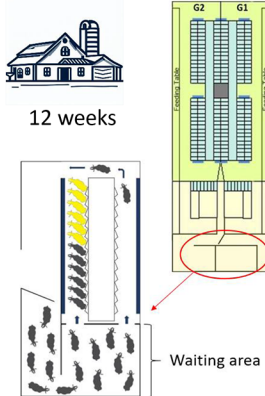


Associations of parity and lactation stage with the order cows enter the milking parlor

Ida Hansson^{1*†} and Svenja Woudstra^{2*}

Graphical Abstract

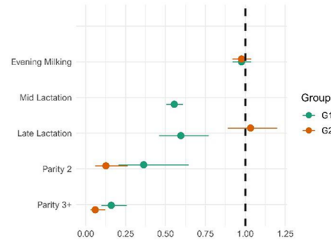
251 cows



Milking Order



1. Order of entrance



Estimated odds ratios and 95% confidence intervals

Conclusions:

Cows do not mix homogeneously. First-parity and early-lactation cows tend to enter the milking parlor earlier compared with the rest of the herd.

Importance for:

- the understanding of mastitis infection dynamics and the design of bio-economic disease transmission models
- future studies on how social contacts affect cows during milking

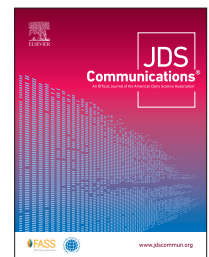
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Summary

This study investigated the milking ordering to a milking parlor in 1 commercial farm with 251 cows over 12 weeks. Two analyses were conducted to determine whether parity and lactation stage were associated with the entrance order and a preference to enter as one of the first cows within one milking line. The milking order turned out to be fairly consistent, and cows in their first parity, as well as cows in early lactation, tended to enter the parlor early. The preference of entering a new milking line first gave similar results, but there were no large individual differences.

Highlights

- Cows do not mix homogeneously during milking.
- Parity 1 and early-lactation cows enter the milking parlor earlier than other cows.
- Cows tend to keep their rank within the milking order constant.
- The results should be considered when planning grouping strategies.



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Associations of parity and lactation stage with the order cows enter the milking parlor

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Abstract: To explore the effect of lactation stage and parity on the milking order of cows, we collected milking order data of all lactating cows ($n = 251$) over a period of 12 wk in one commercial Swedish dairy cow herd using a herringbone milking parlor. Cows were kept in 2 housing groups (G1 and G2) and moved from G1 to G2 at approximately mid lactation. Two analyses were conducted to investigate if lactation stage (early: 2–49 d in milk, mid: 50–179 d in milk, and late ≥ 180 d in milk) and parity are associated with the entrance order to the parlor or a preference of entering a new milking line first. In G1 and G2, cows in first parity entered the milking parlor earlier than cows in higher parities. In addition, in G1 cows in early lactation entered the milking parlor earlier than cows in later lactation. Similar effects were observed for the preference of entering a new milking line first. No effect of mid versus late lactation could be observed in either G1 or G2. The study also found that cows tend to keep their rank within the milking order constant. The results of the study indicate that cows of presumably lower hierarchy (first parity and early lactation) leave the waiting area earlier compared with other cows. This should be considered when planning grouping strategies and preventive measures against mastitis pathogen transmission.

The entrance order of individual cows into a milking parlor, the milking order, is not random, and some cows constantly tend to enter the milking parlor earlier than other cows (Grasso et al., 2007; Vargas-Bello-Pérez et al., 2020). In the study of Sauter-Louis et al. (2004), high-dominance cows tended to be milked first and low-dominance cows last. In other studies, cows with higher milk yield and lower SCC showed a tendency to enter the milking parlor early (Rathore, 1982; Berry and McCarthy, 2012). However, only weak correlations have been reported, and other studies did not find the same correlations for milk yield and social rank (Vargas-Bello-Pérez et al., 2020) as well as SCC (Dias et al., 2019).

