The degree of visual cover and location of birth fluids affect dairy cows' choice of calving site

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ABSTRACT

Under natural conditions, cows seek isolation and visual cover when calving becomes imminent, but the degree of visual cover necessary to provide an attractive calving site is not known. When calving indoors, preparturient cows are attracted to other cows' birth fluids, and this may influence their isolation seeking. Therefore, we aimed to investigate the effect of degree of visual cover of secluded areas and the location of birth fluids on dairy cows' calving site selection. One hundred twenty-four Danish Holstein cows were moved in groups of 8 to 12 to a group pen with access to 6 areas secluded by a barrier of either 3 m (wide barrier) or 1.5 m (narrow barrier). Sixty cows calved within 14 d of having been moved to the group pen and were included in the study. From 3 h before partition and until the cow and calf were removed from the pen within 3 h after parturition, the location and posture of cows were recorded, along with the location of where their birth fluids were discharged. In addition, location and posture of calves were recorded during and up to 3 h after calving. All data were collected from continuous video recordings. Continuous data for location and behavior were analyzed by mixed models, binary data were analyzed by chi-squared analysis, and location of calving relative to where the cow's own or alien birth fluids were discharged was analyzed using logistic regression models. Ten percent of the cows calved in a secluded area, with no effect of barrier width. However, before calving, cows with access to secluded areas with a wide barrier spent more time in a secluded area (22 vs. 7 min/3 h), and more cows were observed lying there (58 vs. 28%) than cows with access to secluded areas with a narrow barrier. Secluded areas with a wide barrier were used by more cows and calves during

the first hour after calving (36 vs. 10%). Among the cows that entered a secluded area after calving, the latency to enter correlated with their calf's latency to enter, suggesting that the 2 sought seclusion together. Seventy-nine percent of the cows calved in proximity (within a 1.25-m radius) to their own or an alien cow's birth fluids. There was no effect of barrier width on the probability of calving in proximity to their own or alien birth fluids. However, the probability of calving in proximity to their own or alien birth fluids was higher than the probability of not doing so. The greater use of secluded areas with a 3-m-wide barrier before and after parturition suggests that these are preferred over areas with a 1.5-m-wide barrier. Only a few cows calved in a secluded area, and more research is needed to explore the environmental and social factors affecting parturient cows' use of designated calving areas. The majority of cows calved close to where their own or alien birth fluids were discharged, suggesting that cows are attracted to these before calving. This may have affected the use of secluded areas as calving sites.

Key words: housing, maternal behavior, transition, parturition

INTRODUCTION

Use of individual maternity pens protects cows from social disturbances during calving and reduces the risk of mis-mothering (Edwards, 1983; Illman and Špinková, 1993) and thus failure of passive transfer of immunoglobulins. One of the challenges of managing individual maternity pens, however, is to move cows into the pens well in time before calving. It is difficult to predict the time of calving based on physical signs (Lange et al., 2017) or automatically monitored changes in behavior (Ouellet et al., 2016). This means that cows are likely to be moved late relative to calving, which in turn increases the risk of prolonged labor (Proudfoot et al., 2013) and thus calving complications. A key question is whether this problem can be solved by attracting the cow to a designated calving area before parturition.

When kept under near-natural conditions, cattle search for an appropriate place to calve isolated from

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the herd and potential predators (reviewed by Rørvang et al., 2018b), and motivation to search for a secluded place to calve is also suggested in dairy cows housed indoors. Dairy cows offered an opportunity to hide behind a 1.5-m-wide barrier in an individual maternity pen predominantly calved behind such a barrier rather than in visual contact with cows in an adjacent group pen, whereas the cows' calving site in similarly sized pens without a barrier was random (Proudfoot et al., 2014b). In the aforementioned study, cows were individually housed and thus separated from the group, but they spent the majority of time visually isolated behind the barrier beginning approximately 1 h before calving. Cows kept outdoors (Lidfors et al., 1994) and cows kept in pairs indoors in a large pen with access to a secluded area (Proudfoot et al., 2014a) were reported to isolate several hours before calving. In the study by Proudfoot et al. (2014b), the cows may have had problems staying behind the 1.5-m-wide barrier during the restless phase before parturition, which is characterized by frequent postural changes (Miedema et al., 2011; Jensen, 2012). A subsequent study, also with cows housed in individual maternity pens adjacent to a group pen housing pregnant cows, found that a 2.5-m-wide barrier was preferred over a 1.5-m-wide barrier, but only by cows with a prolonged calving (Rørvang et al. 2017a), which provides partial support for this suggestion. Thus, a secluded area with a wider barrier may be a more attractive calving site and may better enable cows to hide with their calf than a secluded area with a narrow barrier. The first research question of the present study thus was whether the width of a barrier of a secluded area affects cows' use of it around the time of calving.

