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# A comparative study of social behavior in primiparous and multiparous dairy cows during automatic milking

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ABSTRACT

In dairy farming, social behavior plays a critical role in ensuring welfare and productivity of cows. Understanding social associations in dairy commercial farms could help farmers in creating herd management practices able to consider individual animal needs, in particular in stressful conditions as during regrouping. Here, we investigated the social behavior of 150 dairy cows during milking events in a free traffic barn at an Automatic Milking System (AMS) and we compared their social structure between their first lactation (when they were housed in the primiparous area) and their subsequent lactations in the multiparous area. Data on individual milking traffic and daily milk yield were collected for a period of 5 years and 5 months. To identify and analyze consistent social associations among cows, we utilized the time interval between milking events, which is defined as the time difference between the entry time of one cow and the entry time of the next in the milking robot. Our findings revealed significant social differentiations in both areas, indicating the presence of non-random social structures within the herd. During milking, younger cows exhibited a tendency to form stronger social associations with particular individuals based on genetic relatedness. The correlation observed between genetic relatedness and social behavior in young cows indicates that grouping genetically related individuals could provide advantages. On the other hand, as cows age, they tend to exhibit a higher degree of social connectivity with their herd mates. This suggests that older cows have a wider range of social associations which is not driven by genetic relatedness. We examined the potential influence of social associations on cow productivity, and we found no significant correlation between social behavior and milk yield for primiparous and multiparous cows. At a temporal level, we compared their ego-networks, when they were hosted in the primiparous area and when they were hosted in the multiparous area, and we found a significant level of social stability. Although the factors that influence social behavior in cows may differ with age, our findings indicate the tendency to maintain consistent social relationships over time. Maintaining stable relationships is crucial for enhancing the welfare of cows in social contexts, and this knowledge can promote the development of management practices aimed at supporting their social well-being. Our study highlights the importance of understanding social behavior and dynamics in dairy cows and offers valuable insights that can guide the development of effective herd management practices in the dairy farming industry.

## 1. Introduction

In recent years, there has been a growing awareness of the importance of farm animal welfare, and it is now widely recognized that animal welfare encompasses both the physical and mental wellbeing of animals (Maple and Bloomsmith, 2018). This heightened awareness has led to the emergence of new methods for improving animal farming practices. In this scenario, Precision Livestock Farming (PLF) represents a multidisciplinary field that aims to provide farmers with real-time monitoring and management systems (Berckmans, 2017) which could help in the welfare improvement process. Among the various innovations in PLF, one of the most significant advancements in dairy production is the automatic milking system (AMS). By replacing conventional milking methods, AMS has been shown to improve milking frequency, yield, and reduce labor requirements (Wagner-Storch & Palmer, 2003). Automatic milking is based on cows' voluntary visits to the robot, thus bypassing the necessity to move the animals to the milking parlor two times a day. Therefore, the animals are free to go to

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milking at any time on a daily basis as well as dynamically changing intervals between milking throughout the lactation period (Vijayakumar et al., 2017). Moreover, the cows may benefit from the freedom to control their physical activity, and to reduce potential stress at the time of milking because they are not gathered and crowded as they are in conventional parlors (Miguel-Pacheco et al., 2014).

This new technology provides the opportunity to study the behavior and the welfare of the cows in an environment managed differently than traditional milking systems. In fact, the AMS has modified the daily rhythms and behaviors of the cows during the entire production cycle, and this is of interest both from an AMS efficiency perspective as well as for animal welfare. Thus, the vast amount of data collected from farms utilizing AMS presents a significant opportunity for characterizing herd behavior and optimizing management practices, a potential that is still largely underexploited (Bonora et al., 2018). Since cows in AMS have more freedom to interact with each other at any time throughout the day (eliminating the need to move the animals to the milking parlor as in conventional systems), other factors such as the dominance hierarchy, time of day, and social affiliations may also affect their choices of when and with whom to be milked (Marumo et al., 2022). For example, the utilization of AMS is influenced by social dominance and the pre-existing social hierarchy among cows. As a result, the timing of visits to the AMS can be influenced, leading to the possibility of lower-ranking cows waiting in line in front of the AMS (Ketelaar-de Lauwere et al., 1996).

It is well known that cows are highly social animals, whose welfare is heavily influenced by the opportunity to interact with conspecifics and to create a social environment, despite potential limitations due to bounded space and management practices (Estevez et al., 2007). This species evolved to live in large and structured groups where the individuals present different levels of association, some of which ultimately resulting in lasting social bonds (Gutmann et al., 2015; Boyland et al., 2016). These relationships concur in the creation of a social pattern influenced by various factors, ranging from the management practices employed to the individuals' social preferences (Marino and Allen, 2017; Fielding et al., 2021). In fact, several studies illustrate how cows have individual personalities that, along with factors such as shared experiences, emotional state, and gregariousness, define their own preferences among the herd group (Marino and Allen, 2017; Boyland et al., 2016). Moreover, the possibility to express their preferences during daily activities (e.g., resting, feeding, being milked) has an impact on their stress levels (Marino and Allen, 2017; Bøe and Færevik, 2003). For these reasons, understanding the social dynamics and relationships between cows within the AMS can have a direct impact on their welfare. In particular, knowledge of consistent social associations can inform management practices to promote positive social behavior and reduce stress and aggression among cows.

