainer (henceforth called the observer) observed the cow for the whole test period. Each odour was presented two times in a row for a duration of 2 min each with an inter-trial pause of 2 min. After removal of the first odour the experimental animal again had a 2 min pause without odour (Wesson et al., 2008) before being presented with the next odour. Two stop watches (model 38.2016, TFA Dostmann GmbH & Co. KG, Wertheim, Germany) were used, one to continuously record the occurrence of sniffing behaviour during an odour presentation, and another to time the duration of each odour presentation trial (2 min) and inter-trials pause (2 min). Sniffing behaviour was thus visually monitored and continuously recorded by direct observation (Martin and Bateson, 2007). It was defined as the cow's muzzle being in close proximity of (i.e. less than the length of a cow muzzle; Fig. 1b) or in direct contact with the test bucket, potentially including licking and biting the test bucket. Licking and biting behaviour were recorded separately but alongside the recordings of sniffing behaviour by the same observer using one-zero sampling (Martin and Bateson, 2007). Whenever either licking or biting occurred at any point during the 2-min test, the test cow would be assigned a '1', otherwise a '0'. After each 2-min trial, the odour test bucket was moved back to the preparation room (Fig. 1C) to limit inter-trial contamination. Habituation to the odour was defined as a decrease in sniffing of the odour between the two presentations. Dishabituation on the other hand was defined by a reinstatement of sniffing when a new odour sample was presented.

Table 1
Odour presentation order for all 23 animals (heifers and cows).

1st odour		2nd odour		3rd odour		n
Water 1	Water 2	Coffee 1	Coffee 2	Orange 1	Orange 2	3
		Orange 1	Orange 2	Coffee 1	Coffee 2	4
Orange 1	Orange 2	Coffee 1	Coffee 2	Water 1	Water 2	4
		Water 1	Water 2	Coffee 1	Coffee 2	4
Coffee 1	Coffee 2	Orange 1	Orange 2	Water 1	Water 2	4
		Water 1	Water 2	Orange 1	Orange 2	4

2.6. Statistical analysis

The data comprised 6 repeated measures for each experimental animal; 2 tests per odour i.e. first and second presentation, of 3 odours in total (Table 1).

Normality of data (duration of sniffing, occurrence of biting or licking), assessed by visual inspection of histograms and in a Shapiro-Wilks normality test, could not be assumed and data were analysed using non-parametric methods as described in Siegel and Castellan (1988).

All analyses were performed using the basic platform of the software R version 3.3.1 (2016-06-21, "Bug in Your Hair") and all P-values were evaluated using a significance level of 5%.

2.6.1. Habituation/Dishabituation

A Wilcoxon signed ranks test was used to determine if significant habituation (reduction in sniffing duration) occurred between successive presentations of same odours (one comparison per odour per animal; $n = 23$). When ties were present, data were converted into an exact distribution using the Wilcoxon-Pratt signed-rank test software in the package 'Coin' for the R program (Hothorn et al., 2006). The same procedure was also used to analyse whether reinstatement of sniffing (dishabituation) occurred when a new odour was presented by comparing sniffing durations between successive presentations of different odours (two comparisons per animal, Table 1).

2.6.2. Analysing interest in the odours

When analysing level of interest in each odour, sniffing duration for the first presentation of each odour was compared separately to the sniffing duration for the first presentation of water in a Wilcoxon signed ranks test. Ties were dealt with by conversion to an exact distribution using the Wilcoxon-Pratt signed-rank test software. This analysis was done in order to determine if the animals were able to distinguish between the odours and the odourless control sample, i.e. able to detect the odours. Subsequently, sniffing durations for coffee and orange were compared using the same test (Wilcoxon signed ranks test) to determine which odour elicited the most investigation. The occurrence of licking or biting behaviour was converted into total occurrences per trial for the first presentations of water, coffee and orange, respectively.

2.6.3. Parity effects

To investigate whether an effect of parity occurred, a Wilcoxon-Man-Whitney test was used to compare cow and heifer sniffing durations and a Fisher exact test was used when comparing the occurrences of licking or biting per trial for cows and heifers.

3. Results

3.1. Habituation/Dishabituation

All cows sniffed the same odour significantly less when it was presented the second time and thus habituated to all the odours (Wilcoxon signed ranks test (n = 23): water: $|z| = 4.20$, $P < 0.001$; coffee: $|z| = 4.20$, $P < 0.001$ and orange: $|z| = 4.20$, $P < 0.001$; for the first vs. the second presentation of water, coffee and orange, respectively; Fig. 2A). Sniffing duration increased significantly (dishabituation) after presenting the experimental animal with a new odour (Wilcoxon signed ranks test: water vs. coffee: $|z| = 4.17$, $P < 0.001$ (n = 7); water vs. orange: $|z| = 3.74$, $P < 0.001$ (n = 8); orange vs. coffee: $|z| = 4.20$, $P < 0.001$ (n = 8) and coffee vs. orange: $|z| = 4.17$, $P < 0.001$ (n = 7); for the second presentation vs. first presentation of a new odour; Fig. 2B). All cows sniffed all odours at first presentation and minimum sniffing duration was 2.2 s (median: 18.2, range: 2.2–46.8) for all first presentations.

3.2. Analysing interest in the odours

All cows and heifers sniffed coffee and orange significantly longer than water (Wilcoxon signed ranks test: water vs coffee: $|z| = 4.17$, $P < 0.001$ and water vs orange: $|z| = 3.74$, $P < 0.001$; for the first presentation of water vs the first presentations of coffee and orange, respectively), but the animals also sniffed coffee significantly longer than orange (Wilcoxon signed ranks test: $|z| = 2.28$, $P = 0.021$). Additionally, licking or biting behaviour only occurred when animals were presented with either coffee (13 out of 23 animals) or orange (13 out of 23 animals), never when presented with the water sample (0 out of 23 animals).

3.3. Effect of parity

Parity had no effect on the sniffing duration of any of the odours (Wilcoxon- Man-Whitney test: (cows vs. heifers) water: $w = 65$, $P = 1$, coffee: $w = 50$, $P = 0.38$; and orange: $w = 70$, $P = 0.78$). Cows and heifers were equally likely to lick/bite the samples (Fishers exact test: odds ratio = 3.19, $P = 1$),

4. Discussion

These results show that Habituation/Dishabituation testing is a meaningful paradigm in cattle, and shed light on what bovines are capable of smelling. Cows and heifers habituated to each of the odours on their second presentation, and dishabituation occurred when the animals subsequently had a new odour presented. Both cows and heifers were able to detect and discriminate between water, coffee and orange, with coffee evoking the highest level of interest measured as sniffing duration. Cows and heifers did not differ in sniffing duration, nor licking and biting occurrences, and across parities only coffee and orange samples were licked or bitten.

4.1. The Habituation/Dishabituation test in a social environment

This study is the first to use the Habituation/Dishabituation test on cattle. The Habituation/Dishabituation test was originally designed for rodents confined in an enclosed test arena where the odour is present without interference from other conspecifics (Witt et al., 2009; Yang and Crawley, 2009). In this study we adapted the rodent design to test olfactory capacities of a bigger mammal in a different setting. Advantages of our design were that the animals did not have to be moved from their home environment, they were not confined and always had visual contact with members of the group while tested. Limiting the risk of experiencing stress due to handling, confinement and social isolation during testing, makes the results more reliable as stress reactions can affect the animal's motivation and thus its behaviour (Sapolsky, 2002). The presence of companions can buffer adverse effects of stress. For instance, calves and heifers were reacting less to a novel and fear eliciting situation when they were with a companion than when alone (Færevik et al., 2006; Boissy and Le Neindre, 1990) and horses were less fearful when they had been observing a habituated demonstrator completing a frightening task prior to performing the same task themselves (Christensen et al., 2008; Rørvang et al., 2015a). The disadvantages of keeping animals together in test situations are, however, potential social transmission of fear or induced curiosity caused by reduced fear. The effect of the presence of conspecifics is context specific (e.g. social facilitation in a fear eliciting situation; Rørvang et al., 2015a vs. social facilitation of information in a more complex spatial learning situation; Rørvang et al., 2015b), and it may be speculated that this effect is bigger when the situation is more frightening. From that perspective social transmission of fear is less likely in this particular situation as all animals were habituated to the test buckets and the test situation beforehand, and because the nature of the test was not particularly frightening.

Another issue to consider is the potential that a test contaminated the environment for the subsequent test. As odour molecules may disperse into the air during testing, the animals tested subsequently may have smelled the odours prior to being presented with the odour in the test buckets themselves. However, we tried to account for this by testing a maximum of four animals per day as well as not having any of the animals acclimatizing while testing others in the same area. Additionally, the test procedure was designed to minimize contamination effects within the constraints of the experimental set-up by using small test buckets containing only small amounts of the odour, which was placed in a large barn (927 m^2) with circulating air diluting potential odour contamination.

4.2. Odour interest and effect of parity

The current test design was not a preference test, as the two odours were not presented simultaneously, and thus the measure of interest is not a measure of preference. However, the finding that coffee was investigated more than orange, may indicate which type of odours to look for when trying to enrich the environment of cattle. Moreover, cows and heifers only licked or bit the orange and coffee samples, which could imply that the odours were perceived not only as interesting but also potentially edible. Furthermore, the increased interest evoked by coffee may attract a cow or a heifer to a specific place where coffee odours is present, depending on the level of attractiveness and the distance by which the odour is detectable. Human handling may be reduced if cattle can be moved by means of attraction to a specific odour placed in a specific place. This may be useful in a range of management situations including herding for milking and movements between groups and in relation to calving.

Future studies on using odours as a means of enrichment or in the handling of cattle should aim at investigating the extent to which

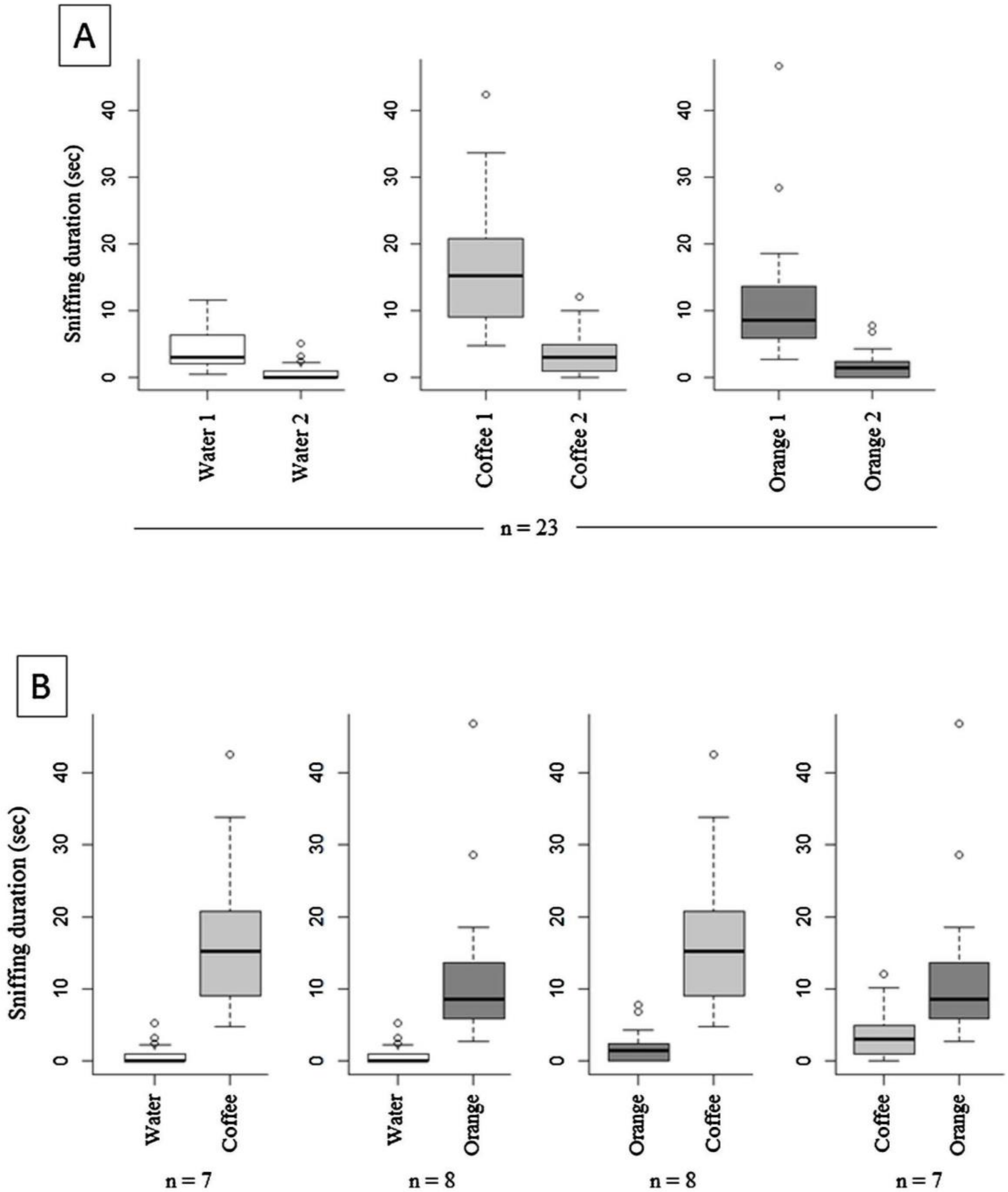

Fig. 2. Sniffing duration measured in seconds per trial. A) Habituation, successive presentations of each of the three odours, with numbers indicating order of presentation. B) Dishabituation of transition between odours; water to coffee, water to orange, orange to coffee and coffee to orange, respectively. Numbers indicate the order of presentation indicated in Table 1.

odours can attract and potentially influence the behaviour of cattle. An experiment investigating olfactory preferences could be followed by a subsequent experiment testing the capacity or level of attraction to these preferred odours in various management situations.

5. Conclusions

This is the first example of a Habituation/Dishabituation test adapted for and applied to cattle. The test showed that cows and heifers can detect and discriminate between different and easily accessible complex odours, and that both cows and heifers were generally most interested in coffee odour. Cows and heifers only licked or bit the orange and coffee samples, which indicates that these odours may have been perceived as edible. This study is the first step in exploring the possibility of using odours when adapting or enriching the environment in which we keep cattle, and further research is greatly needed.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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5.4. Study 4

Paper 4:

The motivation-based calving facility: Social and cognitive factors influence isolation seeking behaviour of Holstein dairy cows at calving.

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RESEARCH ARTICLE

The motivation-based calving facility: Social and cognitive factors influence isolation seeking behaviour of Holstein dairy cows at calving

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Abstract

In order to improve animal welfare it is recommended that dairy farmers move calving cows from the herd to individual pens when calving is imminent. However, the practicality of moving cows has proven a challenge and may lead to disturbance of the cows rather than easing the process of calving. One solution may be to allow the cow to seek isolation prior to calving. This study examined whether pre-parturient dairy cows will isolate in an individual calving pen placed in a group calving setting and whether a closing gate in this individual calving pen will cause more cows to isolate prior to calving. Danish Holstein cows (n = 66) were housed in groups of six in a group pen with access to six individual calving pens connected to the group area. Cows were trained to use one of two isolation opportunities i.e. individual calving pens with functional closing gates (n = 35) allowing only one cow access at a time, or individual calving pens with permanently open gates allowing free cow traffic between group area and individual pen (n = 31). The response variables were calving site, calving behaviour and social behaviour. Unexpectedly, a functional gate did not facilitate isolation seeking, perhaps because the cows were not able to combine a learnt response with the motivation to isolate. Dominant cows had the highest chance of calving in an individual calving pen. If an alien calf was present in the group pen or any of the individual pens, cows were less likely to calve in an individual calving pen. Future studies should allow cows easy access to an individual calving pen and explore what motivates pre-parturient cows to seek isolation in order to facilitate voluntary use of individual calving pens.

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Introduction

Calving is an essential part of commercial milk production and during the transition period (typically defined as three weeks before and three weeks after calving), there are several threats to dairy cow welfare. Calving itself places high demands physically and is associated with pain [1]. Disease and mortality rates are high during this period, with nearly 75% of disease cases occurring within the first month after calving (reviewed in [2]) and 30–50% of post-partum cows being affected [3]. When cows calve in group calving pens they are frequently disturbed

design of this study, data collection/analysis or preparation of the manuscript.

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by other cows and the risk of mismothering is high [4]. In order to protect the calving cow, it is currently recommended that cows calve in individual calving pens to which they are moved well in time before calving (e.g. by law in Denmark by Ministry of Environment and Food of Denmark, Danish Veterinary and Food Administration, Law number. 520, Chapter 4, 26/05/ 2010, [5] and by recommendation of The Canadian Dairy Code of Practice [6]). Cows are, however, often moved too late due to the difficulty of determining the onset of the first stages of labour, which have been suggested to be an appropriate time to move cows to individual calving pens [7,8]. Moving cows later may disturb the process of calving and prolong the second stage of labour [7], leading to an increased risk of calving complications (dystocia) and related diseases [9]. The current international trend towards increased herd size [10] means that farmers have more cows to supervise, making it more difficult to identify the correct time to move cows to individual calving pens. Increased herd size may also result in farmers using group calving facilities more often, which may not be consistent with the choice of the cows. In order to aid identification of imminent calving, devices have been developed to monitor behavioural and physiological changes before calving, such as reduced rumination [11] and increased number of lying bouts [12,13]. In addition, vaginal temperature has been reported to decrease prior to calving [14] and sensors to detect this physiological change are now available. However, these changes are only measurable in the hours prior to calving meaning that many cows are unlikely to be moved in time. Hence, there is a need for a practical solution facilitating the moving of cows well in time before calving. One possible solution may be to develop a motivation-based calving facility, taking advantage of the natural motivation of calving cows to seek isolation.

To design motivation-based calving facilities, knowledge of the behaviour and preferences of calving cows are essential. The natural behaviour of cattle is to stay within the proximity of the herd, and typically cattle synchronize their behaviour [15,16]. However, as parturition approaches, cows become restless [14,17,18] and move away from the group and isolation seeking behaviour have been reported among cows kept in semi-natural [19] and production conditions [20,21]. The underlying evolutionary strategy may be to ensure that the female bonds to her offspring without disturbance from group members, but isolation seeking behaviour may also have developed to protect the newborn against predation as suggested by [22]. In a practical setting, motivation for isolation seeking may be utilized when cows are to be moved into individual calving pens before calving, by allowing cows to move into these pens on their own, and at the same time ensure a timely move to individual housing, as well as ensuring an undisturbed environment.

One aspect of isolation seeking behaviour is the social factors inevitably arising from housing pregnant cows in groups. In addition, individual differences between cows are apparent. Although sparsely studied, dairy cows have different levels of sociability [23] and individual behavioural characteristics [24]. Making a choice of where to calve, may also depend on the personality of the cow. To our knowledge, neither the effects of social factors, nor personality, has been studied previously. Edwards [4] noted that when calving in groups, cows close to parturition were exceedingly attracted to calves (as 14 out of 16 cows licked an alien calf before giving birth themselves). Calves are, however, not the only distraction. In intensive indoor dairy production, pre-parturient cows are often moved to a new group, where the level of aggression can be relatively high [25], especially in relation to accessing limited resources, such as an individual calving pen. Adding to this, maternal aggression (expression of defensive and aggressive behaviour to protect the offspring [26]) is well documented in a number of ungulates (cattle: [27] sheep: [28], pigs: [29]), which may lead to even higher levels of aggression among peri-parturient females. Within a group of pre-parturient cows, social dominance determines the outcome of competition for resources [30]. If individual calving pens are perceived as a limited resource by the cows, the chance of gaining access to a pen is likely higher for dominant individuals. However, possible interactions between social dominance and stage of pregnancy cannot be ruled out. The motivation to seek isolation may also cause cows to avoid social confrontations and become more submissive as calving approaches. What happens in relation to personality and dominance in the pre-parturient cows and whether other factors affect the motivation to isolate before calving, must be determined in order to develop motivation-based calving facilities.