Milking management is one important factor that can influence the udder health situation in dairy cow herds, and mastitis caused by IMI is one of the costliest health disorders in dairy farms (Ruegg, 2017). Pathogens causing mastitis can have either a host-related, contagious transmission pathway, where the udder tissue of infected cows forms the major reservoir, or be mainly transmitted from extra-mammary sites (e.g., feces of cows). Pathogens that show a contagious transmission pathway often cause strong inflammatory reactions measured as SCC, long-lasting infections, and milk yield losses (Heikkilä et al., 2018; Woudstra et al., 2023). Until now, however, it remains unclear if cow characteristics that are associated with the probability of being infected with mastitis pathogens (i.e., parity and lactation stage; Mekonnen et al., 2017; Taponen et al., 2017) also influence the position in the milking order.

Beyond pathogen transmission, understanding cow characteristics that influence the milking order is also essential from an animal behavior and welfare perspective. Sufficient lying time has been considered important for cow welfare (Tucker et al., 2021). Short lying time has, for example, been associated with lameness (Galindo and Broom, 2000), and cows entering the milking parlor

late have also been seen to have an increased risk of lameness (Sauter-Louis et al., 2004). Cows with a long waiting time in the waiting area will also be away from feed for longer, affecting milk production (Dias et al., 2019). In addition, long intervals between milkings also harm milk yield (Stelwagen, 2001), with even only occasionally prolonged intervals influencing the yield (Ayadi et al., 2003). Parity and lactation stage have shown to affect the number of social contacts between dairy cows (Hansson et al., 2023). To be able to further study how social contacts could potentially affect the cows during milking in a parlor, the milking order needs to be investigated more closely.

Previous research has shown that the entrance of cows to the milking parlor does not happen randomly. However, it has not been extensively studied if a cow's parity or lactation stage influences her rank in the milking order. Therefore, the present study aimed to investigate if the order in which cows enter the milking parlor is associated with their lactation stage or parity. In addition, we wanted to explore over a period of 12 wk the cow level variability of positions within the milking order.

Data were collected from one commercial dairy farm in Sweden that housed around 210 lactating dairy cows in a noninsulated freestall barn. The cows were divided into 2 housing groups, G1 and G2, where cows in G1 were primarily in early to mid lactation and cows in G2 were in mid to late lactation. Cows were routinely regrouped from G1 to G2 at approximately 170 DIM when confirmed pregnant or designated for slaughter. The lactating cows were milked in a 2×12 unit herringbone milking parlor (2×12 GEA Euro class 800 with Dematron 75, GEA Farm Technologies, Bönen, Germany) twice daily (start of milking around 0430 and 1630 h). The milking sessions lasted around 1.5 h for each group and G1 was always milked first. Before milking, the cows were

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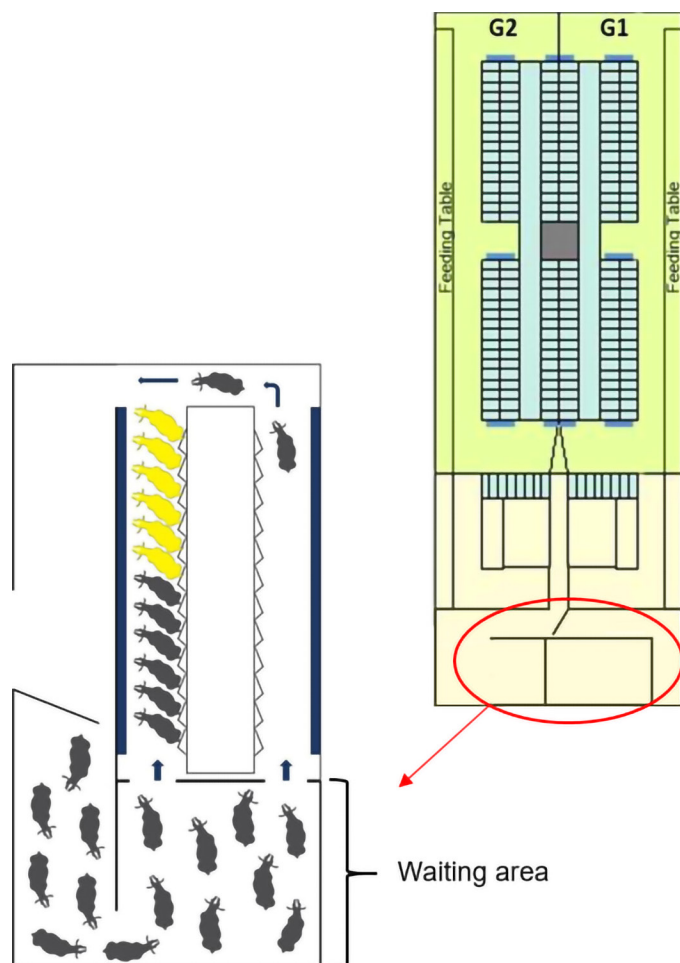