Here, we studied the social associations of 150 dairy cows at an AMS during milking events in a free traffic barn during their first lactation (primiparous phase) and in their subsequent lactations (multiparous phase). Our hypothesis is based on the understanding that cows, as social animals, can form consistent and stable associations during the milking process at the AMS. Cows may develop social preferences during milking procedures, which could influence their overall well-being. This can have a direct impact on milk production, and thus, we hypothesize that the strength of social associations at the AMS may play a role in affecting the cows' physiological state and, consequently, their milk yield. The primary aim of this study was to assess if the social associations of the cows change over time and if they are influenced by age and parity. First, we determined whether the cows showed social preferences in their relationships, and we tested the influence of relatedness in social associations in primiparous and multiparous phases. Second, we evaluated the relationship of strength of these associations and the milk yield. Lastly, we generated two aggregated association networks of the 150 cows, both when they were in the primiparous area and in the multiparous area, and we compared the ego-networks of individuals obtained to determine how stable were their associations over time.

## 2. Materials and methods

### 2.1. Data collection

The study was carried out on a commercial dairy farm in the northern part of Italy (Candiolo, Turin) equipped with Lely A4 Astronaut AMSs (Lely, Maassluis, the Netherlands). The barn was composed of two enclosed rectangular areas, each measuring $45 \times 30$ m. Each area hosted approximately 120 Holstein-Friesian lactating cows and contained two AMSs; the first area was designated for primiparous cows, while the second area housed multiparous cows. The cows were kept indoors in a loose housing system in a cubicle shed throughout their entire production cycle with no access to pasture. The animals were fed ad libitum (fresh feed twice daily at about 06:00 and 14:00 h) with a total mixed ration. During milking, the cows were provided with pellet concentrate through the AMS, which served as an incentive based on the expected daily milk yield. All lactating cows had 24-hour access to the AMS and were milked voluntarily, on average the cows were milked 2–3 times per day. To register individual measurements, an electronic identification collar (Owes-H system, Lely, Maassluis, The Netherlands) was fitted to each cow. This collar records information such as the cow's unique identification number, entrance and exit time from the milking robot, milk yield, milk temperature, and protein, fat, and lactose composition. The data was collected and stored in a management software (T4C "Time-for-Cows" InHerd, Lely, Maassluis, The Netherlands). Data on individual milking traffic and daily milk yield were collected from 150 lactating dairy cows between August 2016 and December 2021. Milking records of the same 150 cows were collected during both their first lactation, when they were housed in the primiparous area, and from their second lactation, when they were housed in the multiparous area, hereafter indicated as 'primiparous' and 'multiparous' cows. Area differentiation was accomplished through the utilization of distinct identification numbers associated with the milking robots.

To identify consistent social associations between cows during milking at the AMS, we utilized the method proposed by Marumo et al. (2022). We calculated the time gap between the entrance time of one cow and the entrance time of a different cow in the same milking robot, on the same day, and in the same area. If the time gap was less than 10 min, we considered it an association between the two cows. This specific time frame was chosen because if a cow entered the milking machine within 10 min of the previous cow entering, it was highly probable that they were queuing at the same time, according to Marumo et al. (2022). In contrast, if the time gap was longer than 10 min, we excluded the event as it was unlikely that the two cows were queuing together. The average length of milking event was 7 min per visit.

Using this approach, we identified a total of 184,923 associations between one cow and the cow following it across the entire dataset, with 89,306 associations when the cows were primiparous and 95,617 when the cows were multiparous. For each pair of cows, we calculated the number of times cow a followed cow b and the number of times cow b followed cow a at the milking robot. Since cow a may follow cow b more frequently than the reverse (for example, cow a may follow cow b 20 times, while cow b follows cow a 18 times), we conducted a correlation analysis using the Spearman rank correlation test for each dyad, including both primiparous and multiparous cows. Additionally, we calculated the percentage of one-sided associations within each dyad. If this percentage approached 50%, we classified the association as symmetric. Our aim was to determine whether the associations exhibited asymmetry or symmetrical reciprocity between cow a and cow b. In the case of observed asymmetry, we treated the network as directional; conversely, in the presence of symmetry, we considered the network as symmetric.