The aim of this study was to examine whether cows kept under conventional dairy farming conditions will isolate in an individual calving pen when they are trained to access this beforehand. We housed 13 groups of six pregnant dairy cows in group pens with access to individual calving pens, allowing either free cow traffic (the gate was permanently open) or access for only one cow at a time (functional mechanical gate). We hypothesized that cows housed with functional gates would be more likely to seek isolation in an individual calving pen prior to calving due to their experience of being alone, behind the closed gate compared to cows housed in groups where calving pens had free cow traffic and thus no gate to keep other cows out. Additionally, we investigated whether other factors, such as personality, dominance, group structure, and presence of alien calves influenced the choice of calving site during 12 to 8 h pre-calving. A personality assessment tests was incorporated into the experimental plan by using Human approach tests and recording social interactions. We hypothesized that subordinate cows would be more likely to seek isolation behind the gates due to increased risk of aggression from dominant cows and that the presence of an alien calf would lower the probability of cows moving into an individual calving pen.

Materials and method

The experiment complied with the current Danish law, except for calving in individual calving pens and except for the cow-calf pairs being separated before 12 hours after calving. The procedures were evaluated prior to the experimental start by the responsible laboratory animal veterinarian from the institutional animal ethics committee at The Department of Animal Science, Aarhus University, Tjele, Denmark. It was confirmed that no ethical oversight was needed.

Housing conditions

The experiment took place at the Danish Cattle Research facility at Aarhus University (Foulum, Denmark) September 2015 to June 2016.

The experimental barn had 3 sections (Fig 1), each consisting of a group area (9 x 9 m) connected to 6 adjacent individual calving pens (4.5 x 3 m each). The individual calving pens were built from 1.3 m high tubular metal bars covered by 1.8 m high grey plastic barriers (barrier width: 10mm, Jyden, Vemb, Denmark), on the two 4.5 m sides as well as 1.5 m of one other side (half of the pen side facing the group area), leaving a 1.5 m opening towards the group area. The fourth side (facing the outer walls of the barn) had a 3.0 m feed bunk (Jyden, Vemb, Denmark). This construction was chosen to offer the cows an opportunity to isolate (Fig 2A and 2D). In the opening into the group area (1.8 x 1.5 m), a mechanical cow gate was installed. The gate was designed to allow access for just one cow at a time by locking mechanically after one cow had entered the pen i.e. opened the gate and passed through (Prototype designed by Jyden, Vemb, Denmark). The cow was always able to go back to the group area, after which, the gate would unlock mechanically from the manipulation when the cow pushed the gate open, allowing another individual to enter. In half of the sections, the experimental calving pens were equipped with open gates (gate permanently open), while the other half of the

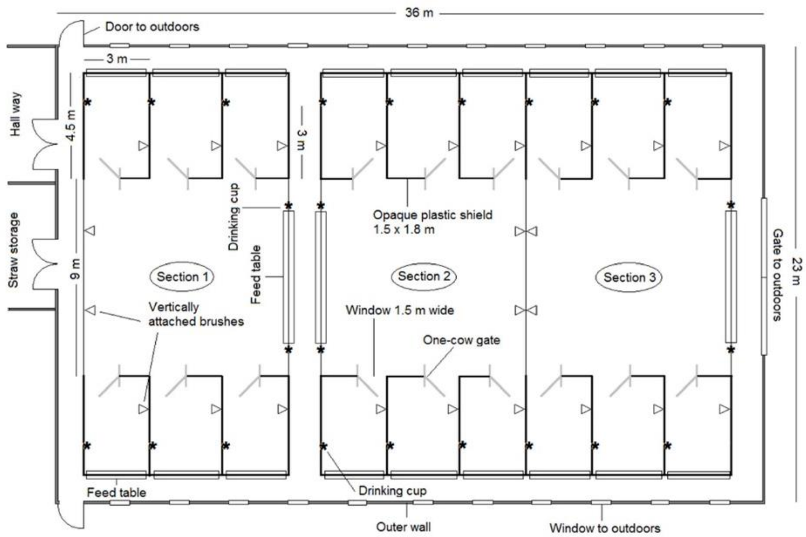

Fig 1. The experimental barn. Top view of the experimental barn, including all three sections. Thick lines around individual calving pens represent covered sides, and the "window" illustrates where the mechanical gate (grey insertion in the "window") was installed in all individual calving pens. Vertically attached brushes, drinking cups and feed tables are shown.

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sections had a functional gate (i.e. gate) (as illustrated in Fig 2A and 2D). The two experimental treatments (permanently open gate; functional gate) were applied to one whole section in a balanced order to control for possible effects of section placement within the barn. The order

Fig 2. The levels of closing the gate. A: The view from an individual calving pen with open gate, representing the start point for all cows (initial training, *step 1*) and the treatment termed "permanently open gate". B: The top bar of the gate being closed (corresponding to training *step 2* for cows housed with "functional gates"). C: The trainer holding the gate, half way open, in order for the cow to see the way out (corresponding to training *step 3* for cows housed with functional gates). D: The view from inside the gated individual calving pens with the gate fully closed (corresponding to training *step 4* for cows housed with "functional gates").

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was (section; treatment: 1; permanently open, 2; functional, 3; functional, 1; functional, 2; permanently open, 3; permanently open, 1; functional, 2; permanently open, 3; functional, 1; permanently open, 2; functional, 3; permanently open, 1; functional).

The floor of all group areas and individual calving pens was covered by approximately 15 cm of sand (Kosand, brand; Dansand, Brædstrup, Denmark; mean grain size 0.322 mm) and approximately 15 cm of barley straw on top as bedding to ensure good lying comfort and a non-slip surface for getting up and lying down behaviour of the cows. New straw was added on a daily basis in the morning between 9.30 and 12.00 am after cleaning (removal of faeces and wet and soiled straw). Cleaning was done to maintain similar bedding quality in the different areas. After each calving, the place where the amniotic sac broke, and the place where the calf was born, was located using video surveillance (described below) and straw as well as sand was fully removed in an area of Ø: 1 m. Afterwards new sand and straw were added. This procedure was used in order to limit the potential influence from leftover birth fluids [31].

Each group area had 6 individual feed bins constituting the feed table (bin width: 75 cm, model 1318–8210, Jyden, Vemb, Denmark) and two self-filling, automatic drinking cups (model 2177–4010, Jyden, Vemb, Denmark). Each individual calving pen had one water cup similar to the ones in the group area.

Cows were only fed in the group area to avoid confounding of feed and isolation motivation in the individual calving pens. During the whole experimental period, a total mixed ration with a forage-to concentrate ratio of 80:20 (%, dry matter basis) was provided for ad libitum intake. Feed was allocated twice daily, in the morning between 9.30 and 12.00 am, and in the afternoon between 5.30 and 6.00 pm.

Animals

Initially, the experiment included a total of 78 multiparous Danish Holstein cows all provided from the Danish Cattle Research facility at Aarhus University (Foulum, Denmark). This sample size was chosen based on a pre hoc power analysis, utilizing the probability of calving in a pen of 0.71 (found in a pilot experiment). Based on the power analysis, we decided to include 6 animals per section meaning that there was one individual pen per cow. The probability of detecting a difference in the experiment was 94% (at least 80% is recommended [32]) when including 72 animals. The total sample size thus consisted of 72 animals determined from the power analysis plus an additional 6 animals included as a buffer. The buffer of animals was included due to the possibility of having to exclude cows due to sickness, calving difficulty or other calving related issues (see exclusion criteria below).

Thirteen section groups were constructed based on expected calving dates in order to ensure a similar dispersion of calvings and allow barn staff time to clean between calvings. Section group cows had at least 1 day between expected calvings and the average dispersion between expected calvings was 6.9 days (range: 1 to 15 days).

Training and tests

Each group of six cows was moved to the experimental section and placed in the group area approximately two weeks prior to the first expected calving. All cows were allowed 24 h to acclimatize to the experimental housing before initiation of training (Fig 3). During this period, all gates were kept permanently open regardless of treatment, and cows could move freely in and out of the individual calving pens. Depending on the gate treatment, a specific training procedure was used (see below).

Personality assessment. On the day of training initiation, a personality assessment of all cows was done based on the cows′ immediate reaction in terms of exploration-avoidance of

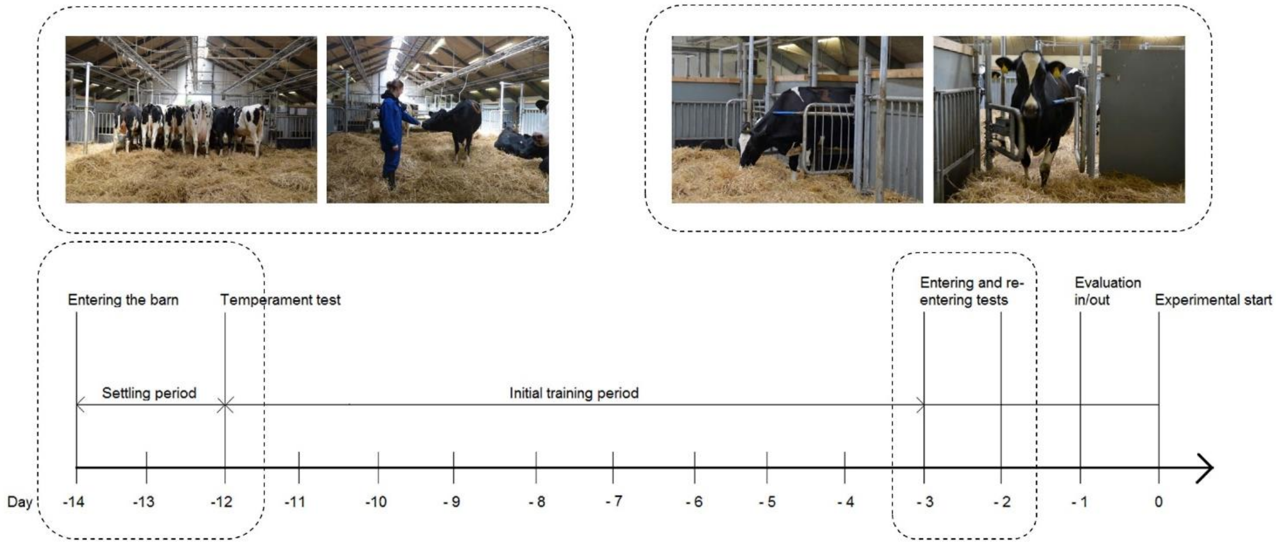

Fig 3. Time line. Days prior to experimental start, indicating all initial procedures and assessments. Moved to the barn (day -14), temperament test (day -12) after a settling period (day -14 to -12), initial training (day -12 to -3), entering and re-entering tests (day -3 and -2) leading to either inclusion or exclusion (day -1) and experimental start (day 0).

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the trainer and the training procedures. This was done in a "section entering test" and a "human approach test", conducted prior to the initiation of training as well as during the first steps of the training procedures (described below) and termed "fearfulness during the initial training". The "section entering test" assessed the cow's immediate reaction to the trainer. When the trainer entered the barn for the first time, the trainer would stand in front of the group area for a few minutes (2 to 5 min) before entering the section. The trainer then climbed the outer walls and entered the middle of the group area. Inside the group area, the trainer walked to the centre, making sure that all cows paid attention, and then scored the cows according to the definitions shown in Table 1. The "human approach test" was initiated by identifying a cow in the group area not eating, drinking or engaging in social interactions, and which was attentive towards the trainer. The trainer would then approach the focal cow from a distance of two cow lengths (approximately 5 m) from an angle of 45° of the front of the cow,

Table 1. Personality assessment.

Test	*Avoiding*	*Explorative*
Section entering test	Cow moves away from the trainer	Cow stands still or approaches the trainer
Human approach test	Avoidance distance more than one cow length from the trainer	Avoidance distance less than one cow length from the trainer
Fearfulness during the initial training	Started on *step 0* and reached no further than *step 1* in first training trial	Started on *step 1* and reached *step 1* or higher in the first training trial

Definition of the "Avoiding" and Explorative" categories according to the three assessments of the dairy cows done during the pre-experimental training period; Entering test, Human approach test and Fearfulness during the initial training. The cut-off for the avoidance distance in the Human approach test was determined before the test. A cow was determined 'shy' when scored in the "avoiding category" for at least two out of three assessments, and likewise determined 'bold' when scored in the "explorative category" for at least two out of three assessments. The initial training steps are described below.

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with an outstretched arm and an even pace (as described by [33]). When the cow moved away from the trainer, the avoidance distance was noted. If the cow did not move, the avoidance distance was left censored as zero (Table 1). The evaluation of "fearfulness towards the initial training" was carried out during the first training bout and consisted of evaluating the reaction of the cow towards the initial training procedure, and the level reached according to the initial training steps described below. Finally, each cow was evaluated as being "explorative" or "avoiding" according to the definitions in Table 1. A cow was determined '*shy*' when scored in the "avoiding" category for at least two out of three assessments, and likewise determined '*bold*' when scored in the "explorative" category for at least two out of three assessments. Boldness was thus defined as an explorative animal, i.e. willing to explore a novel person (the trainer) and a novel situation (the initiation of training) [34].

Initial training. Cows were trained section-wise i.e., trained individually, but with the other five cows of the section present during training. Each training day had two distinct periods of training chosen based on expected level of feeding motivation and disturbances: 08 to 10 am just before feeding, and 01 to 03 pm just before topping up the feed.

Each training lasted a maximum of 8 minutes per cow (determined during a pilot training session with pilot cows prior to the experiment) and the average training duration was 4.8 ± 2.6 min per bout. Training bouts were separated by either one day or 3 to 7 hours. A training bout was always initiated from within the group area, and each cow was trained to enter a randomly chosen individual calving pen (i.e. decided beforehand by a third person rolling a dice). If the chosen individual calving pen was already occupied, the neighbouring pen to the right was chosen. Throughout the successive training bouts, cows were always trained in the pen to the right, during the proceeding training, in order to allow experience with all individual calving pens. No cow was chased out of an individual calving pen in order to be trained, i.e. if a cow was inside an individual calving pen, the trainer would wait until the cow re-entered the group area before initiating her training.

All cows were gradually trained to follow the trainer by reinforcing the approach by positive reinforcement i.e. eating concentrates from a black plastic bucket (12 litres). The trainer would stand in front of the cow and at first allow the cow to eat a mouthful from the bucket without having to move (*step 0*). If the cow was unwilling, or too fearful to eat from the bucket, she was allowed to eat from the bucket while it was standing on the bedding without the trainer present (watching from a 3 m distance). In such cases, the trainer would gradually approach the cow while she was eating from the bucket, until the trainer was able to touch and later lift the bucket. Shortly after completing *step 0*, the trainer moved a few steps away from the cow towards the individual calving pen while still facing the cow and displaying the bucket. When the cow followed, she was again allowed to eat a mouthful upon reaching the bucket and the trainer. At first, the reward would be given after just a few steps in order for the cow to learn to follow. The demand would then rise as training progressed. *Step 1* consisted of the trainer moving backwards through the opening of the individual calving pen, spending a few seconds inside the individual calving pen rewarding the cow and then leaving. When the cow walked willingly through the open gate three times, she had completed *step 1*.

Cows housed with functional gates *(n = 35)*: The gate was gradually closed in three steps: First the top bar was closed (*step 2*), secondly, the gate was closed, but the trainer held the gate halfway open in order for the cow to see the way in and out (*step 3*) and lastly, the gate was fully closed (*step 4*) (Fig 2B, 2C and 2D, respectively). In these steps, the trainer passed through the gate opening, and then faced the cow to entice her to pass. Each time the degree of closing the gate was changed, the procedure of going in and out was repeated until the cow walked willingly through, three times. During the training, the trainer ensured that no other cows followed the focal cow into an individual calving pen at any point. The success criterion for the initial training was that the cow should be able to open the closed gate on her own three times (i.e. two times going into the individual calving pen, and one time going out) without showing any fear-related behaviour (Table 2). For visual of cows being trained to perform step 4, see S1 Video.

Cows housed with permanently open gates *(n = 31)*: All cows were gradually trained to walk with the trainer through the gate opening of the individual calving pen and back again, as described above (*step 1*). The duration of a training bout was determined as the mean duration of a corresponding training bout for cows trained to the functional gates. Likewise, the number of training bouts corresponded to the number of training bouts for cows trained to functional gates, and thus all cows, irrespective of the treatment, were trained for the same duration of time. In this treatment, other cows were allowed to follow the trained cow when she entered an individual calving pen, and likewise allowed to enter the individual calving pen with the focal cow and the trainer. The success criterion for this training was that the cow should be able to enter the individual calving pen (two times) and re-enter the group area (one time) without showing any fear-related behaviour (Table 2).

Entering- and re-entering tests. When a cow complied with either success criterion, she was left inside the individual calving pen to which she was trained as the trainer left through the gate. The cow then had a maximum of 4 hours to re-enter the group area on her own. This test was called *the re-entering test.*

When *the re-entering test* was passed, each cow was observed for 48 hours (on video) to check if she entered any individual calving pen(s) on her own. This period constituted *the entering test.*

When *initial training, the re-entering* as well as the *entering test* was passed successfully, a cow was considered ready for the experiment and thus complied with the overall success criterion (Fig 3).

Training progress and exclusion during training. The training progress was supervised for all 78 cows to make sure all cows complied with the success criterion and to ensure that all were trained for approximately the same amount of time. Seventy-four cows reached the criterion after 4 successive days of training. However, four cows still showed strong fear reactions towards the trainer and the feed bucket upon their fourth training day (eighth training session). These were given two extra training days. Thus, median of training duration was 4 successive days (range 4 to 6 days) until compliance (Fig 4). One cow did not reach the success criterion (housed with functional gates) within these extra days, and was excluded from the experiment. The remaining three cows managed to reach the criterion and were included. Two cows calved during the training period (i.e. before complying with the criterion) and were excluded. In total, 75 cows; 41 housed with functional gates and 34 housed with

Table 2. Description of fear-related behaviour to be absent when evaluating all trained cows irrespective of treatment according to their success criterion.

Behaviour	*Description*
Immobilization	The absence of movement of any limb or head[35]
Moving backwards	The animal is moving backwards by lifting and changing position of both forelegs or all four legs[36]
Flight	The animal runs (and/or jumps) at least 2 meters away from the trainer
Vigilance	Head raised above shoulder height, looking over barrier or side of the pen while upright, ears pointing forwards the direction of the head/eyes

None of the above behaviours could be shown during the last training step (i.e. step 1 or 4 respectively) if the cow were to comply with her criterion.

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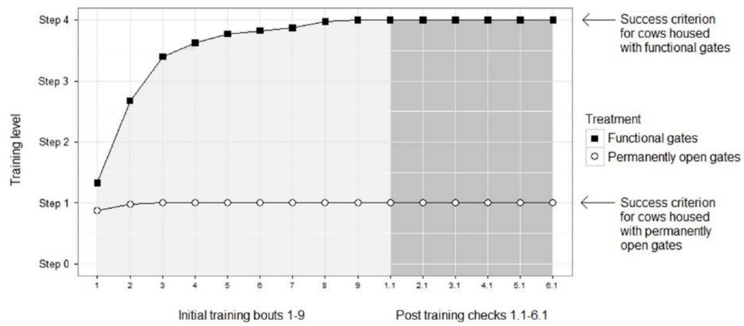

Fig 4. Graphs of mean training level per training bout for each treatment illustrating the progress during the initial training period (divided onto treatment: "Functional gates" and "permanently open gates"). Training level: Step 0 (not able to follow the trainer), step 1 (following the trainer in and out of individual calving pen = success criterion for "permanently open gates") and step 4 (following the trainer in and out of individual calving pen while opening the gate without any help or encouragement = success criterion for "functional gates"). Training bouts 1–9 (2 bouts per day) leading to experimental start (shown in light grey) and subsequent weekly post training checks 1.1 to 1.6 (1 bout per day) during the experiment (shown in dark grey).