Figure 1. A schematic map of the freestall barn and the milking parlor used within the study. The barn holds 2 milking groups, G1 and G2, where the green area in the barn map represents the feeding tables and allocated alleys, and the blue area represents the cubicles and the alleys between the cubicles. The area shown in beige is a separate area with calving boxes, drying off area, and the milking parlor in the bottom of the map. The milking parlor is a 2 × 12 unit herringbone parlor and has a waiting area in front of the 2 entrances of the parlor. The yellow cows in the milking parlor represent the first 6 positions within the left side of the parlor. The figure was partly adapted from Hansson et al. (2023).

gathered in the waiting area in front of the parlor, where they could move freely and alter their position to the entrance of the milking parlor (Figure 1). The cows enter the parlor in a single row to one side of the parlor at a time and are automatically identified at the entrance to the milking parlor. After milking, the cows exit in a single row. Each cow's position within the parlor and the timestamp when the milking cluster detached were transferred from the milking equipment to the farm computer.

Data from 165 milking sessions were collected between August 11, 2020, and November 01, 2020. Records from 251 cows (148 cows in G1 and 154 cows in G2, with 52 of these cows present in both groups during the study period) were collected. Data from one complete milking session was missing due to late data transfer (from the farmer to the researchers). For the 165 milking events

we collected, the proportion of missing records was 1%, and the mean proportion of missing records per cow was also 1%. The total number of observations used in the analyses was 33,237. One reason for missing records was that the milking records of cows that had entered the gradual drying-off process were not included in the files from the farm for some of the weeks. In Sweden, such a gradual drying-off process is common. In the study herd, the gradual drying-off process started on Tuesdays; the cows were moved to a separate area within the barn, and milked subsequently only Wednesday morning, Friday morning, and Monday morning. Then the dried-off cows were moved to another building. They were moved to the calving boxes before calving, and then introduced to the milking groups 24 to 48 h after calving. The milking order was determined based on when a cow finished milking and its position within the parlor. All cows at each milking session and within each group were numbered from first to *n*th order depending on the number of lactating cows daily and the milking spot number in the parlor.

The milking order was divided into 4 groups for each housing group: first, second, third, and last. The first group represents the first batch of cows milked, including both sides of the parlor (positions 1–12 on the right-hand side and 13–24 on the left-hand side), the second group was the second batch of cows (positions renamed as position 25–48 including both sides), the third as the third batch of cows (positions 49–72), and the last group representing positions >72. Thus, there were milking groups 1 to 4 for G1 and again 1 to 4 for G2, reflecting that cows could choose their milking order freely within each housing group, but could not switch freely between housing groups. The milking event was classified as either morning milking (1) or evening milking (2). The cows' positions within the parlor during milking were also categorized into the first 6 positions (1) or the last 6 positions (2) within each side and each milking batch (see Figure 1). In the analysis, records for cows during the drying-off process and less than 2 d after calving were then removed because the cows stayed in a separate area during this time.