### 2.2. Social differentiation

The social differentiation was computed to assess the heterogeneity of associations, i.e., whether associations between cows were more heterogeneous than we would expect given a null hypothesis that all cows associate uniformly. Social differentiation is the measure of the variation in relationships among members of the herd under study to determine the degree of social differentiation in our study, we used the equation proposed by Whitehead (2008):

$$S = \frac{\sqrt{Variance(x_{ab}) - Mean(x_{ab})}}{Mean(x_{ab})}$$

Where $x_{ab}$ is the total number of milkings during which the cow a and the cow b were associated. To evaluate the significance of our findings, we compared the observed social differentiation value with a set of values generated by 1000 null networks using a z-test. Each null network was created by randomizing the nodes of the temporal network derived from the associations between cows during milking events, followed by computing null aggregated networks. The social differentiation was computed both for primiparous and multiparous cows.

### 2.3. Association indices

To determine the strength of associations between two cows, a and b, during milking in relation to the number of opportunities they had to queue together in the same area and time period, we calculated an association index for each pair of individuals. Several techniques have been proposed for assessing dyadic social association indices, including those by Whitehead and Dufault (1999) and Wey et al. (2008). We determined the total number of milkings where a and b were observed together in the same area ($x_{ab}$), as well as the number of milkings where both cows were in the same area but only cow a ($x_a$) or only cow b ($x_b$) was identified by the milking robot. To calculate pairwise social associations, we employed the following formula:

$$AI = \frac{x_{ab}}{x_{ab} + x_a + x_b}$$

The resulting index ranges from 0 (indicating that the two individuals were never associated during milking) to 1 (indicating that they were always associated during milking). A higher index value reflects a stronger level of association between the pair of individuals. We calculated association indices for both primiparous and multiparous cows.

### 2.4. Social associations and relatedness

To determine the degree of genetic relatedness between individuals, we computed pairwise coefficients of relatedness for the entire herd, considering both maternal and paternal pedigrees. The coefficient value ranges between 0 (indicating no genetic relatedness) and 0.5 (indicating either a mother-daughter relationship or full siblings). We utilized the genetic relatedness indices calculated for each cow pair to create a kinship matrix. Additionally, we constructed an association matrix based on the association indices computed for each cow pair throughout the study period. Our objective was to investigate the potential correlation between the association indices and the coefficient of relatedness between pairs of cows. The underlying hypothesis was to uncover a positive relationship between association indices and relatedness. To accomplish this, we utilized the Mantel test (with 1000 permutations) to compare both the kinship and association matrices for both primiparous and multiparous cows.

### 2.5. Social associations and milk quantity

In order to investigate whether social associations at the milking robot had an impact on milk yield, we used multiple linear regression models. These models allowed us to analyze the relationship between the mean and maximum association indices, which were the independent variables, and the cows' average milk yield, which was the dependent variable. The normality of the models' residuals and linearity were assessed graphically (histograms, Q-Q plots, scatterplots) and the normality was also assessed with the Shapiro-Wilk test.

### 2.6. Association networks

We generated time-aggregated, weighted networks of the 150 cows when they were in the primiparous area and in the multiparous area. In these networks, each node represents a dairy cow, and an edge between two nodes (a and b) corresponds to the association index ($AI_{ab}$) between them, calculated over the entire study period of 5 years and 5 months. The degree of a node in a network represents the number of unique individuals that the node has come into contact with. To account for variations in the duration of time cows spent in different areas, we calculated the normalized degree of a node by dividing the total degree for each area by the total number of days each cow spent in that specific area. We also calculated the network density, which reflects the proportion of existing connections in the network relative to the maximum possible connections. The network density can range from 0 to 1, with 0 representing a network with no connections and 1 representing a network with all possible connections. A density value closer to 1 indicates a denser network, with greater cohesion among the nodes. To assess whether the associations between cows in the primiparous and multiparous areas were similar, we calculated the similarities between the two association networks. Specifically, we utilized the Local Cosine Similarity (LCS) (Singhal, 2001) to compare the networks by examining the ego-networks (i.e., the network of a focal node, called "ego" and the nodes to whom ego is directly connected to) of individual nodes. The LCS of a given node (a) was determined by calculating the cosine similarity between the weight vectors associated with node a in each of the two networks. In our study, the weight was measured by the association index (AI). Specifically, we evaluated the networks during two different time periods: the first period (t1) was when cows were hosted in the primiparous area, and the second period (t2) was when cows were hosted in the multiparous area. We denote $AI_{ab,\ t1}$ and $AI_{ab,\ t2}$ the association index of the link between individual *a* and *b* in the network aggregated over t1 and t2 respectively. The local cosine similarity of *a* in the network between t1 and t2 is:

$$LCS \quad (a) = \frac{\sum_b AI_{ab,t1} AI_{ab,t2}}{\sqrt{\sum_b (AI_{ab,t1})^2}\sqrt{\sum_b (AI_{ab,t2})^2}}$$

The LCS is a value that ranges between 0 and 1. A value of 1 indicates that cow a had the same association with exactly the same individuals during both time periods. In contrast, a value of 0 indicates that cow a had completely different associations during t1 and t2 with no overlapping individuals.