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permanently open gates, were included after the training sessions, and they all complied with the success criterion in the subsequent weekly post-training checks (Fig 4).

Measures and analysis

Data editing and video recordings. For each cow the experimental period ended when she calved and each cow-calf pair was removed 5 to12 h after calving, in order to allow the cow time to nurse the calf and for the staff to have time to move them. This relatively rapid removal limited the period where potentially attractive alien calves were present [4]. Cows were excluded from the study if they had a stillborn calf (n = 2), gave birth to twins (n = 1), or had clinical milk fewer, mastitis or retained placenta (diagnosed by the herd veterinarian on the day of calving (n = 4)). Delivery of the calf was assisted if the calf was not born within 4 h after the appearance of the amniotic sac. Assisted calvings also resulted in the cow being excluded from the study (n = 2). Thus, 66 cows could be included in the analysis (35 housed with functional gates (from 7 groups having 5, 5, 5, 5, 4, 5, and 6 cows per group) and 31 housed with permanently open gates (from 6 groups having 4, 6, 5, 5, 6, and 5 cows per group); and 19 and 8 cows entering their second parity, 10 and 16 cows entering their third parity, and 6 and 7 cows entering fourth or later parities, respectively, for the two treatments). The behaviour of the cows was monitored by digital video cameras (model: TVCCD-624, Monacor, Bremen, Germany) mounted above the sections: Two cameras covered each end of each group area and one camera was mounted above each individual calving pen, leading to a total of 8 cameras per section. Recordings were made continuously throughout the whole experimental period, and an experienced technician monitored and stored all video material. The behaviour of each cow was observed continuously for 12 h prior to calving by an experienced observer after termination of the data collection. The same observer performed all video analyses.

Use of the individual calving pens and calving. Location of the cow and the occurrence of behavioural elements (Table 3) were monitored in order to keep track of the calving process and the use of the individual calving pens. The response variable was the location of the birth place (the placing of the cow when the hips of the calf were fully expelled from the birth canal), which also determined the end-point of the observation period. The location of the cow was determined as either within the group area or within an individual calving pen. If a cow was

Table 3. Ethogram of continuously recorded behaviour during the 12-h period prior to calving.

Placement of focal cow	*Description*
In group pen	> 50% of the body placed in the group pen
In a single pen	> 50% of the body placed in a single pen
Behaviour of focal cow	*Description*
Displaced by another cow	The focal cow moves away, more than the length of a cow, after another cow has approached the focal cow rapidly, made a 'head swing' towards the focal cow or butted the focal cow's head or body.
Displacing another cow	The focal cow causes another cow to move away with more than one cow length, by approaching the other cow rapidly, making a ´head swing´ towards the other cow or butting the other cow's head or body.
In contact with the gate	The focal cow is in physical contact with the gate (head, neck and/or shoulder).
Locked gate mistrial	The focal cow places the neck over the gate and pushes the gate with no success of opening it. (Only entering an individual pen with a functional gate)
Missed entering/re-entering	The focal cow opens the gate fully or partially, enters the gateway, but then moves backwards again to the side from where she came, or the cow enters the gateway but then moves backwards to the side from where she came.
Start of rhythmical abdominal contractions	First time the abdominal muscles contracts and release repeatedly in a rhythmic motion, cow can be standing or lying[7].
Other observations	*Description*
Calf´s legs visible	Calf´s legs visible outside the vulva of the focal cow, legs may be covered by the amniotic sac[7].
Another cow is licking the calf legs	Another cow licks the legs of the calf before the calf is born.
Behaviour terminating the observations	*Description*
Calving	The hips of the calf are successfully expelled from the focal cow[7].

The event of calving determined the endpoint of the observation period and thus all cows were observed according to their placement and behaviour within the experimental section group 12 h prior to calving.

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located between the two areas, the head of the cow determined the outcome. This definition was also used when determining where the cow experienced her first sequence of rhythmical abdominal contractions. The duration of the second stage of labour was defined as initiated at the first rhythmical abdominal contraction bout (Table 1) and continuing until the hips of the calf were fully expelled ([7,37]).

Social behaviour. Social behaviour includes displacements with physical contact and displacements without contact (described in Table 3). For each focal cow all displacements given and received were summarized over the 12 h video observation period. Furthermore, it was noted if an alien calf was present within the last 8 h before calving as this is the approximate period where cows have been reported to isolate [19,20]. Within each group, the order of calving was noted. For each calf born, a pregnant heifer (average age and body weight: 21.6 months and 575 ± 48 kg) was added to the group pen after the removal of the cow and her calf.

Gait scores and body weight. Cows were gait-scored when entering and exiting the experiment. The scoring was done by two experienced observers according to the 5-point scoring system developed by Thomsen et al. [38]. Two cows were scored as obviously lame (above score 3) when entering the experiment, but treated for laminitis (hoof washing followed by application of a bandage with salicylic acid treatment) upon entering, and scored 2 and 1, respectively, before calving. Median gait score was 1, when entering as well as exiting the experiment (range 1 to 4 at entry, and range 1 to 3 when exiting). Cows were weighted upon entering the experiment and when leaving the experiment 5 to12 hours after calving, by the use of the same automatic scale (Danvægt, Hinnerup, Denmark). The cows weighed on average (mean ± s.d) 687 ± 75 kg and 715 ± 61 kg when entering the experiment, and 674 ± 56 kg and 692 ± 65 kg when leaving the experiment, for cows housed with functional gates and for cows housed with permanently open gates, respectively. After calving, cows were machine milked once in the experimental pen before being moved from the barn. Rectal temperature was measured at this milking (mean ± s.d.: 38.7 ± 0.8˚C).

Maintenance of bedding and gates

Bedding quality of each individual calving pen and group area was evaluated daily at 08:30–10:00 am prior to removal of manure. The quality of the bedding was scored by the barn staff according to a 5-point scale [39]: (0) dry, no faeces or urea, (1) moist, some parts with faeces ($n < 3$), (2) slightly wet, both dark and light straw and more than 3 parts with faeces, (3) wet, mostly dark straw and faeces, (4) very wet, only dark straw and faeces and urea spread over the whole pen (median: 1, range: 0–4).

The force needed to open a functional gate from inside the individual calving pens and from the group area was measured by an electronic scale (model OCSF300, Scale House ®, 3711 NW 36th St., Miami, FL 33142) at the end of the experiment (5 measures per gate), showing that 12.5 ± 1.8 kg and 12.2 ± 3.6 kg force was needed, respectively.

Statistical analysis

Behavioural variables. We calculated the social dominance as a so-called 'rank ratio' for each cow based on the displacements summarized over the 12 h before calving: the number of individuals being displaced by the focal cow, and the number of individuals displacing the focal cow; the higher the ratio the higher the rank [40]. Afterwards, the ratio was converted into an index (x 100) indicating if the cow was in the dominant or subordinate end of the scale from 1–100 within the group. Calving order was identified as a categorical variable with 6 levels. The presence of an alien calf (a calf born from another cow which was not yet removed before the next calving) during the last 8 hours before calving was categorized as one or zero (present or absent). Similarly, the location where the cow experienced her first sequence of rhythmical abdominal contractions was categorized as 'Group area' or 'Individual Pen'. A preliminary analysis showed positive correlations (based on Mann-Whitney U test; [41]) between personality score (shy, bold) and dominance and thus only one of these two variables could be included in the model. Social dominance was chosen over personality assessment ('shy' or 'bold') as this measurement was based on observations of the cows close to time of calving.

Based on Shapiro-Wilk normality test and visual assessment of histograms, normality of the recorded variables could not be assumed. Hence, all variables were analysed using models and tests not assuming normality. Statistical analyses were performed using the R software, version 3.1.2 (R Core Team 2014, Vienna, Austria) and all p-values evaluated according to a significance level of 5% and 10% for tendencies.

Modelling. Due to the primary response variable having a binary outcome (i.e. calving site), data were analysed using logistic regression and package lme4 [42] in the R software. It was not possible to include season or other time-related measures in the analyses, as the section groups were temporally overlapping to ensure suitable inter-calving intervals within group. We did, however, include the placement of each section group within the barn as a random effect in a mixed effects model. Thus, the full model included the random effect of placement within barn (i.e. section number 1, 2 or 3 in Fig 1) as well as the fixed effects; dominance ratio within group at calving (mean ± s.d.: 64 ± 33), duration of second stage labour (mean ± s.d. (min): 108 ± 51), calving order within group (1, 2, 3, 4, 5 or 6), parity (second, third or older), presence of alien calf (0 or 1) and the location where the cow showed her first rhythmical abdominal contractions (individual pen, or group area). The full model did not show any effect of the random variable "section" (variance and s.d. = 0), which was subsequently excluded. A normal logistic regression model was then fitted to test effects of the explanatory variables. The normal logistic regression model was reduced using a backwards stepwise procedure with $p < 0.1$ as inclusion criteria. The final model included the fixed effects; rank ratio within group at calving, presence of alien calf and the location where the cow showed her first rhythmical abdominal contractions. The model fit was checked using the Hosmer Lemeshow Goodness of fit test [43]. Odds ratios and 95% confidence intervals were constructed from the final parameter estimates by back-transforming, applying the inverse logit function.

Calving location. In order to examine whether cows calved in random locations within each section, and thus were not affected by the location of previous calvings (as suggested by [31]), a 1-sample proportion test with continuity correction was used [44].

Results

All cows displaced other cows but also received displacements from other cows, and thus the social dominance of each cow within each group could be determined through rank ratio calculation without any cows sharing the same level of dominance within a group. Bold cows (n = 34) had significantly higher rank ratios whereas shy cows (n = 32) had lower rank ratios (Mann Whitney U-test: (median; range (rank ratio index)) bold: 93; 6–100 vs. shy: 41; 0–100, W = 806, r = 0.8, $p < 0.001$).

Factors affecting isolation seeking behaviour at calving

Effect of being housed with functional gates. Across the two treatments 34 cows calved in an individual calving pen and 32 in the group area. The odds of calving in an individual calving pen tended to increase when cows were housed in sections with permanently open gates compared to cows housed in sections with functional gates (Table 4).

Table 4. Summary of the output from the final model including 4 fixed effects.

Variable	Levels	No. of animals	*b*	S.E.(*b*)	OR	95% CI (OR)	*p*
Intercept			-10.25	3.75	-	-	
Treatment	Gate	35	0	-			0.068
	No gate	31	3.94	2.16	51.41	1.83; 72.97	
Rank ratio	Continuous	66	0.13	0.045	1.14	1.07; 1.30	0.0035
Presence of alien calf	No	39	0	-			0.069
	Yes	27	-4.67	2.58	0.46	0.011; 1.25	
1st rhythmical abdominal contractions	Group area	32	0	-			0.036
	Individual calving pen	34	3.99	1.91	54.05	2.89; 162.82	

'Treatment' i.e. having a functional gate or a permanently open gate, 'Rank' i.e. the rank ratio index within each group at calving, 'Presence of alien calf' i.e. having an alien calf present within the last 8 h before calving or not and 'where 1st sequence of rhythmical abdominal contractions occurred' i.e. in the group area or in an individual calving pen. Odds ratio for each variable with corresponding 95% confidence intervals and p-values are presented along with coefficients '*b*' and stand errors 'S.E.(*b*)'.

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Effect of dominance. Rank ratio significantly affected choice of calving site; the higher the rank within a group at calving, the higher the odds of calving inside an individual calving pen. For every increase in rank order of 1 the odds of calving inside the individual pen increased by 1.14 (Table 4), and due to the assumption of proportional odds in the model this was the same for each level of rank e.g. rank 33 to 34 and rank 66 to 67.

Effect of location of first rhythmical abdominal contractions. Another factor, influencing the choice of calving site, was the location of the first sequence of rhythmical abdominal contractions. Fifty-one percent of the cows had the first sequence of rhythmical abdominal contractions while being in an individual calving pen and these cows had significantly higher odds of calving inside the individual calving pen than cows showing their first sequence of rhythmical abdominal contractions within the group area (Table 4). Additionally, 10 cows (4 housed with functional gates and 6 housed with permanently open gates) had their first rhythmical abdominal contraction in the group area and then moved to an individual calving pen during the second stage of labour, whereas 6 cows (3 cows housed with functional gates and 3 cows housed with permanently open gates) moved from an individual pen to the group area during the second stage of labour.

Effect of presence of an alien calf. An alien calf was present within the last 8 hours prior to another calving for 41% of the cows. For these cows the odds of calving in an individual calving pen decreased by 0.46 (Table 4).

Three variables had no effect in the full model and were thus stepwise removed from the model: First parity ($p_{parity2} = 0.85$ and $p_{parity3} = 0.62$), then duration of second stage labour (mean ± s.d.: 108.5 ± 51.4 min) ($p = 0.65$) and lastly calving order within group (1 to 6) ($p_2 = 0.89$, $p_3 = 0.16$, $p_4 = 0.49$, $p_5 = 0.38$ and $p_6 = 0.41$). These had no effect on the odds of calving inside an individual calving pen ($p > 0.1$) and were thus not included in the final model.

Calving location

The median dispersion between calvings within group was 6 (range: 0 to 20) days. For 8 out of 13 groups, one or two cows (n = 10) calved in close proximity to the location of the last calving (within one cow length from the site of last calving). When testing these groups separately in the 1-sample proportion test, however, no evidence for selection of calving site due to the site of a previous calving could be found, neither for groups with one cow calving close to another or groups having two cows calving close to a third cow.

Discussion

In this study, we gave cows an opportunity to move to another pen to calve and examined whether isolation seeking behaviour was facilitated by this opportunity to isolate (seek seclusion as well as increase the distance to group members). Additionally, the study included social aspects of housing pre-parturient cows in groups and the effects of these on choice of calving site. We found that 50% of the cows moved away from the group and isolated in an individual calving pen regardless of the presence of a functional gate in the pen or not. Unexpectedly, cows housed with permanently open gates tended to be more likely to calve in the individual calving pens than cows with functional gates. Dominance increased the odds of calving in an individual calving pen whereas the presence of an alien calf, during the last 8 hours prior to calving, decreased the odds.

Contrary to the hypothesis, cows housed with permanently open gates tended to be more likely to calve in the individual calving pens compared to cows housed with functional gates. This implies that the cows experienced the gates as obstacles rather than an advantage for isolating before calving. As we have previously found that cows seeking maximum isolation (75%

versus 50%) at calving had longer second stage labour [39], the fact that there was no effect of the duration of second stage labour on the choice of isolation in this study further supports that the gate may have been an obstacle for the cows. All cows received prior training and complied with a learning criterion for being able to use the gate before being included in the experiment, suggesting that the cows had learnt to use the gate. In none of the treatments did the choice deviate from random. In this study, the cows had to combine a learnt and rather conspicuous response (opening the gate and knowing that no one could follow) with the motivation to isolate. Research on the cognitive abilities of cattle is generally limited, but cows do possess the ability to perform rather complex instrumental conditioning tasks [45], have quite good spatial memory and are able to navigate quite complicated mazes which they memorize for up to 6 weeks [46]. Furthermore, all cows used the pens on their own several times before calving, and approx. 50% ended up calving in one of the pens. Therefore, there is no particular reason to argue that the cows did not learn and memorise the task of opening the gate. Memorising a conspicuous task compared to memorising e.g. a route to a preferred grass patch may however, be of less biological relevance (as argued for horses by [47]). Furthermore, being in labour and experiencing pain may have made it difficult for the cows to recall this knowledge. Not because the task of isolating was not biologically relevant, but because the task of opening a rather conspicuous gate was less biologically relevant in this situation. The gate may also have been perceived as too heavy to manoeuvre, or uncomfortable to pass, especially once the cow was in labour. Being in labour, the abdomen of a pregnant cow may become increasingly sore as contractions arise [1] and thus opening a gate which place pressure on the abdomen may be unpleasant. Furthermore, being in labour pain, cows may have tried to avoid potential conflicts with conspecifics and thus they may have favoured to be able to control their visual field and have the opportunity to withdraw from conspecifics. Therefore, entering a gated pen may have been too risky also because it required effort to leave. In terms of the evolutionary history of ungulates, the trade-off between isolation seeking and having less control in term of executing a flight response to avoid a predator also supports this. Lastly, the distance between the group area and the individual calving pen may have influenced whether cows used the individual pens for calving or not. In a semi-natural setting, Lidfors et al. [19] noted that cows walked a considerable distance away from the group and it is thus possible, that in the present study, the cows perceived the individual calving pens as situated too close (maximum 9 m away) to the rest of the group in order for the pens to be perceived as offering isolation.

Future studies on motivation-based calving facilities may examine whether gates that are less conspicuous and require less effort to open result in more cows calving in them. Potentially, a gate that closes behind the cow when entering the calving pen, without a need for pushing or manipulation may be more appropriate. Also combining a simpler and less heavy gate with a pen that offers a higher level of isolation could be one option to facilitate the use of individual calving pens. In addition, potential effects of distance between individual calving pens and the group-members should be examined. Lastly, more research on learning and memorising of more complex instrumental tasks in cattle is needed in order to understand the capacity of cattle to learn how to perform a task and recall it in the peri-parturient period, where other underlying motivations may be strong.

In the present study social order within the group influenced whether cows chose to calve in the individual calving pens (Table 4). When analysing the variable "rank ratio" an assumption of proportional odds (i.e. that the odds for each increase in rank by a factor 1 would be the same) had to be made, and thus this assumption should be kept in mind when the results are interpreted. Furthermore, when using the term "rank ratio" we had to assume that the social dominance in these groups was linear, which may or may not be the case. However, if groups of pre-calving cows are newly established, or dynamic, there is a risk of aggression due to the establishment of dominance relations [25], especially near defendable resources. Cows become maternally motivated due to hormonal change prior to calving (reviewed in [48]) and as calving approaches an individual calving pen may become a valued site. In the present study, dominant cows may have guarded the individual calving pens leaving them a higher chance of calving there. Also being dominant they were less likely to be displaced from the pens with permanently open gates. This suggestion may be supported by the finding that cows positioned inside an individual calving pen when the second stage of labour was initiated, had a significantly higher probability of calving in the pen. It is possible, that the choice of where to calve was already taken when the second stage of labour was initiated, and thus, if a cow had been guarding an individual calving pen, she was more likely to end up calving there. Moreover, cows that changed their position after initiation of the second stage of labour mainly changed from the group area to an individual calving pen (10 out of 16 cows). These cows were mainly dominant cows (8 out of 10) and all cows moving from an individual calving pen to the group area (n = 6) were subordinate (happened in sections of both experimental treatments, 3 from each), further implying that dominance continued to play a role, even during the second stage of labour. These findings have implications for the design of a motivation-based calving facility as in this case, the facility only succeeded in proving an isolation opportunity for the dominant cows even though there was always one individual calving pen per cow. More research is needed in order to outline how to account for dominance in a motivation-based calving pen design. Additionally, this highlights the role of the social hierarchy in a pre-calving group and points out that farmers may have to consider that some cows need more protection if they are to move away from the group at calving. Another, perhaps surprising result, in relation to this, is that social dominance ("rank ratio") strongly correlated with the personality assessment made prior to the experiment and at least one week prior to calving. This correlation showed that bolder and more explorative cows were also the more dominant while shy cows were more subordinate. This information combined with the above results may be useful for farmers trying to identify cows needing assistance in order to enter individual calving pens and shy cows may even have to be moved manually. In this experiment most shy cows calved in the group area, and protection from competition for individual calving pens may be required. Personality assessment may be a practical tool to assess dominance and to identify cows that may have to be moved manually to an individual calving pen.

Another social aspect of pre-parturient groups, which farmers also need to be aware of, is the presence of alien calves in the group. In this study, the presence of an alien calf lowered the probability of the cows calving inside the individual calving pens, potentially because of an attracting effect of either the calf as such [4] or the amniotic fluids in the fur of the new-born [49]. Combining this knowledge with the described effects of dominance, these social aspects may make a motivation-based calving system difficult to apply unless subordinate cows are moved manually to an adjacent maternity pen.