Dominant cows were more likely to calve in a secluded area than subordinate cows (Rørvang et al., 2018a), and the presence of a newborn calf in either the group pen or any of the secluded areas reduced the likelihood of a cow calving in a secluded area. Birth fluids cover the newborn, and these fluids are thought to contain important chemosensory cues for establishing the maternal bond in many mammalian species (Lévy et al., 1983). In cattle, Edwards (1983) showed that calving cows, as well as newborn calves, have an attracting effect on preparturient cows, and Pinheiro Machado et al. (1997) reported that cows are attracted to amniotic fluid in their feed starting as early as 12 h before parturition. In support of this, a small-scale study suggested that cows favor a birth site close to where a previous calving has taken place (Rørvang et al. 2017b). Likewise, George and Barger (1974) noted in their field observations that cows remained in approximately the same spot as their own amniotic fluid was discharged until the calving was completed. Birth fluids may thus interfere with isolation seeking before

calving, and the second research question of the present study is whether calving site selection of group-housed dairy cows is influenced by discharge locations of a cow's own or alien birth fluids.

MATERIALS AND METHODS

Animals, Housing, and Design

The experiment was conducted on a commercial organic dairy farm in Jutland, Denmark. One hundred twenty-four Danish Holstein cows (59 were going to calve for the first time, and 65 had already calved once or more) were enrolled in the experiment. Cows were moved from the same group of dry cows to the experimental barn during the period from February 14 to May 10, 2017 (wk 7 to 19). Every week on a fixed weekday (Monday), a group of 8 to 12 cows (at least 7 d from expected calving) were moved to 1 of 2 group pens (16 m × 27 m). Except for a 4-m-wide slatted concrete floor area in front of a feed table on the 27-m side, the pens had a compost-bedded pack (a mixture of wood chips and sawdust) including an open area (9 m × 27 m) and 6 secluded areas (each 3 m × 4.5 m). The secluded areas were custom-made by using metal frames fitted with plywood and placed along the partially curtained sidewall of the building. Each secluded area had two 3 m × 1.8 m (width × height) sides and a side facing the open area with an entrance, which allowed the cows free entrance to each of the secluded areas. In 1 of the 2 group pens (group pen 1), this side (hereafter termed "barrier") was 1.8 m high and 1.5 m wide, leaving a 3-m-wide entrance; in the other (group pen 2), this barrier was 1.8 m high and 3.0 m wide, leaving a 1.5-m-wide entrance (Figure 1). For an illustration of the pen with secluded areas with a wide barrier, see Figure 2. The 2 experimental pens were separated by an opaque curtain, and a curtain also separated group pen 2 from the neighboring nonexperimental pen, whereas group pen 1 had an outer wall on one side. Water was available ad libitum from water troughs located in the slatted floor area. All animals had ad libitum access to a TMR, which was provided twice daily.

In uneven-numbered weeks, cows were moved into group pen 1, whereas in even-numbered weeks cows were moved into group pen 2. Within 5 h after parturition, cow and calf were moved to an individual maternity pen in a separate building. Cows that had not calved within 14 d after having been moved to the experimental group pens (groups 1 to 12) or had not calved within 7 d after having been moved to the experimental group pen (group 13) were moved to a maternity pen in a separate building. Seventy-five of the 124 cows calved in one of the experimental pens [10,

Figure 1. Video images of secluded areas (each 3 × 4.5 m) with a 1.5-m-wide barrier and a 3.0-m-wide opening to the open area (top) and secluded areas with a 3.0-m-wide barrier and a 1.5-m-wide opening to the open area (bottom).

6, 10, 5, 7, 8, 5, 5, 8, 4, 2, 4, and 1 cow(s) in groups 1 to 13, respectively]. After each calving, barn staff removed any visible remains of the amniotic sac and placenta when moving the cow and calf from the experimental pen, but the birth fluids absorbed by the bedding were not removed.