### 2.7. Data analysis

We conducted statistical analysis of the data using various tools and software packages. The Spearman rank correlation test, the z-test, the Mann-Whitney U-Test and the association indices were performed using Python packages 'SciPy' v1.2.1, p&lt (Python Software Foundation). To generate a kinship matrix, we used the "kinship2" package in R (R Core Team, 2014). Additionally, the Mantel test was carried out using R software and the "vegan" package v2.5, version 3.1.2 (R Core Team, 2014). The network analysis was performed by using Python packages 'SciPy' v1.2.1 and 'NetworkX' v2.6.3 (Python Software Foundation). The Shapiro-Wilk test and multiple linear regression models were performed using the R software package 'stats', Version 4.2.1 (R Core Team, 2014). The statistical significance was declared at p-value < 0.05.

## 3. Results

### 3.1. Social Differentiation

Our results showed a significant level of social differentiation in both primiparous and multiparous cows (Table 1). Specifically, we found that certain cows established preferred relationships with specific individuals within their herds, while associating less frequently with others ($p < 0.001$ for both primiparous and multiparous cows), indicating the presence of non-random social structures within the groups.

### 3.2. Association indices

Table 2 presents the association index values, while Fig. 1 displays the frequency distribution of primiparous and multiparous cows. Our analysis revealed a statistically significant difference between the association indices of primiparous and multiparous cows (Two-sample permutation test: Z = 0.13.22; p-value < 0.001). Specifically, the youngest cows exhibited higher association indices.

### 3.3. Social associations and relatedness

Results from the Mantel test showed that when the cows were younger (during the first lactation) their association indices were positive and significantly correlated with their relatedness coefficients (Mantel statistics r = 0.03; p-value = 0.003). While we did not find any significant correlation between the relatedness coefficients and the association indices when the cows are hosted in the multiparous area.

### 3.4. Social associations and milk quantity

The regression model showed that the milk yield of both primiparous and multiparous cows was not affected by the social association strength, by demonstrating that there was no relationship between the milk quantity and the social behaviors of the dairy cows, independently by their age and parity (see Supplementary Material, Fig. S1).

### 3.5. Symmetry of associations

To accurately compute the association indices and construct association networks for cows, it is crucial to determine the direction of their associations. We observed a positive and significant correlation between association indices for both primiparous (Spearman rank correlation, rho = 0.70, p-value < 0.001) and multiparous cows (Spearman rank correlation, rho = 0.63, p-value < 0.001). Moreover, we found that the mean percentage of all associations within each dyad that occurred in one direction was 49.75% (SD 17.06%) for primiparous cows and 49.65% (SD 17.94%) for multiparous cows (see Supplementary Material, Fig. S2). These findings enabled us to construct symmetrical association networks, with undirected network edges and symmetric weights on the edges.

**Table 1**
Social differentiation measured in primiparous and multiparous: observed values; median of the distribution of values generated by the 1000 null networks; 95% confidence interval of the distribution of values generated by the 1000 null networks; p-values obtained from the comparison between the observed values and the distribution of values obtained by null model.

| | Observed | Median of null distribution | 95% confidence interval of null distribution | p-value |
|---|---|---|---|---|
| **Primiparous** | 1.06 | 1.02 | 1.01 – 1.02 | < 0.001 |
| **Multiparous** | 1.06 | 0.89 | 0.91 – 0.92 | < 0.001 |

**Table 2**
Values of association indices of primiparous (N = 150) and multiparous cows (N = 150).

| | Mean±SD | median | range |
|---|---|---|---|
| **Primiparous cows** | 0.007 ± 0.005 | 0.006 | 0.0003–0.12 |
| **Multiparous cows** | 0.006 ± 0.004 | 0.005 | 0.0003–0.11 |

### 3.6. Networks structure of the herd

We built symmetrical association networks computed over the entire study period with 150 nodes each. The networks were formed by 6161 and 8635 edges for primiparous and multiparous cows respectively (Fig. 2). The network density, which reflects the proportion of actual connections to possible connections, was higher for multiparous cows (0.77) than for primiparous cows (0.55), indicating that older cows were more socially connected. The mean normalized degree was higher for multiparous cows (0.53) compared to primiparous cows (0.19) in Table 3. We also observed a positive and significant correlation between the normalized degrees of the two periods (Spearman rank correlation, rho = 0.36, p-value < 0.001), indicating that the cows tend to maintain similar levels of social connectivity over time. To assess the distribution of normalized degrees, we used Kernel Density Estimation (KDE) and plotted the smoothed density estimation of the normalized degrees (Fig. 3). The KDE revealed a wider distribution of normalized degrees for multiparous cows (blue line, Fig. 3) compared to primiparous cows (red line, Fig. 3).