The fact that not all cows calved in the calving pens does thus not imply a lack of learning or knowledge of this opportunity, but rather a combination of competition and motivational conflict. For a motivation-based calving facility to function, these issues have to be solved. As discussed above, improving or increasing the quality of the isolation opportunity is important especially when aiming to facilitate isolation seeking at the time where they are motivated to do so. Competition and distractions are problems that may not be easily solved, but if a cow moves to an isolated area before giving birth and if her calf is subsequently retained there, this would nonetheless decrease the level of competition and distractions in the environment of the cows. From this perspective, another possible approach may be to use the presumably attracting effect of birth fluids [31] or amniotic fluid at specific points inside the calving pens. As these substances have previously been shown to attract cows no earlier than 12 h prior to giving birth [49], this may be an option to attract only the cows that are close to calving and move them away from the herd before they (or their calves) become a distraction. Additionally, this attraction may function as a somewhat neutral stimuli for cows that are not yet close to calving.

Conclusions

In this study we could not show that use of a functional gate combined with prior training facilitated isolation seeking, as measured by the use of individual calving pens at the time of calving. This may be due to the rather conspicuous instrumental task of opening the gate or because the cows were trying to avoid a potentially confined situation where executing a flight response may be difficult. Alternatively, the cows may not have been able to combine the learnt response with the motivation to isolate. Social factors had a strong influence on the odds of a cow calving inside an individual calving pen, with dominant cows having the highest odds of occupying an individual calving pen in the moment of calving and the presence of an alien calf reducing the likelihood of cows calving inside the individual calving pen. Therefore, social factors have implications for the functionality of a motivation-based system. More research is needed to fully outline the cognitive basis from which cows are able to learn and recall learnt responses and whether these responses are overruled by other motivations once calving is in progress.

Supporting information

S1 Video. Cow performing the last step in the training procedure for cows housed with functional gates. The video mimics the training situation as this particular cow is not an experimental cow. She was trained on randomly chosen pens and with the five other group members present. Prior to this take, the cow has been successful in all the previous training steps. At this stage the cow is trained to open the fully closed gate on her own and subsequently enter the pen to reach the reward from the trainer facing the cow from inside the pen/the group area (step 4 in the training procedure). The trainer ensured that no other cows followed the focal cow into the individual calving pen at any point.
(MP4)

S1 File. Gate errors.
(DOCX)

S2 File. Data. The data sheet provided for this study includes all necessary information in order to recalculate the results of the study. The sheet has one horizontal line for each individual cow in the experiment and each cow has its own identification number ('cow_number'). All subsequent columns has an explanation line on the top of the sheet and whenever data is not available for the particular cow this is marked with a '.'. Two variables are one/zero variables corresponding to 1 = "yes and 0 = "no".
(XLSX)

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5.5. Study 5

Paper 5:

Pre-partum maternal behavior of domesticated cattle: a comparison with managed, feral, and wild ungulates.

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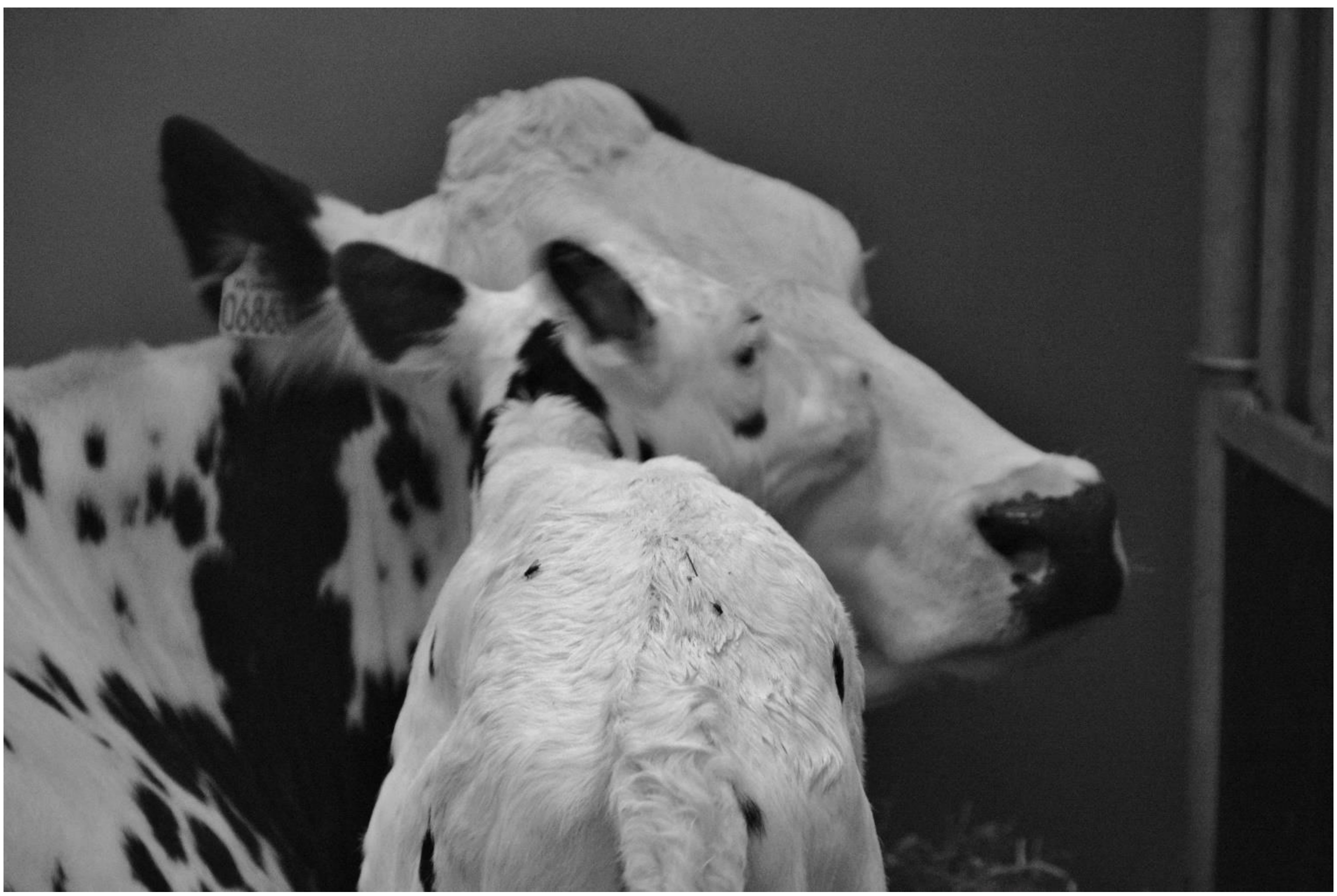

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Prepartum Maternal Behavior of Domesticated Cattle: A Comparison with Managed, Feral, and Wild Ungulates

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The event of giving birth is an essential part of animal production. In dairy cattle production, there are substantial economical and welfare-related challenges arising around the time of parturition, and hence increased focus on efficient management of the calving cow. Drawing on the research literature on prepartum maternal behavior, this review compares cattle to other members of the ungulate clade with the aim of understanding the biological basis of bovine prepartum behavior with main emphasis on dairy cows. Ultimately, this knowledge may be used in future development of housing systems and recommendations for the management of calving cows. Maternal prepartum behavior varies among species, but the final goal of ungulate mothers is the same: ensuring a calm parturition and optimal environment for the onset of postpartum maternal behavior by locating an appropriate birth site, with low risk of predators, disturbances and mistaken identity of offspring. Features of chosen birth sites vary among species and depend largely on the environment, as ungulate females display a considerable ability to adapt to their surroundings. However, within commercial housing conditions in dairy production, the animals' ability to adapt behaviorally appears to be challenged. Confinement alongside high stocking densities leave little room to express birth-site selection behavior, posing a high risk of agonistic social behavior, disturbances, and mismothering, as well as exposure to olfactory cues influencing both prepartum and postpartum maternal behavior. Dairy cows are thus exposed to several factors in a commercial calving environment, which may thwart their maternal motivations and influence their behavior. In addition, prepartum cattle may be more affected by olfactory cues than other ungulate species (e.g., sheep) because they are attracted to birth fluids already before calving. Hence, providing dairy cows with an environment where they can perform the maternal behavior they are motivated for, may aid a calm and secure calving and provide optimal surroundings for postpartum maternal behavior. Future research should focus on designing motivation-based housing systems allowing freedom to express prepartum maternal behavior and investigate in more detail the effects of the environment on the welfare of calving cows and their offspring.

Keywords: behavioral plasticity, birth place, cattle, isolation seeking, maternal behavior, motivation, olfaction, parturition

INTRODUCTION

The event of giving birth is an essential part of animal production. There are substantial economical and welfare-related challenges arising around the time of parturition, and commercial animal production have developed an extensive body of recommendations for housing and managing parturient females. In beef and dairy production, successful management of the calving cow aims to ensure a viable calf with no detrimental effects for the cow. In addition, a smooth transition from dry to lactating is important for dairy cows. To achieve these goals, recommendations state that careful supervision during calving and timely intervention is crucial. Hence, calving cows should be kept in a way that enables the farmer to identify cows in need of assistance. Recent guidelines suggest that cows should calve in individual pens [e.g., by law in Denmark (1) and in The Canadian Dairy Code of Practice (2)] partly based on the finding that cows increase the distance to the herd before calving if they have the opportunity (3). These guidelines appear to be well suited to the behavior of parturient cows, but the motivation underlying this behavior is not known. Are the cows motivated to move away from the herd to avoid other cows, to hide from disturbances in general, or are they attempting to hide from specific threats? If the causal factors underlying the prepartum behavior of parturient cows are not understood, is it then certain that aspects of animal welfare related to behavioral needs and highly motivated behavior are accounted for when cows are kept in individual pens at calving? Keeping cows in individual pens benefit the farmers—and by extension health aspects of dairy cow welfare—due to easier calving supervision and assistance when needed, but does it also satisfy maternal motivation of the cows?

The survival and development of mammalian young depends largely on a strong mother–offspring relationship. The clade Ungulata includes mainly precocial species giving birth to well-developed offspring, capable of moving on their own shortly after birth (4). To protect their vigorous offspring, ungulate mothers exhibit complex behavioral patterns starting in late pregnancy and continuing through parturition and lactation (5). This differs substantially from the normal adult female behavior and functions to provide the young with sufficient nutrition, warmth, protection, comfort, and opportunities for social transmission of information [as reviewed in Ref. (6)]. In the domesticated species, reproductive success has a huge impact on productivity, and thus scientific focus has been mainly on successful parturition and subsequent lactation, and far less on the period leading up to parturition. Both beef and dairy cattle production rely on the cows' ability to reproduce, but it is only in beef cattle production that farmers depend on the ability of the cow to establish a strong and long-lasting bond to her calf, providing it with nutrition and protection until weaning (7). Dairy production is based on the cow's ability to produce milk after removal of the newborn calf (7), and thus selection for maternal behavior in dairy cows may have been relaxed compared with beef cattle due to the reduced need for post-calving maternal investment. However, this does not take into account the inevitable need for prepartum maternal behavior aiming to ensure smooth calvings with few (or preferably no) complications. This will further safeguard strong and healthy calves, as well as healthier and more productive cows with low morbidity (**Figure 1**). Indeed, post-calving success is likely to be dependent on the pre-calving success, which emphasizes the need for appropriate prepartum maternal behavior.

A small body of literature has shed light on the prepartum maternal behavior of the cow, although mainly under production conditions [e.g., Ref. (8, 9)] and only to a lesser extent under semi-natural conditions [e.g., Ref. (3, 10) further details **Table 1**]. To date, there are only few studies on the prepartum maternal behavior of feral cattle [Maremma cattle (11), Masai cattle (12), Chillingham cattle (13), and Camargue cattle (14)] and these were all carried out several decades ago. This may seem surprising as no less than four articles over the last 40 years have pointed out the need for more comparative studies of ancestral and domestic behavior in cattle (15–18). Given that the ancestor of cattle, the Auroch, has been extinct for centuries (19) and the number of feral cattle herds are very limited (**Table 1**), one potential approach to understanding the biology underlying prepartum maternal behavior of domesticated cattle, is by comparison with other ungulate species. Studies of feral cattle may be more likely in the future with the advent of conservation grazing [e.g., Ref. (20)], giving more opportunity to observe prepartum behavior under natural or low-managed conditions.

This review draws on literature from feral and commercial cattle breeds and investigates similarities and dissimilarities to other members of the ungulate clade. With the main emphasis on dairy cows, our aim was to understand the biological basis of prepartum behavior of feral cattle to improve the understanding of motivations underlying and mechanisms causing the behavior seen in domestic cattle today. In the future, this knowledge may benefit the dairy industry and lead to better-adapted housing system designs and recommendations for better prepartum management practice, which improves both efficiency and animal welfare.

WHY ISOLATE?

Many ungulate studies have reported that a proportion of the females are "hiding," "isolating," "being secluded," or "seeking away" from the herd or from other "threats" around the time of parturition. The term "isolation seeking" is commonly used in such studies, but what is termed isolation in one species may differ from what is termed isolation in other species. Irrespectively, the term "isolation seeking" is used to indicate the purpose of the behavior: to hide and seclude the female from disturbances (arising from various threats), thus allowing her to give birth in a calm place, where she subsequently is able to nurse and bond with her young. However, as isolation seeking in one ungulate species may differ from that of other species, the comparison of different ways to achieve the same goal is relevant, especially as the underlying motivations of females of different species may or may not be the same. In the following, isolation behavior is discussed in the context of causality, whereas the hider/follower paradigm is dealt with in Section "The Hider/Follower Paradigm," although some overlap is unavoidable.

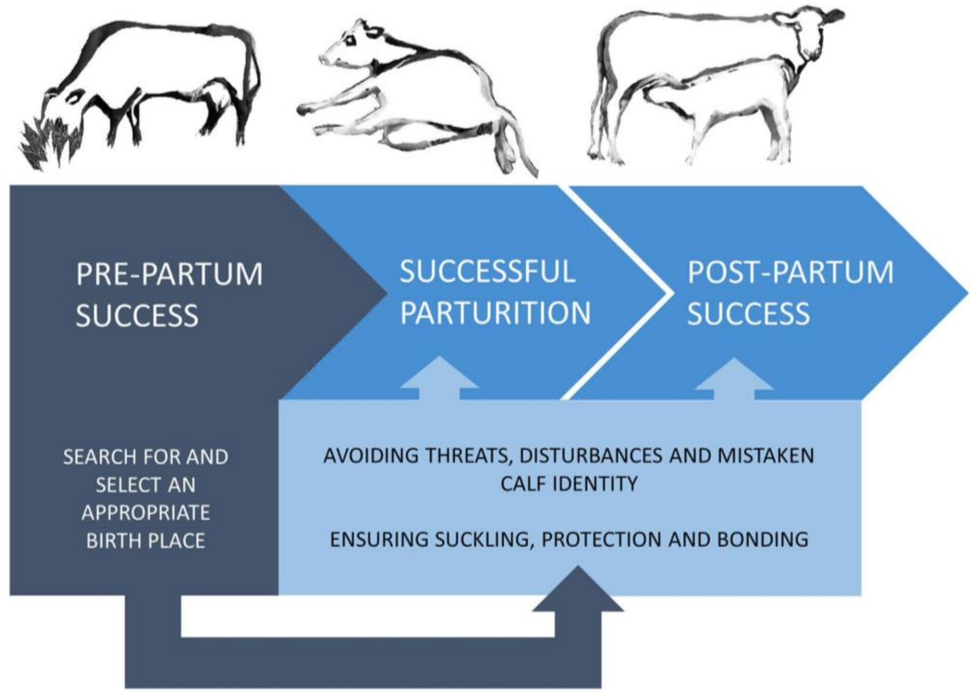

FIGURE 1 | The impact of the prepartum search for and selection of an appropriate birth site. Prepartum success depends on the female's ability to locate an appropriate birth site to ensure and safeguard a calm parturition and optimal surroundings for postpartum maternal behavior by lowering the risk of predators, disturbances, and mistaken identity of offspring. This, in turn, increases the chance of postpartum success.

In sheep, Dwyer and Lawrence (50) suggested that birth-site selection (termed "isolation seeking") varies with increasing degree of domestication. Wild and feral breeds of sheep such as mouflons, Soay, Dall, and bighorn are observed to move away from the herd to rocky and secluded areas (51–55), hence they may not distance themselves from the herd, but seek cover, or a combination of the two. Domesticated breeds such as Merino sheep also distance themselves from the herd, but only when the environment offers a degree of elevation or topographical change, otherwise the ewes give birth within the herd (56). This may be influenced by artificial selection for more sociability in the domestic breeds (50), but is evidently also affected by the environment (see The Hider/Follower Paradigm).

So far, some ungulate studies have sought to explain prepartum isolation seeking behavior of females, and noted that the characteristics of the birth site itself may be less important than the ability to move away from disturbances (57). An example of this can be seen in wild Thomson's gazelles (58): Roberts and Rubenstein showed that if a herd caught up with a parturient female, the newborn fawns were usually killed by jackals, presumably due to the group being too conspicuous. In such cases, being disturbed by the herd can have fatal consequences, and since disturbances during parturition were much more common for non-isolating than for isolating females, the hiding aspect of the isolation seeking behavior appears important for the survival of the offspring in this species. Yet, other studies indicate that fallow deer dams adapt their maternal behavior to the prevailing predator pressure, which even may supersede forage availability (59). This is a sensible priority as a predator is an acute survival threat as opposed to lack of food, which may be tolerated in the short term. Another example of prepartum isolation comes from breeds of domestic sheep, which move to the edges of their enclosure to lamb, thought to be caused by disturbances arising from human activity (50). Likewise, indoor-housed domestic sheep will use a cubicle at lambing when given the opportunity (60, 61). In addition, when disturbed by human activity in the area, elk dams will change their movement pattern, especially if disturbed during calving season (62). Assuming that wild ungulate dams perceive humans as predators, such behavioral flexibility, may have originated from sensitivity to predator pressure (58). Avoiding predators by hiding is an adaptive behavior as it reduces the risk of having the offspring killed and these behaviors may thus be preserved in domestic species. Hence, isolation may be a means to avoid disturbances in general, but more specifically avoid predators or other immediate threats. Irrespectively, in a commercial livestock production environment, where females are surrounded by herd mates, hiding will often be difficult, especially if human activity and other disturbances are frequent. More work is needed to examine whether domestic females are aiming to avoid threats, and whether disturbance may cause artificial isolation opportunities to be less attractive

TABLE 1 | Overview of observations within studies of maternal behavior in cattle with main emphasis on prepartum behavior.