Experimental Animals and Observations

Animals. Among the 75 cows that calved in the experimental pens, 15 were excluded from the experiment due to calving assistance (4) or lost video due to power failure (11). Of the 60 cows available for behavioral recordings precalving, 2 cows were excluded from the data set at calving and after calving due to twin birth and a stillborn calf, respectively, leaving 58 cows avail-

able for behavioral recordings after calving (34 cows with access to pens with a narrow barrier and 24 cows with access to pens with a wide barrier). Because cows and calves were removed from the group pens within the first, second, or third hour of parturition, complete data for hours 1, 2, and 3 after calving included 52, 45, and 36 cows, respectively (30, 27, and 22 cows with narrow and 22, 18, and 14 cows with wide barriers). Due to poor video quality (shadows and insufficient lighting), data for calves included 52, 44, and 34 calves (30, 27, and 22 calves with narrow and 22, 17, and 12 calves with wide barriers) for hours 1, 2, and 3 after calving.

The 2 group pens, each including 6 secluded areas, were monitored by digital video cameras (model TVCCD-624, Monacor, Bremen, Germany) mounted above each of the secluded areas: 6 cameras per group pen. Each camera covered a secluded area and part of the open area in front of this and neighboring secluded areas but not the slatted floor area (Figure 1). Video recordings were stored continuously and backed up weekly on external hard drives. Among the 58 cows for which data were available for behavioral recordings at calving, the location where the cow discharged her birth fluids could be identified on the video for 51 cows.

Choice of Calving in the Open Area or a Secluded Area and Behavior Before and After Parturition. From the video recordings, one experienced observer recorded, for each cow, the exact calving time (when the hips of the calf were fully expelled from the dam), whether cows calved in a secluded area or in the open area, and the time when cow and calf were removed after calving. Furthermore, the observer recorded the location (secluded area 1, 2, 3, 4, 5, 6, or open area) and posture [upright (standing or moving around) or lying (on sternum or flat on one side)] of each cow during 3 h before calving and up to 3 h after parturition. Finally, the observer recorded the location (secluded area 1, 2, 3, 4, 5, 6, or open area) and posture [upright (standing with body supported by 4 legs for at least 5 s or walking), lying (on sternum or flat on one side), or attempting to stand (partially upright with both hind legs extended and at least one front leg bent underneath the body)] of each calf during the period up to 3 h after birth. All locations and postures were recorded continuously (Martin and Bateson, 2007).

Choice of Calving in Relation to Location of Discharge of Own and Alien Birth Fluids. From the video recordings, a second experienced observer recorded the location of the discharge of birth fluids and the location of calving. The discharge of the birth fluids was visible from the video as (1) the amniotic sac breaking outside the cow, (2) a sudden fluid bursting out of the cow when the sac ruptured inside her, or

Figure 2. Floor plan of group pen 2 with secluded areas with a 3.0-m-wide barrier. The dark gray area represents the slatted floor area by the feed manger. The center of the shaded (light gray) circle indicates the calving place of the first cow (light gray cow), and the diameter of this circle indicates one cow length, within which the calving took place (radius of approximately 1.25 m). The black cow illustrates the position of a subsequent calving, and the corresponding 2.5-m-diameter dashed circle covers the area within which her own birth fluids were discharged. The black cow calves within proximity of her own and alien birth fluids. Color version available online.

(3) a thick gel-like thread hanging down from outside the vulva of the lying or standing cow. From the birth fluids discharge location, a circle with a radius of 1.25 m was drawn, with the discharge location as the center (i.e., an area with a diameter of 2.5 m, corresponding to one cow's length; Figure 2) to be able to determine whether a cow calved within close proximity of her own birth fluids discharge or alien birth fluids. The analysis of whether cows calved within a 1.25-m radius of their own birth fluid discharge included all 51 cows available for this observation. The analysis of whether cows calved within a 1.25-m radius of an alien cow's birth fluids discharge included 38 cows [median and range 19.8 (1.8 to 121) h between calving]; 10 cows had to be excluded because they were the first cows to calve in their group, and their birth fluid discharge spot functioned as the "alien" spot for the subsequent calving (excluding groups 2, 12, and 13 because there was no observation of a previous calving for cows in these groups). Additionally, 3 cows were excluded because their particular alien birth fluid discharge spot could not be determined due to power failure (2), or because there had been no previous calving within 5 d (1). The 5-d cut-off was the maximum duration between successive calving events in previous work (Rørvang et al., 2017b).