In order to understand if the associations among cows were maintained the same over time, we computed the Local Cosine Similarity (LCS) values on individual ego-networks between the primiparous and the multiparous period. We found that the median value of the LCS was 0.54 (range = 0.12–0.83). To better understand how much this value can be considered 'large' we compared the values of LCS to a suite of values generated by null networks, i.e., randomized versions of the association networks. In the null networks the topology of the network was unchanged, but the weights of the network were randomly assigned (Farine, 2017). We calculated the LCS distributions from 1000 iterations of the first null model and observed a smaller median value of 0.33. Furthermore, we detected a significant difference between the observed LCS values and those obtained from the null models. Specifically, the LCS values were significantly higher than the random ones (Mann-Whitney U-Test: U =19762; p-value < 0.001) (Fig. 4).

## 4. Discussion

In our study, we examined the social behavior of 150 dairy cows during milking events in 5 years and 5 months and we investigated whether their social structure differed between their first lactation, when they were housed in the primiparous area, and their subsequent lactations in the multiparous area. In particular we investigated their social preferences, how it changed over time and the relationship between social behavior and milk yield. Using Social Network Analysis, we investigated the evolution of social associations in relation to their age and parity.

Our results showed that there was a significant level of social differentiation in both groups of cows, indicating the presence of non-random social structures within the groups. Certain cows established preferred relationships with specific individuals within their herds, while associating less frequently with others. Social differentiation was used as a measure of the variation in relationships among members of the herd (Boyland et al., 2016). The use of social differentiation allowed us to assess the heterogeneity of associations, and the cows under study showed more heterogeneous associations than we would expect given a null hypothesis that all cows associate uniformly. This finding is consistent with previous research on the social behavior of cows, which has suggested that cows exhibit complex social relationships that can

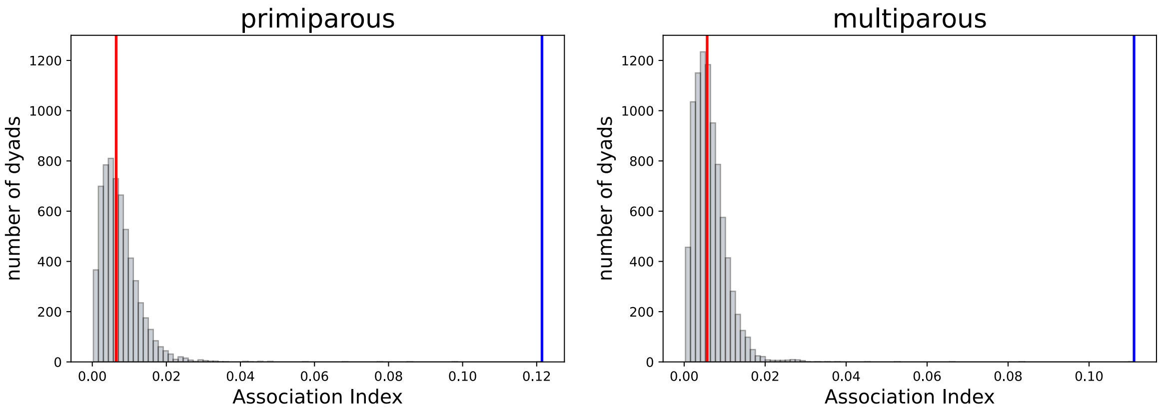


**Fig. 1.** Frequency distribution of the social association index of primiparous and multiparous cows. Vertical red lines indicate the median values and vertical blue lines represent the maximum values.