	Feral cattle	Pasture-kept cattle	Cattle housed in intensive commercial environment (mainly indoors)
Features of the birth site			
Vegetative/visual cover	Differs with habitat[h,k]	Differs with habitat[E] Mainly visual cover[A]	No clear preference[19]
Provides cover from disturbances	*Not studied*	Yes, from herd members[A]	Calving when quiet in the barn[1,5,21] Higher stocking density results in lower "isolation seeking"[17]
Distance to herd	Leave the herd but no defined distance[e,g,h,i] 10–380 m away from the herd[d]	Leave the herd but no defined distance[G]	*Not studied*
Prepartum behavioral changes			
Separation from herd	Yes[a,c,e,g,h,i,j] Only some cows do[b,d,f]	Yes[A,C,E] Only some cows do[F] No[B,D,H]	Yes[4,16] Only some cows do[6] Yes, but depends on calving difficulty[19]
Restlessness	Yes[d]	Yes[A,C,F]	Yes[1,3,4,10,12,21] Varies with calving difficulty[2,19]
Increased walking/searching	Yes[d]	Yes[C,F]	Yes[1,7,12,13,14,19,22]
Lying time	*Not studied*	Unchanged[G]	Lower on the day of calving[10,15] Higher 8 h before calving[3]
Increased transition from standing to lying and *vice versa*	Yes[d]	Yes[A,C,G]	Yes[1,2,3,8,10,12,13,14,15,19,22]
Increased sniffing/exploration	*Not studied*	*Not studied*	Yes[1,14,19,22] No[2]
Increased tail raising	*Not studied*	Yes[C,F]	Yes[2,11,12,13,14]
Licking own body and attention toward abdomen	Yes[d]	Yes[C]	Yes[1,10,12,22] No[11]
Scraping or pawing the ground	Yes[d]	Yes[C]	Yes[1,22]
Less feeding behavior	Yes[d]	*Not studied*	Yes[10,12,13,14]
Reduced rumination	*Not studied*	*Not studied*	Yes[1,3,7]
A role of olfaction			
Licking of own birth fluids	Yes[d]	Yes[A,C,F]	Yes[1,12,14,18,20,22]
Calving at own birth fluid spot	*Not studied*	Yes[A,C]	Yes[18,20]
Mismothering	Not observed[j]	Yes[A,B,F] Not observed[G]	Yes[6,9,19]
Interest and sniffing from other cows during calving	No[j] Yes[f] Only from cows close to calving themselves[d]	Yes[A,B,F] No[G]	Yes[6,9,15,18,19]

The table includes 41 studies separated into the categories: feral (n = 11), pasture-kept (n = 8), and intensive commercial cattle mainly housed indoors (n = 22). Aspects of and behaviors related to three different subjects (features of the birth site, prepartum behavioral changes, and the role of olfaction) are listed. Numbers and characters in superscript indicate the corresponding reference listed at the bottom of the table, with the number in brackets after each reference indicating the order in the reference list. 'Not studied' refers to the authors being unable to find any literature on this specific aspect.

References: [a]Baskin and Stepanov (21); [b]Finger et al. (22); [c]Hall (13); [d]Kiley-Worthington and de la Plain (23); [e]Lent (4); [f]Lidfors and Jensen (24); [g]Reinhardt (25); [h]Reinhardt et al. (12); [i]Schloeth (14); [j]Vitale et al. (11); [A]Aitken et al. (26); [B]Edwards (27); [C]George and Barger (10); [D]Lidfors (28); [E]Lidfors et al. (3); [F]Owens et al. (29); [G]Rice et al. (30); [H]Wood-Gush et al. (31); [1]Arthur (32); [2]Barrier et al. (33); [3]Borchers et al. (34); [4]Dufty (35); [5]Edwards (36); [6]Edwards and Broom (16); [7]Houwing et al. (17); [8]Huzzey et al. (37); [9]Illmann and Špinka (38); [10]Jensen (8); [11]Lange et al. (39); [12]Metz and Metz (40); [13]Miedema et al. (41); [14]Miedema et al. (42); [15]Proudfoot et al. (43); [16]Proudfoot et al. (44); [17]Proudfoot et al. (45); [18]Rørvang et al. (46); [19]Rørvang et al. (47); [20]Selman et al. (48); [21]von Keyserlingk and Weary (49); [22]Wehrend et al. (9).

[as suggested by Rørvang et al. (63)]. The level of disturbances can be high in commercial environments (i.e., from humans and conspecifics), and the use of an artificial hide by the cow may reduce her perceived ability to escape a potential threat; hence, some artificial hides may not provide an attractive birth site.

Another adaptive aspect underlying isolation is a reduced risk of mismothering, i.e., cows licking and nursing calves that are not their own offspring. The immediate licking and sniffing of the young by the dam are part of the typical behavioral repertoire of ungulates enabling the mother to learn the odor and features of her young for later recognition, thereby ensuring that her parental investment is directed toward her own offspring (54, 64). Several studies in domestic cattle have shown that group housed peri-parturient cows may lick alien calves, i.e., calves born from other cows [e.g., Ref. (27)] and cross-fostering (i.e., when a cow adopts an alien calf by allowing it to suckle) has also been reported [e.g., Ref. (65)]. However, observations from feral cattle herds indicate that cows rarely nurse or lick alien calves [Maremma cattle (11), Masai Cattle (12)]. The feral cow and calf may develop a stronger mother–offspring bond, which is established through intensive contact during a sensitive period just after parturition [potentially just a few hours after calving (4, 49)]. This bond may be established quicker in an undisturbed calving environment, which may not be available for domestic cows in a group pen. Calving in a group pen leads to an increased risk of mismothering and failure to obtain colostrum by the calf, thereby challenging the transfer of immunity *via* colostrum intake (27, 38). One reason for the observed mismatches between dairy cows and alien calves may be a weakening of the maternal motivation in dairy breeds. Even though dairy cow maternal behavior may have been modified by genetic change, studies from other domesticated animals [e.g., nest building in pigs (66, 67) and mice (68)] suggest that maternal behavior is preserved despite domestication. Although we cannot exclude that the occurrence of mismothering reported by studies on dairy cattle to some extent is affected by genetic change, a more likely influential factor is disturbances caused by the confined environment. Taken together, the above comparison of prepartum maternal behavior of female ungulates suggest that the behavior described as isolation seeking may be an expression of birth-site selection; functioning to safeguard a calm and secure birth process by avoiding threats and disturbances potentially posing a risk to the survival of the female and the newborn in terms of predation and mismothering whilst at the same time ensuring suckling, bonding, and protection (**Figure 1**).

WHAT ARE THE PROPERTIES OF AN APPROPRIATE BIRTH SITE?

Natural selection favors mothers that display behavior and habitat selection to enhance neonatal survival (59, 69, 70). Hence, in a variable environment, natural selection will favor mothers that are able to modulate and adapt their maternal behavior including habitat selection to the prevailing circumstances. This ability to adapt is evident in an array of maternal behaviors. For example, if ungulates are kept in environments with few options to search actively for an appropriate birth site, the searching behavior displayed by the females may be less pronounced. Due to the scarcity of dairy cow studies on these issues, this section will draw predominately on findings from other ungulate species. Fouda et al. (71) reported that zoo-kept sika deer, a species known to hide their offspring in nature, gave birth within the herd. The authors concluded that this behavior resulted from the lack of suitable sites where a fawn could be hidden. Lott and Galland (72) saw some isolation seeking in American pasture-kept bison. They stated that the bison gave birth away from the herd when vegetative cover offered visual isolation from the herd, whereas calving happened within the herd when visual isolation was not possible. Roberts and Rubenstein (58) found that Thomson's gazelle females spent considerable time searching for a suitable place to give birth (sometimes traveling more than a kilometer) and mainly gave birth in tall grass away from the herd. However, the authors observed that a herd would occasionally catch up with the parturient female, negating the effects of cover availability by their presence. For other ungulates, clear topographical birth-site preferences have been found. Feral goats appear to prefer birth sites protected by an overhead or vertical cover, e.g., trees or hedges (73). Domestic sheep are known to predominantly give birth on slopes and in depressions in the ground or areas close to hedges and walls (74, 75), whereas mountain sheep are attracted to high, rocky areas with cliffs (51, 76). Other species, such as red and fallow deer (59, 77, 78), pronghorn (79), elk (80), wild mouflon sheep (52), and moose (81), favor thick vegetative cover providing visual isolation from conspecifics. For these species, further studies are needed to ascertain if such preferences are expressions of motivation to isolate in terms of distance from the herd or to hide from the herd as well as other disturbances including predators. Cattle do not appear to show clear preferences for specific birth-site types, even though a few studies on dairy cows have tried, without success, to elucidate what features are favored (27, 47). Across studies of bovine birth-site selection, the presence of vegetative cover may play a role (3, 11, 24, 26) for the occurrence of isolation behavior.

DOES SEPARATION DISTANCE FROM THE HERD MATTER?

One important aspect of birth-site selection is the physical distance the parturient female moves away from the herd. In many ungulate species, parturient females distance themselves from the herd [zebra (82); sable antelope (83); bison (72); elk (80); pronghorn (79); horse (84); red deer (77, 85); impala (86); goat (87); various wild sheep breeds (51, 53, 76, 88, 89)], although the exact distance moved by the females has received only modest attention. The only mention of this was by Karsch et al. (76), who found that parturient ewes of wild breeds moved more than 2 km away from the herd. Many studies included distance from the herd as part of the definition of isolation when studying prepartum behavior of females, but only rarely noted the actual distance. For example, Kiley-Worthington and de la Plain (23) observed free-ranging cattle and noted that isolation seeking was rare even though they did not include a definition of the term other than observing cows moving 10–380 m away from the herd. The authors also noted that the herd sometimes moved with the pre-parturient cows, thereby reducing the distance between them, similar to the findings by Roberts and Rubenstein in Thomson's gazelles (58). Another study by Flörcke and Grandin (90) found that red angus beef cows moved 25–1,250 m away from the main herd when calving and the authors further noted that 88% moved more than 100 m away. One complicating aspect of distance between the parturient female and potential threats or disturbances in her environment is the interaction between the distance and the possibility to hide. For example, when ungulates live in flat and barren environments, hiding the offspring becomes difficult irrespective of the maintained distance to threats/disturbances. Blank et al. (91) found that for goitered gazelles in a habitat without vegetative cover, where the mothers were unable to visually hide their offspring, distance between mothers and offspring became crucial for the mother not to attract predators to the young. As the mother was unable to visually hide the offspring, she increased the distance to the offspring, seemingly to compensate for the lack of vegetative cover. This was shown in pronghorn mothers living on mixed-grass prairies, where the mothers separated themselves on average 269 m from the young (92). In other words, these mothers distanced themselves to where the young was hiding when cover was deficient indicating that the increased distance was motivated by protection of offspring from predators. Unfortunately, no data are available for domesticated ungulates kept under natural conditions, but the above findings suggest that cover is an important part of birth-site selection, and that parturient females only relocate long distances in situations where physical cover is limited.

Although isolation, hiding, seclusion, and seeking away can all be part of prepartum behavior of ungulates, it may be more appropriate to use "birth-site selection" to describe the ultimate (functional) causation of the behavior observed. Female ungulates appear to favor birth sites providing protection from predators as well as conspecifics, and the preferences of the dams seem largely to depend on the environment. During the selection of a birth site, physical cover may be an important factor, but in situations where such cover is limited, distance from the herd may become increasingly important.

THE HIDER/FOLLOWER PARADIGM

Within ungulate species, two different peri-parturient types are described in the literature; these are the "hider" and "follower" strategies of ungulate offspring and mothers (4, 51, 87, 93). However, comparative research within ungulate species has shown that the hider–follower dichotomy may be overly simplistic, and that a number of species may be either, depending on the circumstances. Thus, instead of being either/or, in reality, hiding and following strategies may form part of a continuum, and both of these are considered antipredator strategies. Hiders provide protection in terms of hiding the young in covered or secluded habitats after giving birth (4), while followers actively look out for and avoid predation in open habitats by keeping their offspring close (4, 93). Incorporated into the continuum of these two behavioral types is the dependence on the environment, and at present little is known about the extent to which the behavior of an *individual* ungulate mother and young varies depending on variations in the environment.

It is suggested (87) that goats, which are considered typical hider species, were only able to express "true hider characteristics" when kept in their natural environment. In accordance, Tennessen and Hudson (94) found that in domestic goats, early mother–kid contact shared more characteristics with the behavior of follower species. These authors suggested that either the hider characteristics of goats were lost through domestication or maternal behavior changed when the animals were kept in a different environment. Later, studies of goat behavior showed that domestic goats do separate themselves from herd mates before kidding (95). Also, their rather complex hider behavior appears to be largely genetic, making it a highly motivated behavior and thus less prone to evolutionary dilution (96), even though it may be influenced by environmental factors.

Feral populations of ancient cattle breeds living in large and non-managed nature reserves may provide insight into the maternal behavior of non-domesticated cattle. Cows from African and Camargue cattle herds have been observed leaving the herd days or hours before parturition (4, 12, 14, 25). Calves of Chillingham cattle hide after birth (97), whereas calves of Maremma cattle exhibit both hiding and following behavior in the early weeks of life depending on the availability of cover (11). Similarly, studies in domestic cattle seem to support the above suggestion of a lack of a strict hider/follower dichotomy. There are reports of cattle seeking away from the herd before birth when kept in large, open, and non-managed natural environments (4, 11–14, 21, 25), when pasture-kept (3, 10, 26) and when housed under commercial production conditions (44), but many studies report only some cows or no cows separating themselves from their herd mates (**Table 1**). As with sheep, the studies listed in **Table 1** indicate that prepartum separation is more common in feral types of cattle, whereas studies of pasture-kept or indoor-housed cattle rarely report such behavior. This may be due to domestication favoring less fearful, more social animals, which are more stressed by social isolation as suggested for sheep (50) or, perhaps more likely, due to the confined environment in which the animals are usually kept. There is not enough evidence to suggest that domestic cattle display different intermediates of hider and follower strategies although cattle may adapt to the environment they inhabit.

PREPARTUM BEHAVIOR

In wild ungulate species, only few observations on female prepartum behavior have been recorded (as opposed to postpartum behavior studies), which may be caused by the animals not being present near the herd around parturition. Within studies of ungulates kept under commercial housing conditions, most authors describe some of the following behavioral changes occurring as parturition approaches: pacing, pawing, circle walking without an obvious goal, frequent postural changes, and reduced lying duration [domestic goat (96), domestic sheep (98–100), and red deer (85)]. In cattle, similar prepartum behavioral changes have been described (**Table 1**). Restlessness is the behavior most often reported in cows when calving is imminent (**Table 1**). There is, however, a discrepancy in the interpretation of the described restlessness: is it caused by motivation to search for an appropriate birth site, the experience of pain, or is it a sign of frustration? The causation for the restless behavior prepartum and during labor is currently not fully understood. The process of giving birth is most likely painful (101), and pain may therefore be involved in the behavioral changes prepartum. The behaviors observed at this time (reduced lying, increased walking, walking with no obvious goal, reduced eating, pawing, pacing along fences, and more frequent posture changes; **Table 1**) are all often interpreted as signs of restlessness and thus the definition of restlessness varies considerably between studies. So, even though these behaviors may all reflect the same motivation of locating an appropriate birth site, the constraints of the confined environment may cause the restless behavior. Similar arguments have been put forward by Wass et al. (85), who suggested that fence line pacing in pre-parturient red deer may be a result of the hind being thwarted in searching for and locating an appropriate birth site. Other authors have suggested that high stocking density or low inter-individual distance may cause pacing or restlessness due to the inability of the female to distance herself from the herd (85, 98). Studies quantifying prepartum behavioral changes in cattle with the aim of predicting calving time have failed to identify a specific type of behavior, which reliably predicts the timing of calving [e.g., Ref. (34, 102)]. However, it is agreed that a combination of several behavioral indicators improves estimation of calving time, as any one behavioral indicator cannot reliably predict time of calving [e.g., lack of rumination 70% in sensitivity and specificity (103)]. One possible explanation for these findings may be that all behavioral changes at this time are affected by the same underlying motivation, or thwarted motivation, to search for and find an appropriate birth site. If so, the apparent absence of reliable behavioral indicators may reflect different attempts to adapt to the situation, which depends on environmental factors (8, 30, 37, 42, 104, 105).

Focusing on lying behavior, Huzzey et al. (37) measured frequency and duration of standing in cows kept in individual pens on the day of calving, and compared this with the behavior of the same cows before and after calving, when they were group housed in free-stalls. Stocking density remained the same (one cow per stall), but the environment changed markedly on the day of calving, i.e., from group to individual housing. The authors found reduced lying time corresponding to an approximately 2 h reduction, and 80% more standing bouts on the day of calving. Jensen (8) and Miedema et al. (42) also found reductions in lying time on the day of calving (1.3 and 1 h, respectively), as well as increased frequency of lying bouts in the last 6 h before calving. In these studies, the cows had more time (i.e., several days) to adjust to the environment before calving than the cows studied by Huzzey et al. (37) (which had 24 h or less). In contrast to these findings from indoor calving studies, a recent study by Rice et al. (30) found no reduction in lying time and only an increase in lying bouts 3–4 h before calving in cows calving on large pasture. Therefore, it is possible that the behavioral responses observed as calving approaches are signs of failed behavioral attempts to adapt to the confined environment. If so, restlessness may be a sign of frustration resulting from the cow not being able to search for and find an appropriate birth site, rather than a sign of stress or pain induced by parturition *per se*. One might argue that as birth-site selection behavior is observed in large and open environments, restlessness may be seen in the confined environment because the calving cow moves as if she was in the large environment. The behavior is similar, but the environment affects its expression and hence its interpretation.

Frustration from being prevented from performing prepartum maternal behavior has been documented in at least one ungulate, the domestic pig. Crating of parturient sows, as is typically done in commercial housing systems, prevents the choice of nesting site [feral sows will walk kilometers to choose an appropriate nesting site (66)] and prevents the performance of natural prepartum nest building mainly due to lack of space and lack of nesting materials (106, 107). The higher activity level measured pre-farrowing, such as frequent changes between standing and lying (108, 109), is most likely a reflection of the inability to search for a nesting site. Abnormal behaviors such as bar biting (110–112), rooting the floor, and sham chewing (111, 113) are also seen in the period leading up to farrowing. Moreover, loose housed sows provided with pre-formed nests still perform nest building behavior (114) and thus achieving the goal of having a nest does not satisfy this behavioral need. The high activity level and the abnormal behaviors may reflect the same underlying cause as the restlessness seen in cattle and many sow studies suggest that these are signs or out-lets of frustration arising from not being able to express the highly motivated prepartum maternal behavior. This view is further supported by the findings that preventing sows from nest building activities results in decreased oxytocin levels (113, 115), increased cortisol concentrations (111, 116), and increased heart rate (117), leading several authors to propose that impairment of natural behavior during the prepartum period results in compromised welfare of sows (111, 117–119). Also, confined sows have longer farrowing durations and longer inter-piglet birth intervals, thereby challenging the vitality of the offspring (107). Such measurements are not available within studies of prepartum behavior of cattle but we do know from work on social isolation and lying deprivation [measured as ACTH increase in Ref. (120)] that non-parturient cows show signs of frustration. In cows, more studies of the consequences of allowing the possibility to perform prepartum maternal behavior are needed to understand the motivational background of the prepartum behavior observed in cows in commercial production systems. Such studies would enable evaluation of whether and when motivation-based systems mitigates the expression of prepartum behavior, thereby improving the welfare of calving cows and their calves.

A POSSIBLE ROLE FOR OLFACTION?

Olfaction is an aspect of maternal behavior in cattle which has received little scientific attention until now. In many ungulate species, birth fluids are attractive and consumed by parturient females, e.g., domestic and wild sheep (4, 56, 98, 121), horses, pigs and goats (122), sable antelopes (123), and red deer (85), but this behavior has only been studied sparsely in relation to ungulate mothers' selection of birth site. However, the attractiveness of birth fluids is closely related to parturition. In sheep, the attraction has been shown to last for a few hours after lambing (121), whereas cows show signs of attraction as early as 12 h before calving lasting for at least 24-h postpartum [the duration of the study (124)]. George and Barger (10) found that parturient cows remained within the same area where their amniotic fluids were discharged until calving had been completed, and recently Rørvang et al. (46) suggested that cows predominantly would calve at the spot where another cow had previously calved. Attraction to olfactory cues therefore appears to have implications for the prepartum maternal behavior of cattle. Maternally motivated cows kept in groups are inevitably affected by the odor cues in the birth fluids of other cows even before giving birth themselves, and this may be exacerbated when housing conditions prevent cows from avoiding these odors. In addition, the attractiveness of these odors may reduce the likelihood of a cow moving away to find a birth site elsewhere, which may make artificial hides less attractive (63). Based on the above, we suggest that olfactory cues need to be considered in future prepartum maternal behavior studies and are likely to influence the use of any calving facility provided.