Other Measures. In a previous study (Rørvang et al., 2018a), the presence of a newborn alien calf within 8 h of calving tended to reduce the likelihood of a cow calving in a secluded area. Therefore, cows that calved

within 8 h after another cow calving were identified. The secluded areas were placed along the outer wall with windows. As temperature and light intensity (Grandin, 1980) may affect how readily cattle move into new areas, light intensity (lx) and temperature were measured at a height of 1 m above floor level in the center of each of the groups and secluded areas weekly from wk 12 to 18, and values for the secluded areas were averaged within week. For light intensity, the median and range was 471 (272 to 885), 424 (134 to 693), 480 (217 to 985), and 341 (113 to 683) lx for secluded areas in group pen 1, the open area in group pen 1, secluded areas in group pen 2, and the open area in group pen 2, respectively. For temperature, the median and range was 9.2 (1.8 to 15.1), 9.1 (2.1 to 15.0), 9.2 (1.7 to 15.1), and 8.8 (1.7 to 14.9) °C for secluded areas in group pen 1, the open area in group pen 1, secluded areas in group pen 2, and the open area in group pen 2, respectively.

Statistical Analysis

Choice of Calving in the Open Area or a Secluded Area and Behavior Before and After Parturition. Whether the cow calved in the open area or in a secluded area was analyzed by chi-squared analysis for the effect of treatment (narrow or wide) and parity (primiparous or multiparous), respectively. Before parturition, lying time and the number of lying bouts were square root-transformed before analysis to be able

to assume normal distribution as assessed by visual inspection of residual plots. These variables were analyzed by a mixed model including treatment (narrow or wide barrier), parity (primiparous or multiparous), presence of an alien calf within 8 h of parturition (0, 1), and hours from calving (−3, −2, −1) as fixed effects, whereas the number of cows in group pen at parturition [(2, 3, 4, ..., 12), median 7 (interquartile range 5 to 8)] was included as a covariate. Group (1, 2, 3, ..., 13) was included as a random effect, and the covariance between repeated observations on cow was modeled as autoregressive (based on Akaike information criterion). Denominator degrees of freedom were calculated using the Kenward-Roger approximation.

Because of many zero observations in the individual hour before parturition, the duration of time spent in any of the 6 secluded areas was summarized over the 3 h before parturition, and it was noted whether the cow had been lying in a secluded area within the 3 h before parturition or not. These 2 variables were analyzed for the whole 3-h period before parturition. The duration of time spent (either standing or lying) in any of the 6 secluded areas was transformed by the natural log (to be able to assume normal distribution, assessed as described above) and analyzed using a mixed model including treatment (narrow or wide barrier), parity (primiparous or multiparous), and presence of an alien calf within 8 h of parturition (0, 1) as fixed effects. The number of cows in the pen at parturition (2, 3, 4, ..., 12) was included as a covariate, whereas group (1, 2, 3, ..., 13) was included as a random effect.

Not all cows lay down in a secluded area before parturition, and this variable was transformed into a binary variable and analyzed by chi-squared analysis. After parturition, lying duration and number of lying bouts of cows and calves, as well as the duration of calves' attempts to stand were analyzed using a similar mixed model as described above for data collected before parturition (after parturition, hours from calving were 1, 2, and 3). The only exception was that for time spent upright and for time attempting to stand, the covariance between repeated observations on calf was modeled as unstructured, because only this covariance structure allowed for the estimation of the random effect of group.

The number of cows, the number of calves, and the number of cows together with their calves observed in either of the 2 types of secluded areas in each of the 3 h after parturition were analyzed by chi-squared analysis. The latency from parturition until entering a secluded area was calculated for each cow and each calf. For those cows where both cow and calf entered a secluded area, cows' latency to enter a secluded area was cor-

related with that of their calves using Spearman rank correlations.

The above statistical analyses were performed with SAS software (version 9.4; SAS Institute Inc., Cary, NC).