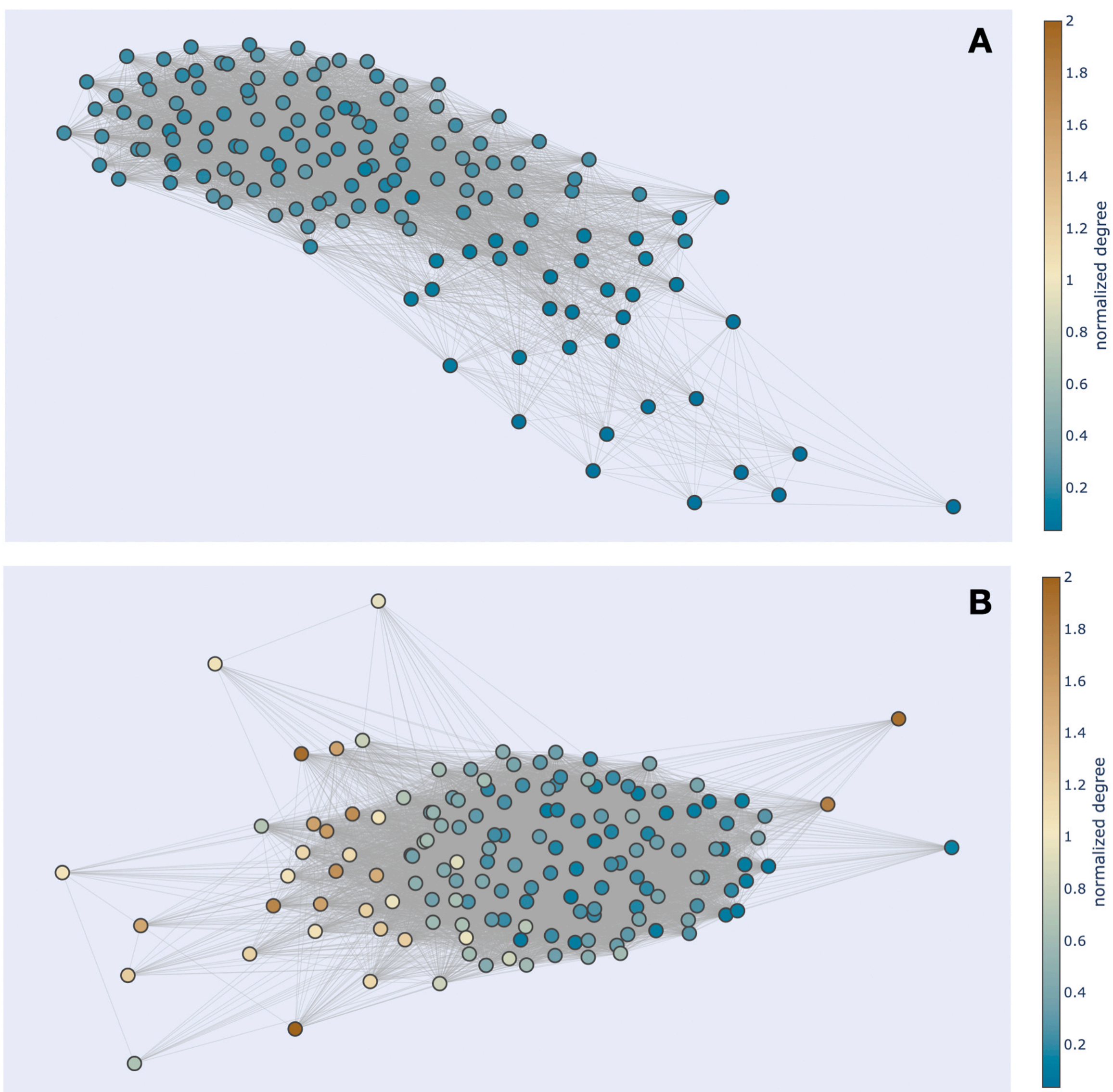


**Fig. 2.** Aggregated association networks of primiparous (panel A: 150 nodes and 6161 edges) and multiparous (panel B: 150 nodes and 8635 edges) dairy cows. Nodes are color-coded according to the normalized degree. The edge weight corresponds to the association index between two nodes.

**Table 3**
Normalized degree of primiparous (N = 150) and multiparous cows (N = 150).

| | mean±SD | median | range |
|---|---|---|---|
| **Primiparous** | 0.19 ± 0.068 | 0.20 | 0.035–0.308 |
| **Multiparous** | 0.53 ± 0.46 | 0.35 | 0.08–2.00 |

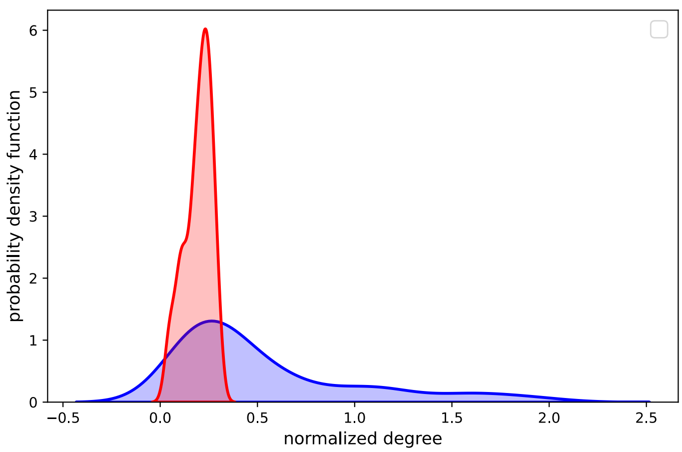


**Fig. 3.** Kernel Density Estimation of the normalized degrees of the primiparous (red line) and multiparous (blue line) dairy cows.