Olfactory cues, however, are not only important for the prepartum behavior of female ungulates. In sheep, the role of olfaction is essential for the onset of lamb-directed maternal behavior, at least for inexperienced mothers (125, 126). For instance, Basiouni and Gonyou (125) showed that fostering of alien lambs to parturient females was possible only if the lambs were covered by jackets soaked in amniotic fluid. Adult domestic goats show interest in alien newborn kids (95), and in farmed red deer such attention can be rather intense and even increase if stocking density is high [in addition, more mismothering occur in this situation (85)]. As mentioned earlier, also cattle studies have reported attention from cows toward and licking of alien calves, especially in commercial housing conditions (**Table 1**). Studies from free-ranging cattle, however, often do not report cows showing interest in alien calves', which may be explained by the cows seeking away from the herd (11). Hiding or separating from conspecifics probably lowers the risk of mothers interacting with alien offspring in general, but physical cover may not suffice to keep maternally motivated cows away if they can smell a calf. Recently, we offered pregnant group housed cows an opportunity to select an individual pen as birth site. The presence of a newborn alien calf in the group pen reduced the likelihood of the cows using this opportunity (63), most likely because the newborn calf's coat contained birth fluids. Hence, olfaction and odors are likely to be important for the onset and direction of maternal behavior also in cattle.

Commercial dairy cow housing conditions often mean high stocking densities in a relatively barren environment offering few options of selecting a birth site away from other cows as well as more disturbances from human activities and conspecifics. Taken together, this means that pre-parturient cows housed in groups are in close proximity to olfactory stimuli important for maternal behavior, i.e., birth fluids from other cows and their calves. Unlike sheep, cattle show a preference for birth fluids also before parturition, and prepartum cows have been reported to nurse alien calves (59, 62), observations which may explain the higher occurrence of mismothering in commercial housing (**Table 1**). This may also introduce a higher risk of mismothering when cows calve in group pens when compared with parturient sheep, as ewes are not attracted to birth fluids until after parturition (121). In addition, group housing may increase the risk of agonistic social interactions limiting the access of bovine mothers to their own calves. Mismothering and lack of contact between cow–calf increases the risk of colostrum and maternal care being allocated to alien calves, leading to failure of passive transfer of immunity from the mother to her biological offspring. The importance of olfaction and odors thus need to be taken into consideration in the design of housing facilities for parturient cattle (127), especially in relation to group housing. Implications of group housing of calving cows need to be critically addressed as this type of management is quite common [for example, 70% of US dairy operations (128)], and particularly if cows and calves are to remain together post-calving. Keeping parturient cows in groups is normally associated with early cow–calf separation (2) and thus if early calf nursing and cow–calf bonding are to be ensured, housing of parturient cows in individual calving pens appears to be necessary.

CONCLUSION AND PERSPECTIVES

Drawing on research literature on prepartum maternal behavior, this review compared cattle to other members of the ungulate clade with the aim of understanding the biological basis of bovine prepartum behavior with main emphasis on dairy cows. Prepartum success depends on the female's ability to locate an appropriate birth site to ensure and safeguard a calm parturition and optimal surroundings for postpartum maternal behavior by lowering the risk of predators, disturbances, and mistaken identity of offspring. At present, the motivations of cows underlying the apparent prepartum isolation seeking behavior have not been fully explored. In addition, traditional concepts of ungulate maternal behavior such as the hider/follower-dichotomy appear overly simplistic. Based on the reviewed literature, we suggest that more scientific focus should be given to the prepartum maternal behavior (i.e., the phase of birth-site selection) in dairy cows, as they are exposed to several factors in a commercial calving environment, which may thwart their maternal motivations and influence their behavior and welfare. One such factor is olfactory cues, which may exert stronger effects on prepartum cows than other ungulate species as cows are attracted to birth fluids already before parturition. Providing dairy cows with an environment where they can perform the prepartum maternal behavior for which they are motivated, may facilitate postpartum maternal behavior and success. Further research focusing on motivation-based housing of peri-parturient cows is needed to ascertain the importance of degree of movement and distance from the group within the constraints of dairy housing systems. These studies should include effects on the welfare of calving cows and their offspring. Ultimately, this knowledge may be used in future development of more suitable housing and management systems for calving cows.

AUTHOR CONTRIBUTIONS

All authors contributed to the initial idea and early discussions underlying this review. MR did the main literature search and selection and wrote the first draft of the manuscript, including figure and table.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6. General discussion

This thesis investigated the maternal behaviour and use of maternity pens in parturient dairy cows. Preferences and motivations of parturient dairy cows as well as sensory modalities affecting pre-partum maternal behaviour were examined through the five studies underlying the thesis. The thesis was initiated by an introductory review of the topic 'maternal behaviour of parturient dairy cows'. Gaps in the literature were identified in the background chapter and some of these gaps were explored in the studies underlying the thesis, aiming to answer the research questions stated in Chapter 3.

The studies underlying this thesis have collectively shown that several factors influence the motivation and behaviour of parturient cows besides the expected motivation to calve while isolated. Future design of motivation-based calving facilities may therefore be a more challenging task than originally expected. Below, this chapter presents a critical discussion of the term 'isolation seeking behaviour' in order to facilitate a common understanding and terminology when describing this aspect of pre-partum maternal behaviour. This is followed by a chapter discussing factors influencing the expression of pre-partum isolation including effects of features in the environment, social factors, olfaction and individuality. Collectively, the discussion aims to expand on the knowledge obtained from the studies underlying the thesis, to allow a better understanding of pre-partum maternal behaviour of dairy cows, suggest future research and discuss future recommendations for calving facilities and management practices of parturient dairy cows.

6.1. What constitutes an appropriate birth site?

Collectively, the findings from Studies 1, 4 and 5 indicate that there is more to a cows' perception of an appropriate birth site than initially assumed. From the findings in Study 5, it seems important to 'go unnoticed', presumably to reduce or eliminate the risk of disturbances from predators and conspecifics. The Chapters below aim to discuss what might constitute an appropriate birth site and what factors modulates the perception of a birth-site. The Chapters critically discuss how to describe pre-partum maternal behaviour, how social and physical factors affects the behaviour and pre-partum motivations as well as provide a critical discussion of the influence of olfaction and individuality.

6.1.1. Describing pre-partum maternal behaviour of cattle

One important outcome of the work underlying this thesis, also formulated in the conclusions from Study 5, is that the definition of the commonly used term 'isolation seeking' seems to lack specificity. The term has been widely used to describe the goal of the ungulate maternal pre-partum behaviour: To separate and/or hide from disturbances (arising from various threats including conspecifics and predators), thereby allowing the parturient female to give birth in a calm place, with subsequent opportunity to nurse and bond with the young (Study 5). In previous studies, it has not necessarily been described which of these aspects were in focus. Proudfoot et al. (2014b) and Lidfors et al. (1994) both concluded that cows isolated themselves from the group when calving, but Proudfoot et al. (2014b) observed a preference for physical cover inside a maternity pen when housed indoors, whereas Lidfors et al. (1994) observed spatial separation or distancing on pasture. The preference for physical cover in the indoor-housed cows may, however, include an additional aspect. Hiding behind a barrier in a maternity pen, where the group is not able to enter does not necessarily indicate active moving away from the group, even though this may be a consequence of entering the maternity pen. Spatial separation on pasture may, likewise, include preference for physical cover but this was not how Lidfors et al. (1994) described isolation. In order to specify, more precisely, the motivations underlying pre-partum maternal behaviour, additional terms may be used when describing the different aspects of the behaviour expressed by parturient females (Table 1).

Table 1. Specific aspects of ungulate pre-partum maternal behaviour and corresponding descriptions. The behaviour, the underlying motivation and the proximate goal with explanatory questions.

	Question	*Description*
The behaviour	What the females does?	Separates and/or hides herself
	How does she do it?	By means of distance and physical cover, both on a continuous scale (Figure 9)
The motivation	Why this behaviour?	To avoid disturbances from various threats such as predators and/or conspecifics
The proximate goal	What is she trying to achieve?	To ensure an environment allowing calm and secure parturition, and subsequent successful bonding and nursing Equalling an appropriate birth site

The table describes different aspects of pre-partum maternal behaviour of ungulates based on findings from the literature reviewed in Study 5 and results from Study 1 and 4: The behaviour, the underlying motivation, and the proximate goal of the behaviour. The behaviour can be divided into hiding and/or separating by means of a combination of distance and physical cover (Table 1 and Figure 9). Physical cover refers to the female reducing or eliminating the risk of disturbance from potential threats (predators and/or conspecifics) by means of physically hiding herself. Distancing arise from the same motivation but the female separates (and hides) by means of distance from the potential threat, thereby reducing the need for physical cover. One way of illustrating this graphically may be by use of motivational isoclines as McFarland and Sibly (1975). The goal of the female, to locate an appropriate birth site, can be achieved by any combination of the two environmental aspects (distance and physical cover, Figure 9). When there is a high level of physical cover, distance may be less important and thus separating spatially may not be as pronounced as separating by means of physical cover, and vice versa. Ultimately, both aspects of the behaviour arise from the same motivation serving the same proximate goal: locating an appropriate birth site (Table 1). The terms introduced above and in Figure 9 will be used in the remainder of the thesis.

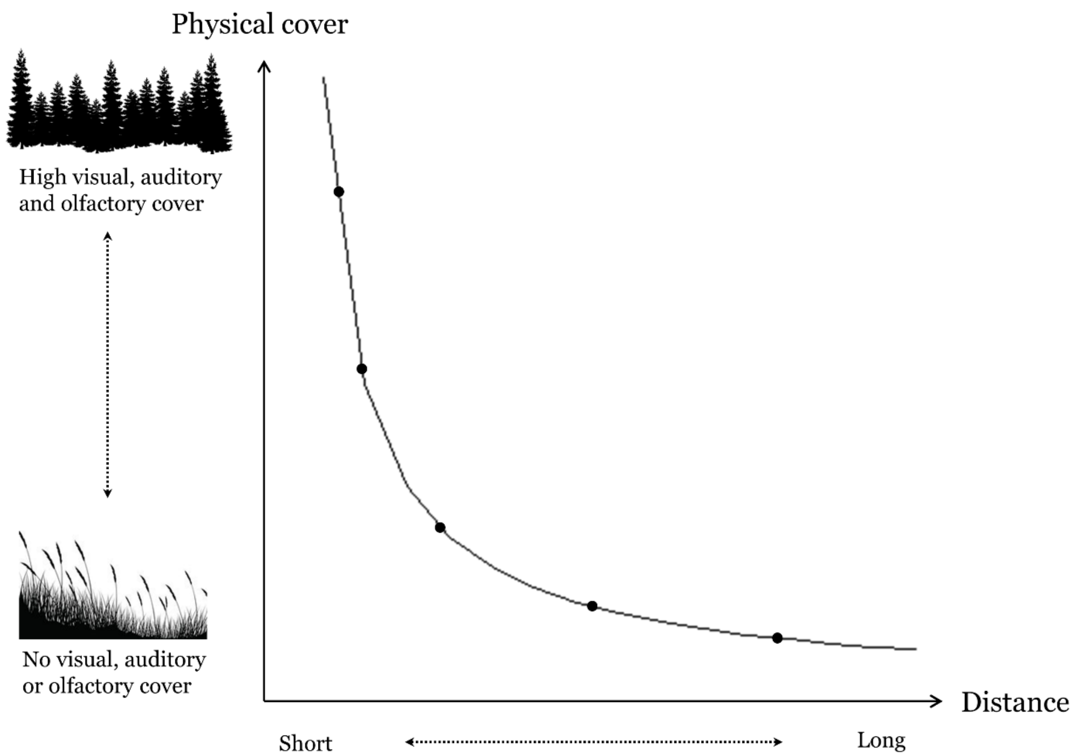

Figure 9. Graphical two-dimensional illustration of the suggested relation between motivation for distance and physical cover (adapted from McFarland and Sibly, 1975). Distance and physical cover seen as complementary (inverse correlated). Physical cover may be various features of the particular environment e.g. trees and bushes on pasture or barriers in a barn. A high degree of physical cover reduces the motivation for distancing and vice versa.

The points on the isocline (x*y) all illustrate the same degree (level of) of isolation (even if it is of different quality), and the isocline joining these points is a motivational isocline for isolation.

6.1.2. Calving duration as an indicator of an appropriate calving environment

Calving is often divided into three stages (Ball and Peters 2004; Wehrend et al. 2006): The 1st stage of labour begins with the dilatation of the cervix and ends with the rupture of the chorionallantois usually inside the vagina. This process usually lasts 6 – 24 hours (marking the start of the 2nd stage of labour). The 2nd stage of labour thus starts with contractions and lasts until the calf is fully expelled (Noakes et al., 2001), which lasts about 30 min to 4 hours (e.g. Berglund et al., 1987). The 3rd stage of labour covers the expulsion of the foetal membranes and the placenta, after the calf is born (marking the end of the 2nd stage of labour), which usually lasts for 6 hours and is termed pathological when exceeding 24 hours.

Difficulty in giving birth is typically termed dystocia in cattle, and is defined as a difficult or prolonged calving process (Mee 2008, Barrier 2012c). The 2nd stage of labour (as defined above) is commonly used to determine when to assist the calving and thus accordingly determining whether or not the calving process is prolonged and/or difficult. The most prevalent risk factor for a prolonged calving is foetal-pelvic incompatibility (Meijering 1984; Mee 2008), but other factors affecting the expulsion of the foetus also adds to this: 1) weak labour (i.e. lack of contractions), 2) incomplete dilatation of the cervix and vagina due to stenosis and 3) uterine torsion. Environmental disturbance has also been shown to increase the risk of prolonged calving. For instance, overcrowded calving areas have led to increased occurrence of vulva constriction (Dufty 1981), and generally, pain and/or stress have led to impairment of the labour process by partially blocking the oxytocin release, which is crucial for the onset of contractions (Ehrenreich et al. 195; Taverne 1992; Lawrence et al. 1997). Therefore, calving progress may provide an indicator for the environment in which the cows are calving. Previously, duration of 2nd stage labour has been used as an indicator of calving progress in various studies (Table 2). This thesis has been among the first to include indicators of calving progress in the assessment of whether or not the specific calving environment was perceived as appropriate (Studies 1 and 4). Based on the study by Proudfoot et al. (2013) suggesting that disturbing a calving may prolong the calving process, the present Studies 1 and 4 included calving duration as a possible indicator of whether or not the particular calving environment was perceived as appropriate or not. Table 2 lists durations of 2nd stage labour reported from earlier studies and Studies 1 and 4.

Table 2. Duration of 2nd stage labour of dairy cows from Barrier et al. (2012b), Miedema et al. (2011b), Studies 1, 4, Proudfoot et al. (2013), and Campler et al. (2015).

		Duration of 2nd stage labour (min)	Variation	N
Barrier et al. (2012b)	Unassisted	55 (median)	27-97 (range)	258
Miedema et al. (2011b)	Unassisted	27 (median)	21-40 (range)	12
Study 1	Overall	94 (median)	76-137 (range)	37
	Cows calving in 75% visual isolation	124 (median)	88-161 (range)	15
	Cows calving in 50% visual isolation	79 (median)	63-113 (range)	22
Study 4	Overall	100 (median)	77-134 (range)	66
	Cows calving in an individual maternity pen	109 (median)	83-140 (range)	34
	Cows calving in the group area	90 (median)	70-127 (range)	32
Proudfoot et al. (2013)	Cows moved before labour	Approx. 60		16
	Cows moved early stage 1 labour	Approx. <60		17
	Cows moved late stage 1 labour	Approx. 90		9
Campler et al. (2015)	Overall (assisted while lying and unassisted)	114 (median)	79-151 (range)	121

In Studies 1 and 4, duration of 2nd stage labour was defined as the period from the first abdominal contractions (either standing or lying) until delivery of the calf. This definition has been used previously by Proudfoot et al. (2013) and Campler et al. (2015). Other definitions have been used, as for example Barrier et al. (2012b) defined 2nd stage labour as the period from when the calf´s feet was visible until the calf was born and Miedema et al. 2011b used the period from the bursting of the amnion until delivery of the calf to define this phase of calving. Miedema et al. (2011b) and Barrier et al. (2012b) found lower median durations of calvings as compared to the median durations reported by Studies 1, 4, Proudfoot et al. (2013) and Campler et al. (2015) which may be due to the different definitions of 2nd stage of labour used. The overall median durations of 2nd stage labour did not differ between Studies 1 and 4 and this duration is numerically longer than reported by Proudfoot et al. (2013) (Table 2) despite the use of identical definitions. Campler et al. (2015) found a median duration of 114 min, which is similar to the present Studies 1 and 4. An explanation for these differences, obtained with same definitions, may be the determination of behavioural indicators initiating 2nd stage labour. Future studies investigating inter-observer reliability when determining the onset of the 2nd stage of labour would thus be advantageous even within studies using the same definitions.

Another explanation for the differences in the duration of 2nd stage labour evident from Table 2 may be related to the experimental setups. Comparison of Proudfoot et al. (2013), Campler et al. (2015), and the present Studies 1 and 4, suggest that the physical calving environment as well as the level of human disturbance differed, hence potentially affecting calving progression (as reported by e.g. Proudfoot et al. 2013, movement/disturbance during late 1st stage labour led to a prolonged 2nd stage labour). In their study, Proudfoot et al. (2013) presented the cows with a choice of calving behind a physical cover or not (calving in the open or shielded side of the individual maternity pen). In the study by Campler et al. (2015), all cows were manually moved to an individual maternity pen for calving but with no mentioning of the specific design of the pen sides. In Study 1, cows had three choices of physical cover inside an individual maternity pen, and within each pen, a choice of an open or a shielded side. In Study 4, the calving environment offered cows additional space to move and more choices of where to calve. Cows could calve in various areas within the 9 m x 9 m group area and in the shielded or open area within each of the six available individual maternity pens. Studies 1 and 4 thus offered cows a higher degree of space and freedom to choose, but resulted in longer 2nd stage labour durations as compared to Proudfoot et al. (2013). Frequent changes of calving site may have implicated the calving process. Cows in Study 1 were observed changing individual maternity pen frequently (mean ± s.d.: 61 ± 30 pen changes 12 h prior to calving) by walking back and forth on the rubber aisle. This was partially due to feeding alongside the feeding manger, but cows also entered the individual maternity pens after feeding bouts. Cows in Study 4 also changed pen often (defined as changes between group area and individual maternity pen; mean ± s.d.: 16 ± 12 changes 12 h prior to calving) indicating some degree of unrest. Conversely, the duration of 2nd stage labour was the longest in Campler et al. (2015) where no choices of calving site were offered. These numbers contradict that frequent calving site changes or freedom to choose could be a causal factor. The environment offered by Campler et al. (2015), however, differed from Studies 1 and 4 in terms of frequent disturbance from humans and machinery disturbances (personal communication with the author). Therefore, disturbances (in terms of humans and machinery) of the cows during calving might be a more likely explanation for the length of the 2nd stage labour durations reported by Campler et al. (2015). In light of this, level of disturbance (regardless of origin) may be an important factor in order for the cow to perceive a specific maternity pen as appropriate (also argued in Barrier 2012c).