Choice of Calving in Relation to Location of Discharge of Own or Alien Birth Fluids. To investigate whether the treatment (narrow or wide barrier) influenced whether cows chose to calve close to their own or close to alien birth fluids [(0, 1); did or did not calve within a radius of 1.25 m of own or alien fluids], 2 logistic regression models were applied separately. Each full model included the fixed effects of treatment (narrow or wide barrier) and parity (primiparous or multiparous), and the random effect of group (1, 3, 4, 5, 6, 7, 8, 9, 10, 11). Parity was found to have no effect ($P > 0.1$) and was excluded from both models. The final models thus included the fixed effect of treatment and the random effect of group.

In a post hoc analysis, the count from the 2 categorical variables [calving within a 1.25-m radius of own birth fluids (1, 0) and calving within a 1.25-m radius of alien birth fluids (1, 0)] was used to calculate probabilities that were subsequently compared in an Exact binomial test to analyze whether the probability of calving within a 1.25-m radius of a birth fluid discharge (own or alien) was greater than the probability of not doing so. Finally, a Fisher's exact test was carried out to test whether calving within a 1.25-m radius of alien birth fluids discharge also meant calving within a 1.25-m radius of a cow's own birth fluids discharge or vice versa.

These statistical analyses were performed using the R software, version 3.4.0 (R Core Team, 2014) with the additional packages *xlscx* (Dragulescu, 2014) and *lme4* (Bates et al., 2015).

RESULTS

Choice of Calving in the Open Area or a Secluded Area and Behavior Before and After Parturition

Only 6 of the 58 cows for which data for the moment of calving were available calved in a secluded area (3/34 and 3/24 for cows with a narrow and a wide barrier, respectively), and this was not affected by treatment or parity.

Before calving, lying time did not differ between hours (median 17 min; interquartile range 6 to 32 min), but the number of lying bouts per hour increased with each hour approaching parturition [square root-transformed estimates ($\pm$ SE): 1.30, 1.42, and 1.77 ($\pm$ 0.104); $F_{2,114} = 10.49$; $P < 0.001$]. Back-transformed means were 1.7,

2.0, and 3.1 lying bouts per hour for -3, -2, and -1 h, respectively.

During the 3 h before parturition, wide barriers in the pen resulted in more cows lying down in a secluded area [58% (14/24) vs. 28% (10/36); chi-squared (df = 1) = 5.60; $P < 0.05$] and in cows spending more time in a secluded area [log-transformed estimates ($\pm$ SE): 3.05 ($\pm$ 0.36) vs. 1.94 ($\pm$ 0.33); $F_{1,11} = 6.36$; $P < 0.05$] than did the narrow barriers in the pen. Back-transformed estimates were 21 and 7 min/3 h. Neither parity, the presence of an alien calf, nor the number of cows in the pen at parturition affected any of the mentioned variables.

After parturition, primiparous cows lay down less [square root-transformed estimates ($\pm$ SE): 3.39 ($\pm$ 0.35) vs. 4.43 ($\pm$ 0.34) min/h; $F_{1,43} = 5.76$; $P < 0.05$; back-transformed estimates were 11.49 vs. 19.62 min/h] and had a lower number of lying bouts [square root-transformed estimates ($\pm$ SE): 0.62 vs. 0.95 (0.10) bouts/h; $F_{1,47} = 6.01$; $P < 0.05$; back-transformed were 0.38 vs. 0.90 bouts/h] than multiparous cows. Lying time was lowest in the second hour, and the number of lying bouts was lowest during the first hour (Table 1).

For calves, no effect of treatment was found on lying time, time spent upright, or the time spent attempting to stand. However, with hour after birth, calves spent less time attempting to stand and more time standing upright (Table 1). Neither the presence of an alien calf nor the number of cows in the pen at parturition affected any of the mentioned variables.

In the group pen with wide barriers, more cows and more cow-calf pairs were observed in a secluded area than in the group pen with the narrow barriers during the first hour after parturition (Table 2); in the second and third hours after parturition, no effect of treatment on these 2 variables was seen. Parity did not affect the number of cows or cow-calf pairs observed in a secluded area during the first 3 h after parturition. However, a tendency for more first-parity cows in a secluded area was noticed during the second hour after parturition (12/24 vs. 5/21; chi-squared (df = 1) = 3.27; $P = 0.07$). There tended to be more calves in secluded areas with a wide barrier during the second hour after birth (Table 2).