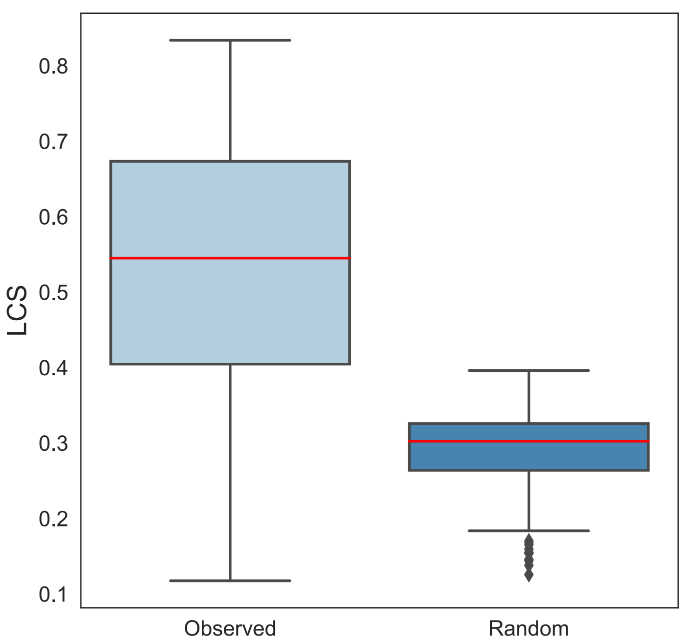


**Fig. 4.** Local Cosine Similarity (LCS) ego-networks. Distributions of LCS measured for each node of the observed association network compared to those obtained from a randomized version of the association networks (1000 realizations for each null model). In each box the red line marks the median and the extremities of the box correspond to the 25 and 75 percentiles, the whiskers give the 5 and 95 percentiles of each distribution.

also vary depending on the management system (Boyland et al., 2016; Fielding et al., 2019).

The association indices showed slightly higher maximum values for primiparous cows compared to multiparous cows, and the range of association indices was similar for both groups. However, our analysis revealed a significant difference between primiparous and multiparous cows, with significantly higher association indices observed in primiparous cows. According to previous studies (McLennan, 2013), our results indicate that younger cows exhibit a greater tendency to form stronger social associations with specific individuals during milking, in comparison to older cows. This may be because cows during their first lactation are still in the process of establishing their social network within the herd and may have a greater need for social support. As they are new to the group, they may be more motivated to interact with others to form social bonds and to establish their position in the social hierarchy within the herd (Reinhardt, Reinhardt, 1975; Kondo and Hurnik, 1990). This may involve spending more time grooming, resting, or interacting with specific individuals during milking events. On the other hand, the cows in the subsequent lactation have already established their social rank within the herd and may not require social support from specific individuals.

Moreover, the factors that influence the strength of associations changed over time. Indeed, we investigated the potential correlation between association indices and coefficients of relatedness for both primiparous and multiparous cows. The results from the Mantel test showed that when the cows were younger (during the first lactation), their association indices were positive and significantly correlated with their relatedness coefficients. However, we did not find any correlation between the relatedness coefficients and the association indices when the cows were hosted in the multiparous area. The dairy cows involved in our study underwent separation from their mothers shortly after birth and were individually housed in single boxes until the weaning period. Subsequently, they were introduced into social groups; however, it is important to note that these groups were not specifically formed based on kin relationships. This information is significant when considering the social dynamics and associations observed in the study, as the cows' early social experiences were independent of familial ties. The positive correlation between association indices and relatedness coefficients in primiparous cows suggests that younger animals have stronger social associations when they are genetically related. This finding is consistent with previous studies, which have shown that kinship can play an important role in the formation of social relationships in many animal species (Smith et al., 2010; Schülke et al., 2013; Wey et al., 2010). Several studies have investigated the relationship between relatedness and social behavior in ungulates (Coltman et al., 2003; Albery et al., 2021). For instance, Godde et al. (2015) found that social aggregation in goats is influenced by relatedness, with closely related individuals forming more cohesive social groups. In sheep, association preferences are influenced by kinship, with a greater tendency to associate with siblings and half-siblings compared to unrelated individuals (Ozella et al., 2022). Similarly, in cattle it was found that social relationships are strongly predicted by relatedness and social aggregation is influenced by relatedness, with closely related cows tending to form more cohesive social groups (Coulon et al., 2010). The absence of correlation between relatedness coefficients and association indices in multiparous cows could potentially be attributed to several factors. One of these factors could be that, as cows age, their reliance on relationships based on kinship tends to diminish as part of their ontogeny or growth and development process. Our findings can have significant implications for the management of dairy herds. The observed correlation between genetic relatedness and social behavior in young cows suggests that grouping genetically related individuals together may be advantageous. By promoting stronger social associations, cows may experience red uced stress levels and improved welfare.