6.1.3. The influence of physical and social factors

6.1.3.1. The physical and spacial appearance of the calving environment

In contrast to the hypothesis, results from Study 4 showed no overall effect of having a gate inserted to the individual maternity pens or not. Based on literate and the findings of Study 1, the hypothesis was that cows would prefer to calve in the individual maternity pens of Study 4 (and especially so in the pens with gates), particularly cows with longer 2nd stage labour, as it would allow them isolation in an environment without disturbances from the group members. Results from Study 4, however, indicated no effect of choice of calving site on the duration of 2nd stage labour. In addition, a similar proportion of the cows with longer duration of 2nd stage labour in Study 4 (duration over 130 minutes, as defined in Study 1) chose to calve in the group area and in the individual maternity pens. An explanation for why some cows in Study 4 chose to calve in the group area, may be the physical appearance or design of the individual, partially covered maternity pens, accessible via a gate. In Study 4, cows had to comply with a learning criterion of how to manipulate the gate, and thus all cows learnt how to enter and leave the individual calving pens. Moreover, all cows had entered the pens and had been lying down in a pen before calving (further details in Chapter 5.4.). Nevertheless, cows may not necessarily make the connection that being inside an individual calving pen with a gate ensures being alone at calving. When entering and leaving the individual calving pen, the cow must manipulate the gate, thereby potentially reducing the speed and the distance with which she is able to escape a threat (illustrated in Figure 11B). Being in a confined space during calving reduces the possibility for executing flight responses in order to avoid predators or other threats. This may have reduced the attractiveness of the individual calving pens. The topographical and spacial appearance of the calving environment in Study 4 also differs from the environments offered in other studies by e.g. Kiley-Worthington and de la Plain (1983) and Flörcke and Grandin (2014) (Figure 11a). Study 5 clearly indicated that physical characteristics of the birth site may be less important than the ability to move away from potential threats and disturbances in ungulate females. Assuming that wild ungulate females perceive humans as predators, such behavioural responses may originally have been developed from sensitivity to predator pressure (Roberts and Rubenstein, 2014). Therefore, avoidance of disturbances by means of isolation is an adaptive behaviour as it reduces the risk of having the offspring killed, and such behavioural reactions may have been preserved in domestic species. If parturient cows aim to avoid threats and disturbances (regardless of origin), a confined, indoor space with barriers may not be optimal in terms of being able to escape, even though it offers physical cover. Insertion of a gate at the entrance of such physical, indoor and confined space may have added to the limited possibility for flight responses (illustrated by the blue areas in Figure 11A and B, respectively).

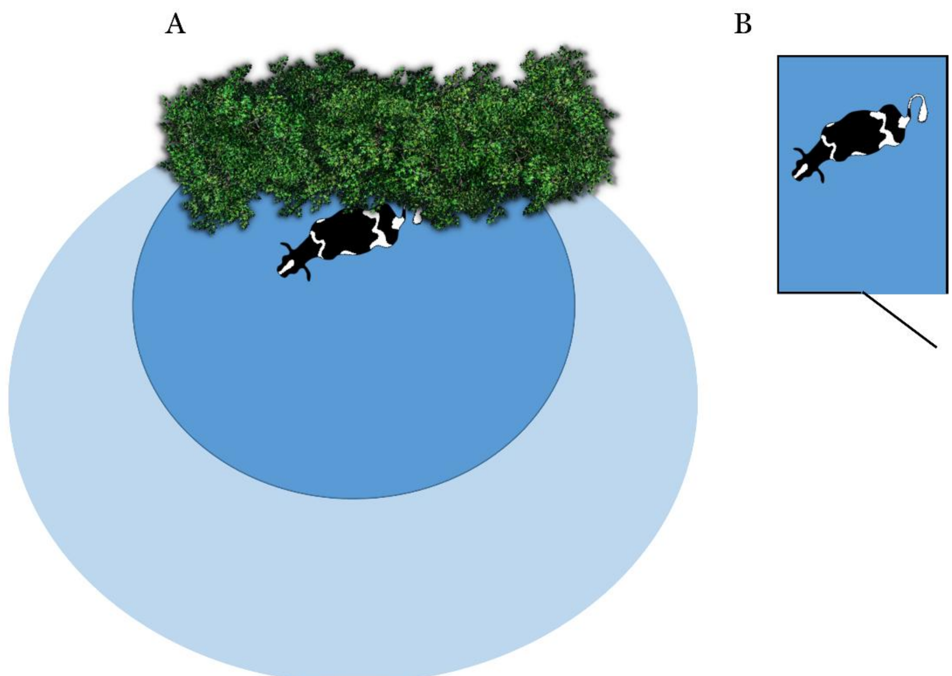

Figure 11. Illustration of a cow calving A) under spatial, outdoor conditions with isolation opportunity behind trees or bushes, and B) indoor more confined conditions (as in Study 4) with isolation opportunity inside an individual maternity pen with a gate. The blue areas represent the theoretical area in which it is possible for the cow to avoid potential threats and disturbances. Even though the cow in B) may have a gate, which prevents other cows from entering, her perception of the particular site may not include this aspect. Instead, the confined space and reduced avoidance opportunity (blue area) in B) may result in the cow not perceiving B) as an appropriate calving site. In that case, A) may be a better site in terms of the cow perceiving it as appropriate for both hiding and being able to escape.

6.1.3.2. The role of conspecifics in the calving environment

The above discussion emphasized the importance of disturbance in the decision making when choosing a calving site. Results from Studies 4 and 5 suggested that conspecifics may also act as disturbing factors in a calving environment. Social dominance significantly affected calving location in Study 4. Dominant cows had a higher chance of occupying an individual maternity pen and vice versa. Additionally, the limited space within the calving facility (the cows were able to move maximum 9 meters away depending on the location of the group members) meant that cows were within close proximity to other group members and thus risked agonistic interactions close by the calving site (no matter what specific site was chosen). As argued above, although all cows complied with a learning criterion for using the individual maternity pens, they may not have learned that being in a pen behind a gate also meant not having other group members entering. Therefore, cows may not have perceived the offered individual calving pens as appropriate isolation if other conspecifics were located nearby. Furthermore, if the motivation for isolation in terms of separating 9 m (or less) from the group was greater than the motivation for isolation in terms of entering a semi-covered pen, this may have resulted in the high number of cows calving in the group pen. Other authors have also argued that the ability to escape from disturbances may be more important for the choice of birth site, than the characteristics of the site itself (Murphy et al., 1994). Such priorities also make sense in terms of reducing the risk of mis-mothering by increasing the probability that a maternal investment is directed towards own offspring (Alexander and Shillito, 1977; Espmark, 1971). A calm and isolated environment, offering the cow an opportunity to learn the odour and features of her calf would benefit later recognition, thereby suggesting that isolating from conspecifics at calving is adaptive. In this respect, the presence of group members within a calving facility may influence the cow's perception of a given calving site.

The effect of social dominance may additionally explain the observed preference for a high level of physical cover by cows with prolonged 2nd stage labour in Study 1. Based on the reports of cattle sometimes following parturient cows when they leave the herd to calve (Kiley-Worthington and de la Plain, 1983; Roberts and Rubenstein, 2014) and the effect of social dominance (Study 4), it is likely that group behaviour in Study 1 may have affected the behaviour of the cows inside the maternity unit. If the parturient cows (Study 1) were motivated to avoid disturbance from group members, presence of these in front of an individual maternity pen may have been a reason for why the cow isolated behind the barrier. This is supported by the finding that cows choosing 75% isolation (individual maternity pen C) had significantly longer duration of 2nd stage labour. Contrarily, if a cow did not perceive the barrier as sufficient cover, presence of group members near the pen may have limited the level or quality of isolation behind the barrier. The latter may explain why no overall preference for any of the three designs were found. In this case, inclusion of information about the position of group members in the statistical modelling in Studies 1 and 4 could potentially have added information on the effects of the social environment but this was not included in Study 4. In Study 1, effects of group placement were monitored and analysed for the initial 40% of the cows studied, however, showing no effect of the placing of group members on the choice of individual maternity pen A (tall and narrow), B (low and wide), or C (tall and wide) (Delautre, 2014). Future studies may thus benefit from including such measurements.

6.1.3.3. The role of disturbance

The collective results from Studies 1 and 4 imply that physical cover in terms of a barrier covering, or partially covering an individual maternity pen, may not be sufficient in order to make the parturient cows choosing to cave inside the pen. When placed in an indoor, commercial, group setting, a barrier or a partially covered maternity pen may not be perceived as an appropriate calving site due to environmental disturbances of various origin (human interference, machinery or conspecifics). Adding more space and thereby increasing the distance between individuals and offering more freedom to move away from the group may improve the attractiveness of the isolation opportunity offered behind barriers. For instance, Rice et al. (2017) observed dairy cows calving on 32 m^2 of orchard grass and fescue in groups ranging in size from two to 18 cows, at the time of calving. In this study, no changes in activity level was found prior to calving, but an increase in number of lying bouts was reported between 3-4 h prior to calving without lying bout duration changing. Proudfoot et al. (2013) accordingly found a reduced lying bout duration (approx. 12 min per bout) in the hour before calving when cows were moved in late 1st stage labour (transitioning to 2nd stage labour) compared to when cows were moved well in time before calving (approx. 25 min per bout). Rice et al. (2017) likewise reports a reduction in lying bout duration in the hour prior to calving (approx. 35 min per bout) but this duration is longer than any other studies conducted indoor. Barrier et al. (2012b) reports increased number of lying bouts 6 hours prior to calving, with the 2 hour period just before calving being the period with the highest frequency of transitions from lying to standing (5.8 bouts per hour). Possibly, behavioural responses observed as calving becomes imminent, may be signs of failed behavioural attempts to adapt to a confined environment with a high frequency of disturbances. Theoretically, external factors may shift the motivation isocline upwards (Figure 10A) meaning that adding larger distances could be necessary if the degree of physical cover is constant and vice versa. Further studies investigating the effect of adding distance to indoor calving facilities are therefore needed. However, from this discussion it is clear that effects of disturbances are impossible to ignore no matter the origin, and it is possible that disturbances will outweigh potential improvement of isolation opportunities in terms of e.g. increased distance.

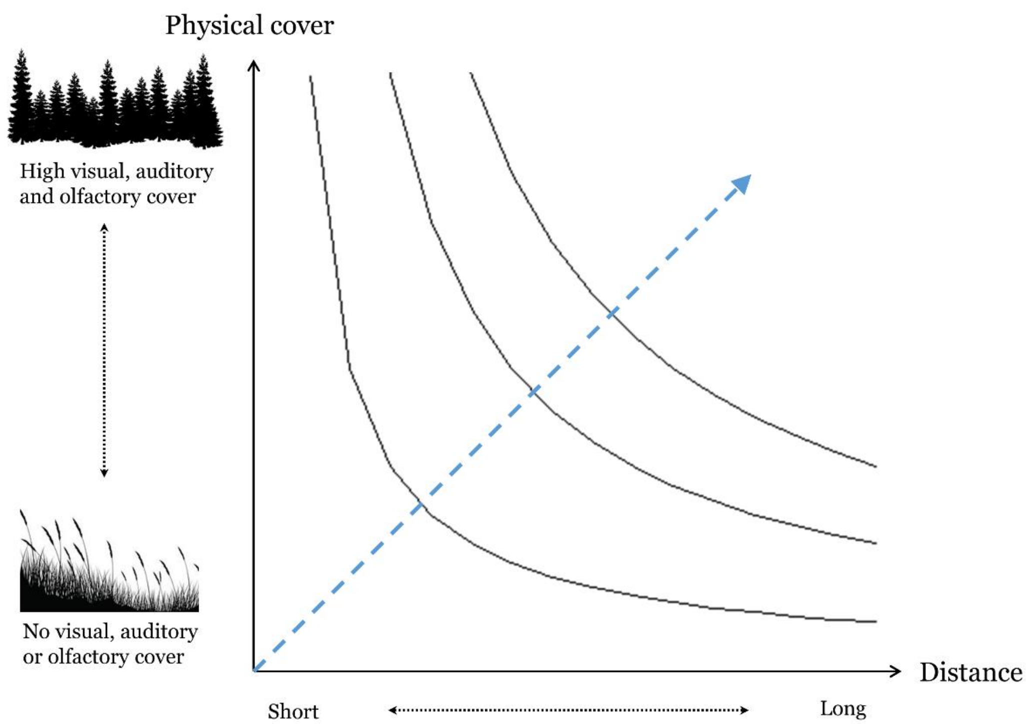

Figure 10A. Motivation for isolation represented as in Figure 9, but including the influence of external disturbances (after Baerends et al. 1955). Higher level of disturbance will shift the isocline in the direction of the arrow, meaning that higher level of disturbance will increase the motivation for isolation by means of distance and physical cover. See Figure 9 for more details.

Figure 10A illustrates the shift in motivation for isolation with increasing level of disturbance, illustrated by the blue arrow shifting the motivation isoclines upwards. This relation can also be seen in a three-dimensional space (as according to McFarland and Sibly, 1975), where disturbance represents the third dimension (z) modulating the motivation for isolation (Figure 10B). The demand for distance and physical cover increases with level of disturbance and if the given environment has a limit to distance and physical cover available, increasing disturbance will also result in decreasing variation of combinations by which the female can achieve isolation i.e. the goal of the behaviour. There may thus be a critical point where the female is incapable of locating an appropriate birth site, which fulfils her motivation to isolate (illustrated in Figure 10B, as the top point of the plane – the dark red point).

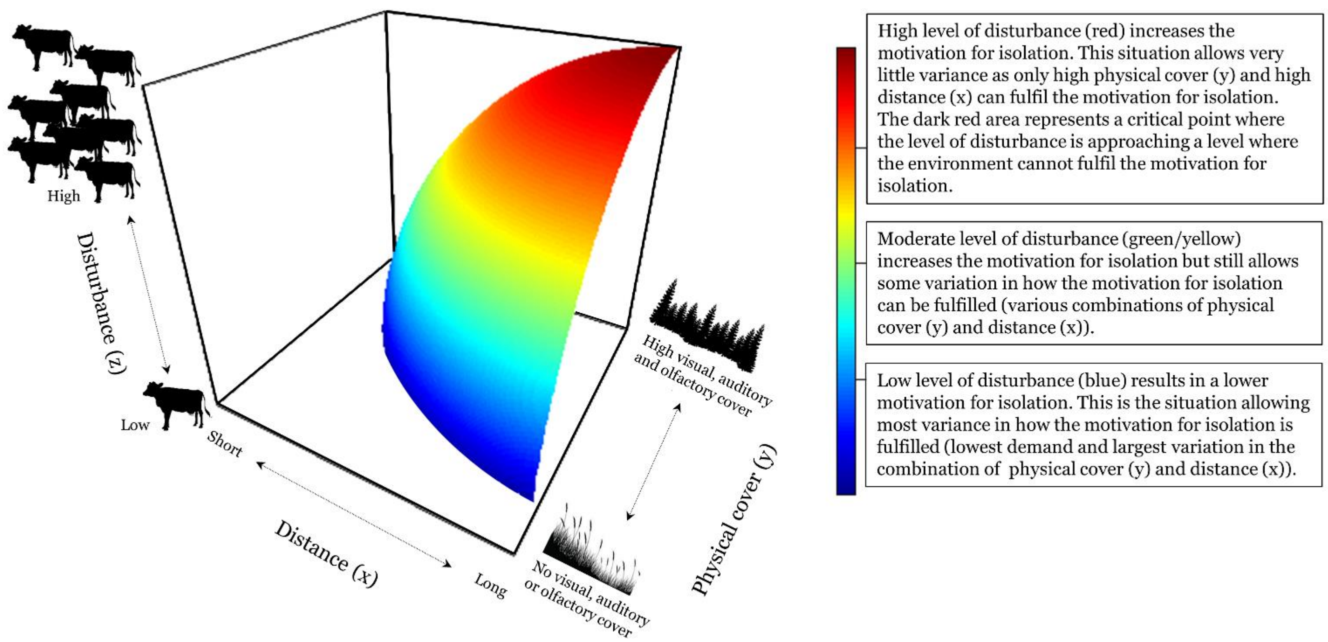

Figure 10B. Simulation of how level of disturbance affects the cow's motivation for isolation as a combination of distance and physical cover (Figure 10A in a three-dimensional space including all motivation isoclines). The plane represents the minimum range of combinations of distance and physical cover fulfilling the cow's motivation for isolation at a given level of disturbance. Increasing level of disturbance increase the motivation for distance and physical cover illustrated by the slope of the 3D plane. Blue areas represent the lowest level of disturbance with corresponding lowest motivation for isolation (large variation in how distance and physical cover can fulfil isolation motivation). Moderate level of disturbance in the green and yellow areas represent the corresponding increased motivation for isolation, and at the highest level of disturbance in the red area, motivation for isolation is at a maximum (low variation in how isolation motivation can be fulfilled). The area below the plane represents situations (combinations of disturbance, distance and physical cover) where the motivation for isolation may be fulfilled by the environment. The area above the plane conversely, represents when the motivation for isolation may not be fulfilled by the environment. The dark red point where x, y and z intersects at the maximum, represents the critical point where the level of disturbance exceeds the isolation opportunities given by the environment (the cow cannot achieve her goal of locating an appropriate birth site).

Study 4 investigated if a motivation-based calving facility might aid the movement of parturient cows into individual maternity pens solely on their pre-partum motivation to isolate, but did not yield a solution to ensure all cows calving in such pens. It may thus be an advantage for future research to focus on 1) facilitating entrance to individual maternity pens, 2) optimizing the design in terms of adding distance and 3) exploring other motivations, which may allow controlling pre-partum maternal behaviour. Concerning 1), avoiding cow-operated entrances to the pens would probably be beneficial. Using, for instance, a sensor-controlled gate, which opens when the cow approaches may aid the entrance to the pens. As with cow-operated gates, this solution demands some training or habituation of the cows to the system, which may be a challenge in practise. The solution also poses a risk of having cows not due to calve occupying the individual maternity pens. Another less technical solution (concerning both 1) and 2) may be to offer manoeuvrable calving hides, which the farmer can place to offer an isolation opportunity and then close around the parturient cow when she has chosen a place to calve. This may ensure that all cows end up calving inside an individual maternity pen (limiting disturbance from conspecifics), but may in turn disturb the cows in the process (human disturbance). As Study 5 suggested, a possibility for physical cover and/or spatial distance in order to avoid being disturbed might be key when aiming to optimize the design of calving facilities. The practicality of designing such isolation opportunity within indoor commercial housing of parturient cows is however still a challenge. Generally, future development of maternity pens for cows would benefit from research into the decision making of parturient cows. Do cows weigh up a) degree of physical cover and distance, b) risk of disturbances and threats, and c) ability to escape, and if so, how do they obtain the information needed to make this decision?

6.1.4. The influence of odour cues

In addition to the effects of the social and physical factors of a calving environment mentioned above, collective results from Studies 2, 4 and 5 emphasized a role of olfaction in the onset and direction of pre-partum maternal behaviour in dairy cows. Although often ignored in a production setting, olfaction has implications for cattle behaviour and probably also for the cows' use of maternity pens (Study 4). This underlines the need for future studies to consider odour cues when studying the behaviour of pre-partum cows in general and when aiming to exploit the motivations of parturient cows in the management.

The small-scale study preceding Study 4 (Study 2) indicated that presence of birth fluids originating from other calving cows influenced the selection of calving site. Although effects from other environmental factors (such as light intensity, bedding quality, and features of the design of the group calving area), which could not be controlled for, cannot be excluded, Study 2 suggested that chemo signals or odour cues in birth fluids directed the attention of the cow towards the source. This result is in line with previous findings from Pinheiro Machado et al.