Of the 52 cow-calf pairs available for behavioral observations at calving, 6 pairs had a latency to enter a secluded area of zero because of the calf having been born in the secluded area. Seventeen pairs were removed before either of the pair entered a secluded area. Among these, 2, 6, and 5 pairs did not enter before they were removed within the first, second, and third hour, respectively, whereas the final 4 pairs had not entered before they were removed after the third hour. Six pairs were removed after the calf had entered but

Table 1. The effect of hour after parturition (1, 2, and 3) on the duration of lying and the number of lying bouts of the cow and the duration of lying, upright, and attempting to stand of the calf

Posture	Hour 1		Hour 2		Hour 3		P-value
	Mean	SEM	Mean	SEM	Mean	SEM	
Posture of the cow							
Lying (min/h) ¹	5.00 ^a (25.00)	0.36	2.78 ^c (7.73)	0.38	3.99 ^b (15.92)	0.44	$F_{2,67} = 12.98$ <0.001
Lying bouts (no./h) ¹	0.45 ^a (0.20)	0.10	0.89 ^b (0.79)	0.10	1.00 ^b (1.00)	0.11	$F_{2,70} = 12.80$ <0.001
Posture of the calf							
Lying (min/h)	38.76 ^a	2.76	32.53 ^c	2.93	29.93 ^b	3.47	$F_{2,62} = 3.11$ 0.06
Upright (min/h) ¹	2.07 ^a (4.28)	0.33	4.31 ^b (18.58)	0.41	4.77 ^b (22.75)	0.49	$F_{2,37} = 23.60$ <0.001
Attempting to stand (min/h) ¹	2.87 ^a (8.24)	0.31	1.09 ^b (1.19)	0.28	0.37 ^c (0.14)	0.17	$F_{2,45} = 21.63$ <0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Transformed by square root; back-transformed values are indicated in parentheses.

Table 2. The effect of hour after parturition (1, 2, and 3) on the number of cows, calves, and cow-calf pairs observed in a secluded area (no. of animals in secluded area/no. of animals available for observation) in group pens with secluded areas with a narrow and a wide barrier, respectively

No. of animals	Hour	Barrier		χ^2 (df = 1)	<i>P</i> -value
		Wide	Narrow		
Cows					
n = 52	1	8/22	3/30	5.29	<0.05
n = 45	2	10/18	9/27	2.19	0.14
n = 36	3	8/14	11/22	0.18	0.68
Calves					
n = 52	1	9/22	6/30	2.70	0.10
n = 44	2	10/17	9/27	2.76	<0.10
n = 34	3	8/12	13/22	0.19	0.66
Cow-calf-pairs					
n = 52	1	8/22	3/30	5.30	<0.05
n = 45	2	9/18	8/27	1.91	0.17
n = 36	3	8/14	11/22	0.18	0.68

before the cow entered: 4 calves entered within the first hour without the cow following (before they were both removed within the second hour) and 2 calves entered within the second hour without the cow following (before they were both removed within the third hour). Finally, 1 cow entered within the first hour without the calf following, before they were both removed within the second hour. The latencies of the remaining 29 cow-calf pairs, where both cow and calf entered a secluded area, were correlated (Spearman $r = 0.82$; $P < 0.001$), which is illustrated in Figure 3.

Choice of Calving in Relation to Location of Discharge of Own and Alien Birth Fluids

Overall, 47 out of 51 cows (23 primiparous and 24 multiparous) calved within one cow's length (in an area within a radius of 1.25 m) of where their own birth fluids were discharged (Table 3). Additionally, 32 out of 38 cows (13 primiparous and 19 multiparous) calved within one cow's length of where alien birth fluids had been discharged less than 5 d previously (Table 3).

There was no effect of barrier width on the probability of calving within a cow's length of own birth fluids (logistic regression, estimate $\pm$ SE: 0.71 ± 1.19 , $P = 0.55$) or on the probability of calving within a cow's length of alien birth fluids (logistic regression, estimate $\pm$ SE: 1.10 ± 1.16 , $P = 0.34$).

The probability of calving within close proximity of own birth fluids [Exact binomial test, estimate (95% CI): 0.92 (0.81 ; 0.98), $P < 0.001$] was overall higher than the probability of not doing so, and likewise for calving within close proximity of alien birth fluids [Exact binomial test, estimate (95% CI): 0.84 (0.69 ; 0.94), $P < 0.001$].

Last, 30 out of 38 animals calved within a cow's length of own and alien birth fluids discharge [Fisher's Exact test, odds ratio (95% CI): 6.89 (0.40 , 121.68), $P = 0.11$], implying that calving in close proximity to alien birth fluids discharge did not necessarily mean calving within close proximity of a cow's own birth fluid discharge and vice versa.