The number of records per cow ranged between 14 and 165, with a mean of 148 and a median of 164 records. Parity ranged between 1 to 6 and 1 to 7 in G1 and G2, respectively, where cows were classified as parity 1 (41 cows in G1 and 44 in G2), parity 2 (42 in G1 and 51 in G2), or parity 3+ (65 in G1 and 60 in G2). Each cow was categorized into one of 3 lactation stages depending on the current DIM: early (2–49 DIM), mid (50–179 DIM), or late (≥ 180 DIM) lactation. During the study period, 83, 112, and 29 cows in early, mid, or late lactation, respectively, were for a minimum of 10 milkings in G1. Accordingly, 7, 49, and 130 cows in early, mid, or late lactation, respectively, were in G2 for at least 10 milkings. Since the 7 cows in early lactation in G2 were exceptional cases and most of them in higher parities (e.g., cows with specific problems designated for slaughter from start of lactation on), they were removed from the first analysis when the model was fitted group-wise.

The statistical analyses were performed with the statistical software R version 4.0.3 (R Core Team, 2020). Two different analyses were conducted to investigate if lactation stage and parity are associated with the entrance order to the parlor and the variability of position within the milking order:

First, an ordinal logistic regression model with milking order group (first, second, third, or last) as response variable and parity,

lactation stage, and milking event as fixed effects and cow ID as random effect was used to investigate associations of cow characteristics with the overall entrance order. One model per milking group (G1 and G2) was fitted using the `clmm` function in the `ordinal` package (Christensen, 2022). The proportional odds assumption was graphically assessed according to the recommendations from Harrell (2001) and concluded to hold. Second, a logistic regression model with the first 6 positions as the response variable and parity, lactation stage, group, and milking event as fixed effects, and cow ID as a random effect was used to investigate if specific cow characteristics are associated with a preference to enter as one of the first cows within each milking line. One model, including records from both milking groups, was fitted. The logistic regression model was analyzed with the `glmer` function in the `lme4` package (Bates et al., 2015). The intraclass correlation (ICC) was estimated for each model as the proportion of the variance of the individual random effects in relation to the total variance (i.e., the sum of the residual variance and the variance of individual random effects). The residual variance is assumed to be $\pi^2/3$ for all 3 models (Nakagawa and Schielzeth, 2010).

In the first model, we found an effect of parity on the order of entrance to the milking parlor in G1 and G2, and an effect of lactation stage in G1 (Figure 2), where it seems that first-parity cows and cows in early lactation tend to be first in the milking order. In G1, cows in parity 2 had 64% lower odds of being in higher rather than lower categories of the outcome compared with cows in parity 1 (odds ratio [OR]: 0.36, 95% CI: 0.20–0.65), and cows in parity 3+ had 84% lower odds of being in higher rather than lower categories of the outcome compared with cows in parity 1 (OR [CI]: 0.16 [0.10–0.26]). In G2, cows in parity 2 had 87% lower odds of being in higher rather than lower categories of the outcome compared with cows in parity 1 (OR [CI]: 0.13 [0.06–0.26]), and cows in parity 3+ had 94% lower odds of being in higher rather than lower categories of the outcome compared with cows in parity 1 (OR [CI]: 0.06 [0.03–0.12]). These results show that cows in parity 1 have higher odds of entering the milking parlor early than cows in higher parities. However, there was no difference in entrance order between cows in parity 2 and parity 3+, indicating that the behavior of cows in these categories is more similar.

Berry and McCarthy (2012) found a nonlinear relationship between milking order and parity, where the youngest and oldest parity cows entered the parlor last. They argued that the younger cows might be more hesitant to the milking system and probably have a lower social rank, which has also been seen as correlated with milking order (Sauter-Louis et al., 2004). However, Melin et al. (2006) concluded that cows most likely find it rewarding to be milked and that cows of low social rank within an automatic milking system monitored every chance to advance in the milking queue by staying close to the milking unit. In the study herd, we also observed that cows of higher parity used different cubicle areas than first parity cows, which preferred areas with less cow traffic (Churakov et al., 2021). The waiting area in front of milking parlors is usually full at the beginning of milking. First-parity cows, with often lower rank in the hierarchy, might therefore prefer to enter the milking parlor early and leave the crowded waiting area. Furthermore, it has been observed that cows with similar attributes tend to stay together and create preferential bonds (Boylund et al., 2016; Marina et al., 2023), which might also explain the cows' division by parity within the milking order.