(1997) who found that parturient cows were attracted to birth fluids (specifically amniotic fluid) mixed in the feed. The reviewed literature of Study 5 confirmed that olfactory cues originating from birth fluids play a role in the onset and direction of pre-partum maternal behaviour of ungulates. Moreover, Study 5 indicated that the behaviour of parturient cows might be more affected by olfactory cues than e.g. sheep (Levy et al., 1983) due to the attraction occurring already before parturition. Hence, housing parturient cows in groups is likely to influence the pre-partum motivations and behaviour of other parturient group members. Study 2 illustrated the influence on calving-site selection and Study 4 illustrated the impact of pre-partum attraction, as the presence of newborn calves attracted the attention of pre-parturient cows, potentially competing with their motivation to enter the individual maternity pens offered in Study 4. Other studies have also illustrated the impact of the pre-partum attraction to alien newborn calves in terms of mistaken identity of offspring when cows were calving in groups (Edwards, 1983; Hudson, 1977). Conversely, mis-mothering is rarely reported for cows calving in feral conditions (e.g. Vitale et al., 1986; Kiley-Worthington and de la Plain, 1983). Pre-partum attraction towards birth fluids may thus result in maternal behaviour being directed towards alien calves and locations of previous calvings, which has implications when aiming to control the behaviour of parturient cows. Attraction towards birth fluids may, however, also represent future possibilities. If targeted placement of birth fluids can control calving sites of dairy cows kept indoors, placing birth fluids in individual maternity pens may stimulate entry to individual maternity pens. Moreover, combining the use of birth fluids with a motivation-based calving facility (e.g. the facility designed for Study 4) may be advantageous if maternal attraction towards birth fluids is superior to other pre-partum maternal motivations such as e.g. the motivation to isolate. Pinheiro Machado et al. (1997) showed attraction of parturient cows specifically to amniotic fluids, and thus the attracting compound in birth fluids may be contained within the amniotic fluid (the fluid contained within the amniotic sac, surrounding the foetus). It is currently unknown what specific compound elicits the attraction in cows, which may be both an innate meaningful (induced by a pheromone) or a learned response (signature mixtures) (Wyatt, 2010). Identification of either of these may be complicated as an innate effect of a pheromone can be to induce learning of non-pheromonal substance (e.g. signature mixtures), which then come to have attractive properties, as a result of learning. Additionally, attraction to odour cues can also be a result of learning due to a non-olfactory conditioned stimulus, e.g. from rats as olfactory conditioning following tactile stimulation of rat neonates

(Miller and Spear 2008). There may thus be unexploited potential of using odours to control the behaviour of parturient cows (either as innate responses or through learning), which could be a valid focus for future research. An experiment investigating olfactory preferences could be followed by a subsequent experiment testing the capacity or level of attraction to these preferred odours e.g. in relation to calving site selection. Such an experiment could for instance be targeted placement of donor birth fluids within the bedding of the maternity pen or calving facility. One suggested hypothesis would then be that the increasing attraction towards birth fluids as calving approaches results in cows deciding to calve in close proximity to the donor birth fluids. An additional future aspect would be to test if a first exposure to the olfactory cues is needed i.e. test whether it is an innate response to a pheromone or a learned response to a signature odour (Brennan and Kendrick, 2006). The first maternal experience has lasting consequences at least in sheep where the development of selective recognition of own lambs occurs more rapidly for subsequent lambings (Keverne et al., 1993; Kendrick, 1994), therefore it is possible that older cows may be easier to manipulate and that heifers may need a first exposure. As a last practical remark, it is also important to test if procedures such as freezing and thawing of birth fluids affects the attractive properties of the fluid, if future experiments aim to collect and store birth fluids for future use. Such preceding investigation is needed in order for the above mentioned experiments to yield a credible outcome.

Although olfaction may play a crucial role in various aspects of cattle management in general (reviewed in Archunan et al. 2014), only limited knowledge exists on the olfactory capacities of cattle (sequencing of the bovine olfactory subgenome: Lee et al., 2013, feed preference trials including aspects of odour and novelty: Corley et al., 1999 and Herskin et al., 2003). Study 3 represents one of the first studies aiming to explore olfactory capacities in cattle. The study showed that cows and heifers were able to detect and distinguish between complex odours, and that they directed attention towards specific complex odours. These results are important in future research aiming to utilize odours in management of cattle and illustrate that cattle are able to detect complex and easily accessible odours. A next step could be to explore whether cows can be positively conditioned to odour cues and whether such conditioning may result in a maternity pen being perceived as more attractive if the conditioned odour cue is present in the pen. Based on these impressions, utilizing odour cues for cattle may not only be relevant in relation to managing parturient cows. As olfaction is a main sensory modality playing a central role in relation to both social and sexual behaviour in many livestock species (Brown and

Macdonald, 1985; Wyatt, 2003) there may be unexploited potential to utilize odour cues in the management of animals in various contexts (Nielsen et al., 2015). Results from Study 3, show that cows are not only interested in novel odours but also that some odours evoke more interest than others. These results thus opens up new aspects to the possibilities for using odour cues in management of cattle. Enriching the environment in which cattle are kept, may therefore be possible by the use of odour cues. For instance, controlling behaviour of cattle e.g. moving cows between barns, may be possible by use of odour cues. More research is needed to progress and expand further on this.

6.1.5. The influence of individuality

The main basis for the current project was the hypothesis that cows in a production setting would isolate themselves from the group when offered the opportunity. From the collective findings of Studies 1-5, it is suggested that many other factors may influence behaviour of parturient cows. Additional to these findings is the aspect of individual differences between cows, which also potentially influences expression of pre-partum maternal behaviour. Across animal species, individuals differ in their behaviour in terms of risk taking, particularly in novel situations or under challenging circumstances (Wilson et al., 1994; Boissy, 1995; Gosling, 2001). For instance, pigs that struggle a lot when being restrained in supine position for 1 minute (termed 'high resisters') were less affected by their general housing conditions, but also less successful in reversal learning tasks (Bolhuis et al., 2004), whereas for pigs that did not struggle (termed 'low resisters') the opposite relation was found. Personality may thus reflect the capacity of an individual to cope with the environment in which it is kept. Modern dairy cows have been selected for generations to function within the current production systems; however, individual differences between cows are apparent. Although sparsely studied, dairy cows have different levels of sociability (Gibbons et al., 2010) and individual behavioural characteristics in spontaneous situations (Schrader, 2002). Making a choice of an appropriate calving site through assessment of different aspects of isolation, may thus also depend on the personality of the cow. Results from Study 4 showed that a personality assessment of being either bold or shy correlated with social dominance. Dominant cows were bolder, whereas subordinate cows were more shy, and social dominance significantly affected how the individual cows managed calving in the calving facility - the higher the dominance (boldness) the higher the chance of calving inside an individual maternity pen. Additionally, Stehulová et al. (2013) have shown that cows of Gasconne origin (beef cattle breed) differ with respect to protective maternal care and nursing behaviour, and Lidfors et al. (1994) noted large individual variation in terms of how cows express pre-partum isolation behaviour. Individuality may therefore be worth considering when developing future motivation-based calving facilities. If pre-partum maternal behaviour is influenced by personality, this inevitably adds to how an appropriate calving site is perceived and how this motivation can be modulated. More studies on the effects of cattle personalities on the use of calving facilities are needed to clarify this. Closer examination of aspects of personalities may aid the understanding of the background for the behavioural differences seen and help explain some of the variance seen in studies of pre-partum maternal behaviour. In light of the findings from Study 4, controlling cow pre-partum maternal behaviour by mimicking optimal calving site conditions may be complicated as cows may differ in terms of what they perceive appropriate. Hence, another solution to achieve more consistent behavioural responses may be the use of maternal derived odour cues. Pre-partum maternal attraction towards olfactory cues are dependent on the late gestation hormonal environment (Brennan and Kendrick, 2006), and it is unlikely that fundamental differences occur between mammalian species with regards to neural, hormonal and neurochemical control of maternal responses (as argued by Kendrick et al., 1997). As a result, individual differences may be less pronounced, as has for instance been shown in humans (Fleming, 1990). A future study investigating individual differences in connection with studying manipulation of pre-partum maternal behaviour using odour cues would allow further clarification on this matter.

6.2. Effects of an inappropriate calving environment: why bother?

6.2.1. Potential adaptive aspects of locating an appropriate calving environment

Throughout the above discussion, locating an appropriate birth site has been suggested to be adaptive in terms of aspects related to the proximate goal of the behaviour (Table 1: '*To ensure an environment allowing calm and secure parturition, and subsequent successful bonding and nursing. Equalling an appropriate birth site*'). Firstly, avoiding threats and disturbances seem a high priority for parturient cows and highlights the need for going 'unnoticed' when calving. This behaviour may originally have been developed in order to avoid threats, which potentially could kill the offspring. Secondly, this behaviour is probably adaptive in terms of ensuring proper bonding between cow and calf, as insufficient bonding may result in mismothering (for further details see Study 5, Chapter 5.5.). The discussion also emphasised that an appropriate birth site may not be present within commercial, indoor housing of parturient dairy cows. Being housed in an environment where the animal is not able to perform behaviour, which it is motivated to perform, have been shown to cause frustration and abnormal behaviour (e.g. Duncan, 1970; Lawrence et al., 1997), both of which are often considered key parts of animal welfare. Although an inappropriate calving environment may affect maternal behaviour and welfare, such effects have not yet been studied.

6.2.2. What is animal welfare?

Because of increasing ethical concerns about animal products, animal production systems are increasingly becoming a focus of amplified public scrutiny (European Commission, 2007). Animal welfare is a multifactorial concept due to the many aspects of the term including scientific, ethical, and economic issues as well as religious, cultural, and trade considerations (Robertson, 2015; Weary et al., 2015). The World Organisation for Animal Health (OIE) defines animal welfare as *'how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.'* (OIE, 2017). In a classical paper, Fraser et al. (1997) emphasized that perception of animal welfare is influenced by value-based concerns about what makes up a good life for an animal (which may not be the same for everyone). Fraser et al. (1997) therefore suggested three different concerns for animal welfare based on data on public concerns on animal welfare: 1) the biological functioning, 2) affective states and 3) natural living. People that are concerned about animal functioning focus mainly on measures of biological health in relation to diseases, injury, and on reproductive problems. Although concerns about animal suffering have been presented since the 1960ies, scientific understanding of affective states of animals is a relatively new and active area of research (Dawkins 2008), which has focused mainly on negative affect such as pain and fear. Concerns about natural living refers to the animal's ability to perform natural behaviour and live naturally (reviewed in von Keyserlingk et al., 2009; Ventura et al., 2015), and often includes at least some outdoor access. Naturalness is however open to interpretation (Vetouli et al., 2012), as many questions remain about which aspects of natural living are considered central by people who value naturalness as important for animal welfare. Fraser et al. (1997) recommended that indicators from all three concerns are included when assessing animal welfare. This is possible when using the animal welfare definition by OIE as all three concerns contribute to the state of the animal coping with it's environment. In order for new housing facilities and management practises to be implemented, and not only be science-based, it is suggested to be important to consider indicators of animal welfare from more than one of these concerns (preferably all). Otherwise, new initiatives may end up failing as for example enriched cages for layers as argued by Weary et al. (2015). Scientific evidence suggest that enriched cages improve the welfare of layers, but as society apparently did not want cages in general, implementation of this initiative has failed. Within this thesis, evaluation of animal welfare or the concept of animal welfare has not been a main focus. Aspects of commercial indoor housing of parturient cows is however likely to affect their welfare and below I will use the definition of animal welfare suggested by OIE to discuss welfare of parturient cows, with main focus on affective states.

6.2.3. Implications for animal welfare of parturient dairy cows

One step in relation to understand the welfare impact of different calving environments could be to examine whether calving in an appropriate calving environment (as perceived by the cow) represents a behavioural need. A behavioural need can be defined as 'a behaviour, which the animals are highly motivated to perform, and that lack of suitable opportunity to perform this behaviour results in abnormal behaviour and stress responses' (inspired from Jensen and Pedersen, 2008). Additionally, animals will work in order to be able to perform the behaviour and the behaviour is likewise a part of the natural behavioural repertoire (e.g. Mason et al. 1998; Tucker et al. 2018). Unsatisfied behavioural needs are often (but not always) associated with negative affective states (Broom, 2014) and, hence, animal welfare will be lower when a behavioural need is not satisfied. Likewise, satisfied behavioural needs are associated with positive affective states (Broom, 2014) and there may thus be improved welfare to gain from satisfying cows' needs e.g. in terms of providing a suitable calving environment. Further studies focusing on the different concerns as well as studies investigating whether the requirements for the behaviour to be a behavioural need is needed to clarify this. From the studies underlying this thesis, it may be suggested that low or no availability of physical cover and/or spatial distance (e.g. inability to enter an individual maternity pen, as for example for the subordinate cows in Study 4), could result in thwarted pre-partum motivation (i.e. unsatisfied behavioural need) and hence lead to frustration – a negative affective state. This may further lead to prolonged calving durations (as argued in Chapter 6.1.2.), which is associated with increased pain - another negative affective state (Mainau and Manteca, 2011), as well as increased inflammation (Proudfoot et al. 2013). However, in Study 4, the duration of the 2nd stage labour was not affected by calving location, which might contradict this suggestion. Nevertheless, there could be other explanations for this mis-match. It is possible that the lack of effect on calving duration was due to cows not being affected by calving location. Alternatively, the lack of effect may indicate that neither of the environments (individual maternity pen or group area) were perceived as being appropriate. From the results of Study 5, it is possible that inability to locate an appropriate calving site may have led to frustration expressed as e.g. restlessness. The causation of pre-partum restless behaviour is not currently fully understood. The process of giving birth is most likely painful (Mainau and Manteca 2011), which may cause restless behaviour. However, there are other possible explanations for the pre-partum occurrence of restless behaviour. In studies of pigs under production conditions, higher activity level measured pre-farrowing as frequent posture changes (Hansen and Curtis, 1980; Heckt et al. 1988) and abnormal behaviours such as bar biting (Jensen, 1988; Lawrence et al., 1997; Yun et al., 2015), rooting the floor and sham chewing (Lawrence et al., 1997; Damm et al., 2003), have been interpreted as restlessness inferring out-lets of frustration from not being able to express pre-partum maternal behaviour. Preventing sows from nest building activities accordingly resulted in decreased oxytocin levels (Damm et al., 2003; Yun et al., 2014), increased cortisol concentrations (Lawrence et al., 1997; Jarvis et al., 2002) and increased heart rate (Yun and Valros 2015). From work on social isolation and lying deprivation in non-parturient cows, Munksgaard and Simonsen (1996) found increased plasma concentration of ACTH in the cows deprived from lying and social contact and suggested this to be a sign of frustration. The cows in Studies 1 and 4 might, therefore, have been frustrated from being in an environment, which they did not perceive as appropriate, potentially leading to a prolonging of the duration of the 2nd stage of labour. However, further studies are needed to verify this suggestion. It could be advantageous to include measurements of frustration in parturient cows, but these are currently not available, and thus more studies of the consequences of allowing dairy cows the possibility to perform pre-partum maternal behaviour are needed. Including measurements of physiological indicators such as oxytocin, heart rate and cortisol (or ACTH), as well as behavioural measurements of frustration and more measurements of calving progress, would enable evaluation of current calving facilities and management, in relation to animal welfare.

6.2.4. Implications for maternal bonding

The above discussion emphasizes that keeping cows in individual maternity pens pre-partum may not provide an outlet for their pre-partum maternal motivation, as this confinement may not offer an opportunity to move away from disturbances (i.e. humans, machinery and/or conspecifics). Nevertheless, calving in an individual maternity pen may be a better solution than calving in a small-scale group area in terms of ensuring the bonding between cow and calf. As results from Study 4 indicate, alien calves are attractive to cows, and the presence of the former led to fewer cows calving in the individual maternity pens. Calving in groups, therefore, may lead to increased contact between non-related cows and calves during the sensitive period where bonding occurs. Immediate licking and sniffing of the offspring post-partum is essential in the establishment of the maternal bond allowing the mother and the offspring to recognise each other (Alexander and Shillito 1977; Espmark 1971). When newborn offspring is not being licked, it poses a risk of being rejected by the mother (Klopfer et al. 1964; Hudson and Mullord 1977). Additionally, lack of licking is associated with interruption of the maternal behaviour, possibly due to the dam not learning the odour of her offspring (Kendrick et al. 1997). Hudson and Mullord (1977) suggested that a short sensitive period immediately after calving may be present. If cows were given the opportunity to be in contact with their calves for 5 minutes immediately after calving, a bond was formed, persisting for up to 12 hours. The authors noted that this may take place regardless of the calf being an alien calf or the cow's own offspring. This may be why a higher prevalence of mis-mothering is seen in indoor housing of parturient dairy cows as compared to feral cattle (reviewed in Study 5 and suggested in von Keyserlingk and Weary, 2007). It is, therefore, likely that interruption of licking and sniffing between cow and calf can interfere with bonding, increasing the risk of mis-mothering. Because cows are attracted to birth fluids and newborn calves (potentially explained by birth fluids in the fur of the newborn) prior to their own calving, they risk being in close proximity to an alien calf at this stage. If a cow and her calf fail to bond, it may be easier for other cows to gain access to the calf, potentially leading to rejection of their own calf after calving. As bonding is part of the proximate maternal goal from Table 1, mis-mothering is likely to cause frustration in the cow (negative affective state) as discussed above. Therefore, failure to bond may have implications for the welfare of parturient cows. Likewise, failure to bond may have welfare implications for the calf in terms of biological functioning, as it may cause inability to obtain colostrum and limit the chances of maternal care after birth. Licking of the calf within the first few hours after calving is important for stimulating activity, breathing, circulation, urination and defecation (Metz and Metz, 1986). Timely provision of colostrum is important as the calf absorbs immunoglobulins from the milk and receiving colostrum 12 hours or later after birth have been shown to result in low immunoglobulins in the serum (Sangild, 2003). Calving in individual maternity pens may, therefore, be an advantageous solution to ensure achievement of this aspect of the maternal goal and to safeguard welfare.

The motivation-based calving facility designed in Study 4, was developed in the aims of limiting the need for intervention by the farmer (see Chapter 2.4.4. and Chapter 4.4.). In order to be successful for the farmers, a motivation-based calving facility would require a minimum of human intervention in terms of moving cows to individual maternity pens, while ensuring that all cows are moved at the right time (when the motivation shifts, see Chapter 2.4.4.). Additionally, in order to be successful in terms of animal welfare the facility should provide an outlet for the motivations of parturient cows i.e. the cow should be able to locate an appropriate calving site. Furthermore, risk of mismatches between dam and calf would be lowered and post-partum joined housing of cow and calf would even be a realistic future possibility (Johnsen et al., 2016). Study 4, however, showed that, so far, the system did not succeed in moving all cows to individual maternity pens. The collective studies underlying this thesis achieved new insights and suggested aspects to the mechanisms underlying pre-partum maternal behaviour of cattle, which may be beneficial for future development of motivation-based calving facilities. Among the suggestions to pass on to future studies are that 1) easing the entry and exit to/from individual maternity pens is probably an advantage; 2) increasing the distance between group and individual maternity pens may be beneficial; and 3) combining such optimised motivation-based designs with olfactory cues may facilitate the use of the pens at the right time without farmers having to interfere.

7. Conclusions

This thesis aimed to obtain new knowledge about the behaviour of parturient cows, factors affecting this behaviour and the use of maternity pens. The collective findings from the studies underlying this thesis suggest that several factors influence pre-partum maternal behaviour of dairy cows and their use of maternity pens.

a. Overall, no preference for a certain level of physical cover was found in parturient dairy cows, but a higher level of physical cover was chosen by cows with prolonged calving.

b. Calving site was influenced by the site of a previous calving potentially due to attracting effects of birth fluids. This attraction may represent a future possibility to control pre-partum maternal behaviour of dairy cows and facilitate entry to individual maternity pens.

c. Parturient cows and heifers were able to detect and distinguish between complex odours and some odours evoked more attention than others. There may be unexploited potential to use odours in managing dairy cows not only around calving.

d. Insertion of a gate at the entrance of an individual maternity pen did not increase the proportion of cows calving in the pens. High social dominance increased the probability of a cow calving in a pen, whereas presence of alien calves decreased the probability of a cow calving in a pen.

e. The causation of pre-partum maternal behaviour of cattle is suggested to be the motivation to locate an appropriate calving site, by means of isolation achieved through a combination of distance and physical cover. Based on literature, the motivation for isolation may increase with increasing level of disturbance.

Within a commercial dairy production environment, parturient cows can be affected by a number of factors, suggested to modulate their pre-partum maternel behaviour. The physical environment, disturbances from being housed in groups as well as olfactory cues influence the expression of pre-partum maternal behaviour of parturient cows. The collective results from this thesis can be used in the development of future calving facilities and improvement of welfare of parturient cows.

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