DISCUSSION

The first research question was whether the width of the barrier to a secluded area affected cows' use of

Figure 3. A plot of calves' latency (y-axis) to enter a secluded area against their dams' latency (x-axis) to enter the pen after parturition. Data for animals in pens with secluded areas with a narrow barrier are indicated as squares, whereas data for animals in pens with secluded areas with a wide barrier are indicated as circles.

Table 3. Probabilities separated into response (yes or no) in terms of calving close to own or alien birth fluids or not

Group and response	Overall	Divided by treatment ¹		Treatment probability	
		Narrow	Wide	Narrow	Wide
Match own amniotic fluids spot/calving spot (n = 51)					
Yes	47	28	19	0.90	0.95
No	4	3	1	0.097	0.05
Match alien amniotic fluids spot/calving spot (n = 38)					
Yes	32	20	12	0.80	0.92
No	6	5	1	0.20	0.08

¹Sample size (n) of animals calving within close proximity (diameter of ~2.5 m) to own or alien birth fluids discharge, and separated into response including number within each treatment (narrow or wide barrier).

the secluded area around the time of calving. Before calving, cows with access to secluded areas with a wide barrier spent, on average, 22 min there during the final 3 h before parturition compared with 7 min in cows with access to secluded areas with a narrow barrier. As expected, cows spent more time in secluded areas that offered the highest level of visual cover. However, it is surprising that the cows did not spend more time in the secluded areas as they approached calving and that only 6 out of 58 cows calved in a secluded area. By comparison, approximately 50% of the cows calved in a secluded area accessible from an open area in the study by Rørvang et al. (2018a). The discrepancy between studies may be due to a lower availability of secluded areas in the present study where there were 6 areas for up to 12 cows (median 7 cows) compared with 6 areas for 6 cows in Rørvang et al. (2018a). In addition, no water was available in the secluded areas. In sows, placing water and feed in a small pen designated for farrowing increased the number of sows farrowing there (Haskell and Hutson, 1994), and offering water in a secluded area may increase parturient cows' use of it. Furthermore, in the present study, secluded areas were placed along the outer wall, which was partially curtained. The temperatures were within the same range in the secluded areas as in the open areas, but the mean light intensities were numerically higher in the secluded areas than in the group areas. Thus, placement along a curtained outer wall may have made the secluded areas less attractive as birth sites. Selected birth sites in other ungulate species may have visual cover of various types, ranging from a slight topographical change to a dense cover of trees and bushes (reviewed by Rørvang et al., 2018b). Vegetation provides a different quality of cover than plywood barriers because it may conceal the cow while she retains an outlook to and view of the surroundings, an effect that may be achieved by venetian blinds (see an example in poultry in Newberry and Shackleton, 1997). A cover in the form of an overhang may also make a secluded area more attractive, as suggested by Lidfors et al. (1994).

The secluded areas in the present study may have been situated too close to where the other cows stayed. Among beef cattle kept outdoors, cows increased their distance from other cows on the day of calving (Swain et al., 2015), and most cows calved at some distance from the main herd (Flörcke and Grandin, 2014). In the present study, the space allowance in the open area was 20 m²/cow when the maximum 12 cows were in the pen. Cows were removed from the pen once they had calved, but the number of cows present in the pen at the time of calving had no effect on the use of the secluded areas. However, this study was not designed to assess the effect of space allowance and accessibility of secluded areas. Future studies should investigate the effects of these factors, as well as aspects of cover, placement in a building, and distance to main feeding and resting areas for dairy cows' use of designated calving areas when housed indoors in a group setting.