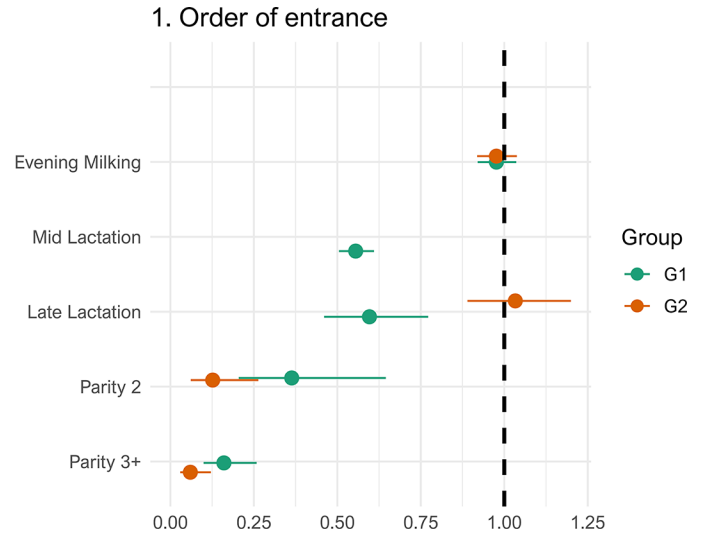


Figure 2. Estimated odds ratios and 95% CI of the entrance order model estimated with an ordinal logistic regression model (large dots = estimated odds ratios; error bars = range of 95% CI; G1 = group 1, G2 = group 2).

In G1, cows in mid lactation had 45% lower odds of being in higher rather than lower categories of the outcome compared with cows in early lactation (OR [CI]: 0.55 [0.50–0.61]), and cows in late lactation had 40% lower odds of being in higher rather than lower categories of the outcome compared with cows in early lactation (OR [CI]: 0.60 [0.46–0.77]). These results show that cows in early lactation have higher odds of entering the milking parlor early than in mid and late lactation. There were no differences between the entrance order of mid- and late-lactation cows in G1. This matches the results of G2, where we did not include any early-lactation cows in the analysis and did not see any effect of the lactation stage when comparing mid- and late-lactation cows. In G1, 61 cows went from early to mid lactation during the study period. To capture the behavior of cows concerning the entrance order when they transitioned from one lactation stage to another, we ran an additional model for G1, where we treated the lactation stage as a continuous trait. The model showed same results and similar effect sizes for all the coefficients as the previous model, and the estimated odds ratio for DIM was OR [CI]: 0.992 [0.990–0.993], which means that with a 1 unit increase in DIM, the odds of being in higher rather than lower categories of the outcome decreases by 0.8%.

That early-lactation cows show a different behavior than cows in later lactation could perhaps be related to the time the cows have spent in the group and their familiarity with the other cows, which seem to affect the number of contacts the cows have with each other (Gygax et al., 2010; Hansson et al., 2023). This might explain why early-lactation cows seem to have similar behavior as subordinate first-parity cows. The ICC was 0.48 in G1 and 0.49 in G2, which shows that there is variation between cows regarding the order in which the cows enter the milking parlor and indicates that the milking order is somewhat consistent.

In the second model, we saw a parity and lactation stage effect on the probability of being milked in the first 6 positions or the last 6 positions within one milking batch (Figure 3). Cows in parity 2