We also investigated whether social associations at the milking robot had an impact on milk yield in dairy cows. Multiple linear regression models were utilized to analyze the relationship between the mean and maximum association indices (which represented the strength of the social interactions of the cows), and the cows' average milk yield. Surprisingly, the results showed that there was no relationship between milk yield and social behavior in both primiparous and multiparous cows. Our findings contrast with previous studies that have shown a positive correlation between social behavior and milk yield (Boyland, 2015), and a significant decrease in milk production after long-term separation of cows from their preferred bonded partners (McLennan, 2013). Additionally, a study by Fukasawa, Tsukada (2010) found that social behavior, such as grooming and licking, was positively associated with milk yield in dairy cows housed in groups. It is possible that differences in study design, management practices, and cow characteristics may have contributed to these discrepant results compared to our study. However, the recent study by Marumo et al. (2022) also investigated the relationship between social behavior and milk production and their findings agreed with the results of our study, as they also found no significant correlation between social behavior and milk yield in either primiparous or multiparous cows.

Through the use of Social Network Analysis (SNA) we assessed the level of connectivity within the group of cows with regards to their age and parity, as well as the stability of their social associations. SNA has become a valuable tool for investigating and evaluating the complexity of social relationships, providing a more precise understanding of the social life and group structure of animals (Croft et al., 2008). SNA has been widely used in research to investigate the social behavior and structure of cattle herds (Natale et al., 2009; Boyland et al., 2016), as well as to understand disease transmission dynamics within these populations (Fielding et al., 2021; Fielding et al., 2020; de Freslon et al., 2019).

The results of our study showed a significant effect of age and parity on the number of social associations among cows. Specifically, we found that multiparous cows had a higher network density compared to primiparous cows, indicating that older cows tended to be more socially connected with their herd mates. This finding is consistent with previous research indicating that social relationships among cows become more stable and long-lasting as they age and gain more experience in social interactions (McLennan, 2013; Gutmann et al., 2015). The larger number of edges observed in the multiparous cow network also suggests that social connections among cows become more complex with age. This complexity may reflect the ability of older cows to form more sophisticated social structures, such as hierarchies and sub-groups, within their herd. Our analysis also revealed that multiparous cows had a broader distribution of normalized degrees than primiparous cows. This result suggests that older cows tended to have a greater tendency to interact with a larger number of individuals, which may contribute to increased social complexity within the herd. Furthermore, the positive correlation between the normalized degrees of the two periods indicates that cows tend to maintain consistent levels of social connectivity over time. This could suggest the importance of stability in social relationships for the well-being of cows. However, the apparent contradiction of stable ego networks and heightened social complexity in multiparous cows can be explained by recognizing that while the overall relationship structure remains stable, the number and variety of connections might increase. Stability in social relationships may involve maintaining core associations while incorporating new connections.

To further investigate this aspect, we utilized the LCS method to compare the individual networks of cows (i.e., ego-networks) in both primiparous and multiparous periods and we found a significant high degree of similarity between the two networks. LCS has been utilized in previous studies to evaluate social stability in various species, including ruminants, providing a reliable measure of the degree of association between individuals within a group (Gelardi et al., 2019; Ozella et al., 2022). By providing insights into the stability of social associations over time, the LCS method can inform the development of management practices that support the social well-being of animals. In particular, our results can have important implications for the welfare of cows in social contexts. Future research could further investigate the factors that contribute to the maintenance of social associations in cows, as well as the potential benefits of social stability for cow health and productivity.

In conclusion, our study demonstrated that factors such as age and parity may influence the social behavior of cows during milking events, however, further research is necessary to fully comprehend social interactions in other areas of the barn and at different times. Indeed, social behavior is a complex and multifaceted phenomenon, and there may be several factors that contribute to the observed differences between primiparous and multiparous cows. Our study is characterized by several limitations. Primarily, associations observed at the milking robot lack contextual information about the nature of these contacts. The act of one cow following another may not necessarily denote an exclusively positive association. Moreover, it's important to note that our results are confined to a specific area within the barn and a particular timeframe. An exciting area for future research could involve utilizing cameras capable of automatically detection of social interactions across the entire area to infer the social behavior of primiparous and multiparous cows during various daily activities. Finally, we suggest that studying the social organization of dairy cows could help farmers in creating more tailored herd management practices that consider individual animal needs, potentially leading to improved productivity and well-being.

## CRediT authorship contribution statement

Laura Ozella, Elena Diaz Vicuna, Claudio Forte: the conception and design of the study, or acquisition of data, or analysis and interpretation of data. Laura Ozella, Claudio Forte, Mario Giacobini, Achille Schiavone: drafting the article or revising it critically for important intellectual content.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.106065.

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