In spite of the low usage of the secluded areas, more cows were lying in a secluded area before parturition when the secluded areas had a wide barrier (>50%) than when the secluded areas had a narrow barrier (<30%). This suggests that secluded areas with a wide barrier, although not selected more often as a birth site, were investigated more and might have been viewed by cows as a more suitable place to rest before parturition than secluded areas with a narrow barrier. In addition, during the first hour after parturition, only cows in pens with wide barriers moved to a secluded area within the first hour after calving, suggesting that cows found a wide barrier more attractive than a narrow barrier. For calves, there was only a tendency for more calves in a secluded area in pens with wide barriers during the second hour after calving, and, perhaps, calves found the 2 barrier types equally suitable to hide behind. During the first hour after parturition, the dam licks her calf intensively, which stimulates the calf to stand and to suckle, typically occurring during the second hour after birth (reviewed by von Keyserlingk and Weary, 2007). In accordance with this, the calf was upright for more time during the second and third hours compared with

the first hour after birth in the present study. The association of latency to enter the secluded area between the dam and her calf suggests that the 2 sought seclusion together in these areas. We can only speculate as to why the dam and calf moved into the secluded areas after parturition. Numerically, more calves than cows entered a secluded area within the short time span available to do so, and, possibly, calves especially found a secluded area attractive and were therefore driving this behavior. In feral cattle, the newborn calf remains hidden in vegetation for the first few days of life while the dam grazes nearby (e.g., Vitale et al., 1986), and newborn calves may be motivated to seek seclusion. Providing hiding places for calves when cows calve in a group setting may provide an opportunity to perform this natural behavior and thus be beneficial for calf welfare, but more studies investigating the motivation for hiding behavior in newborn dairy calves are needed to clarify this.

The second research question of this study was to investigate whether calving site selection in group-housed dairy cows was influenced by discharge locations of their own and alien birth fluids. This study showed that cows housed in groups chose to calve close to both their own birth fluids discharge location and that of alien birth fluids. These results are similar to the results of a small-scale study by Rørvang et al. (2017b) and add support to the hypothesis that olfactory cues in birth fluids become increasingly attractive to the preparturient cow. As parturition approaches, the female becomes increasingly responsive toward olfactory cues in the birth fluids, which are thought to direct her attention toward her newborn, thereby facilitating maternal behavior [e.g., rodents (Kristal, 1991), sheep (Lévy et al., 1983; Dwyer, 2014), cats, horses, pigs, and goats (Fabre-Nys et al., 1993)]. As cattle are motivated to distance themselves from the herd before calving (reviewed by Rørvang et al., 2018b), there may not have been a biological selection toward attraction to their own birth fluids in particular, resulting in an attraction also toward all birth fluids when housed in indoor group calving environments, where cows are not able to distance themselves from other cows.

The attraction toward alien birth fluids may result in cows discharging their own birth fluids within the same area and thus increases the attraction to this specific place and, subsequently, increases the likelihood of the cow calving there. The possibility that visual or physical cues affected calving site selection cannot be ruled out, but as the probabilities of calving within one cow's length of alien birth fluids were high (~90%), this suggests that olfaction may outweigh the effects of other cues. Pinheiro Machado et al. (1997) found that attraction toward amniotic fluid occurred 12 h before calving

and not before that time, which makes it unlikely that olfactory cues were attractive to cows that were not close to calving.

We were unable to include an effect of parity in the analysis due to too little variation and the fact that the proportion of animals not calving within close proximity of either their own or alien birth fluids was low. This, however, also implies that parity had very little or no effect on the attraction to birth fluids, and it is unlikely that a first exposure to birth fluids is needed in order for cattle to develop or learn an attraction, which is the case for some odors (Brennan and Kendrick, 2006). The attraction to birth fluids may thus be innate, but more studies are called for to clarify this.

The attraction to birth fluids before calving may have interfered with the cows' use of the secluded areas and may explain why only ~10% of the cows calved in a secluded area in the present study. In the study by Rørvang et al. (2018a), where ~50% of the cows calved in a secluded area, deep bedding containing birth fluids was cleaned out after each calving. Identifying where birth fluids are discharged in the bedding and removing this bedding requires surveillance and labor and is unlikely to be a practical solution. However, the effect of birth fluids also represents an opportunity to use this to control parturient cows' calving site, but this also requires further investigation.

CONCLUSIONS

Only a few cows calved in the secluded areas irrespective of barrier width. However, when cows had access to secluded areas with a wide barrier of 3 m, they used these areas more before parturition, and more cows and calves also used the areas after parturition than when they had access to secluded areas with a narrow barrier of 1.5 m. This suggests that a secluded area with a 3-m-wide barrier is preferred before and after calving to an area with a 1.5-m-wide barrier. Most cows calved in proximity to their own and alien birth fluids, and this occurred in both primiparous and multiparous cows. This suggests that cows are attracted to their own and alien birth fluids before calving, and this attraction occurred without a first exposure.

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