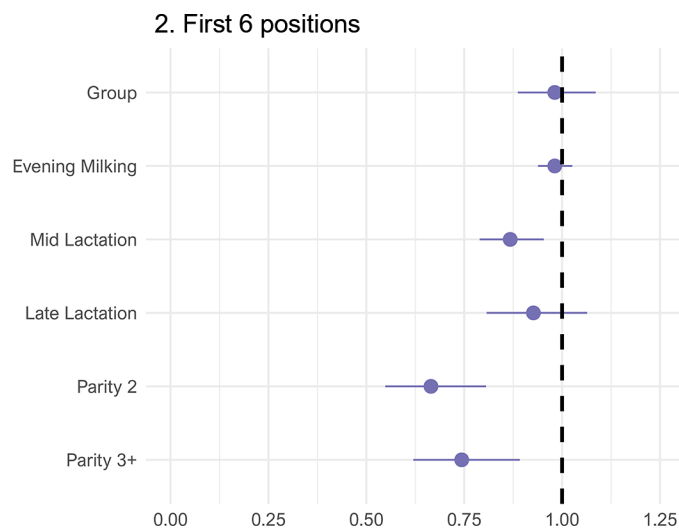


Figure 3. Estimated odds ratios and 95% CI of the first 6 positions model estimated with logistic regression (large dots = estimated odds ratios; error bars = range of 95% CI).

had 34% lower odds of being in the first 6 positions than those in parity 1 (OR [CI]: 0.66 [0.55–0.81]). Cows in parity 3+ had 26% lower odds of being in the first 6 positions than cows in parity 1 (OR [CI]: 0.74 [0.62–0.90]). There was no difference between the cows in parity 2 and parity 3+. Cows in mid lactation had 13% lower odds of being in the first 6 positions than early-lactation cows (OR [CI]: 0.87 [0.79–0.95]). There was, however, no difference between cows in early and late lactation or between cows in mid and late lactation. These results align with the results from our first model. However, the effects are of lower magnitude, and the ICC was only 0.09, indicating no consistency for individual cows to be in the first or last 6 positions. In both models we considered the milking event time (morning vs. evening) to control for an effect of the time of milking. We did not expect any effect, and this was confirmed for models 1 and 2. The data from the present study show that in the study herd, cow characteristics were associated with their order in the milking parlor. Cows in later lactation and higher parities tended to be later in the milking order and it is known that these animals are also those more likely to be infected with mastitis pathogens (Mekonnen et al., 2017; Taponen et al., 2017). That older cows and those later in lactation seem to be later in the milking order might contribute to their higher probability of having IMI. However, several mastitis-causing pathogens cause long-lasting infections and the higher probability of being infected at later stages of lactation might also simply be the result of low spontaneous cure rates, at least for some pathogens (Woudstra et al., 2023).

One of the measures regularly named in the literature for the prevention of the transmission of mastitis pathogens is the implementation of a milking order based on the cow's infection status (sometimes deducted from SCC measurements; Nielsen and Emanuelson, 2013). However, for this the milking herd needs to be split into separate housing groups, which is often difficult to implement, especially in smaller herds. Additionally, a study by Hansson et al. (2011) has shown only a limited economic effect

of this measure. If cows in their first lactation and those that are still early in lactation anyhow show a tendency of coming first into the milking parlor, this might contribute to the limited effect of implementing a milking order. Another important reason, however, is probably that it is not possible to correctly identify all infected animals and that spread of contagious pathogens can still occur in a group of presumably uninfected cows. Knowing that cows do not mix homogeneously during milking with regard to parity and lactation stage contributes to a further understanding of mastitis infection dynamics and needs to be taken into account when designing bio-economic disease transmission models like those of Gussmann et al. (2018). Such bio-economic models could also be used in the future to quantify the impact of the here observed effects of parity and lactation stage compared with assuming a random milking order. However, the present study was conducted in one herd only, and similar studies need to be repeated in a larger number of herds to confirm our findings and investigate further the management factors influencing the order in which cows with certain characteristics enter the milking parlor.

In conclusion, the present study indicates that cows do not enter the milking parlor in a random order and that first-parity cows and those earlier in lactation tend to enter the milking parlor earlier compared with the rest of the herd. These results indicate that early-lactation and first-parity cows have a higher motivation to leave the waiting area early. In addition, the results of the present study should be considered when simulating the effect of preventive measures against the transmission of contagious mastitis pathogens or planning these for dairy farms.

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Notes

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The authors declare that according to the Swedish Animal Welfare Act, no ethical approval is needed for this type of study.

The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: G1 = housing group 1; G2 = housing group 2; ICC = intraclass correlation; OR = odds ratio.