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Longitudinal changes of stem-like cells in colostrum and milk of dairy cows

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Research Article

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Abstract

This study aimed to identify and quantify the various stem-like cell types in dairy cows' colostrum and milk at the onset of lactation. Five second parity Holstein cows were monitored from calving until the seventh-day postpartum. Mammary secretions were collected immediately after calving, then every 3 h until 12 h during day (d) 0, and during morning milking on d 1, d 2, d 4 and d 7. Cells were prepared from mammary secretions and analysed by flow cytometry using relevant cellular markers. The highest total and viable cell concentrations were observed in colostrum collected at calving and up to 6 h, with these concentrations decreasing substantially in samples collected later at d 0. Then, the concentrations of both total and viable cell populations continued to slowly decrease until d 7, the kinetic curves reaching a baseline plateau. Flow cytometry showed that the $CD49f^{pos}CD24^{pos}$ population, which identifies mammary epithelial stem cells, represented about 0.9% of viable cells at calving and about 0.1% 12 h later, the mammary epithelial stem cell concentration therefore being at its highest level in the very first colostrum. In contrast, the percentage of mesenchymal stem-like cells, defined as the population of $CD34^{neg}CD105^{pos}CD90^{pos}CD29^{pos}$ cells, was roughly constant (≈0.3%) during the first two milkings and decreased mainly during the first day to a basal level close to 0. Concerning haematopoietic stem-like cells, defined as the $CD45^{neg}CD34^{pos}CD117^{pos}CD90^{pos}$ cell population, they were only observed in the colostrum collected at calving. All the types of stem cells studied here were therefore only present in substantial quantities in the colostrum of the very first hours after calving, a period during which the calf's intestine is permeable, possibly allowing the transfer and integration of these cells in the tissues of the newborn calf.

On many dairy farms around the world, it is common to separate the calf from its mother immediately or within the first few hours of birth. This routine practice aims to address several animal health concerns by preventing transmission of disease to the calf, or injuring from cows, or even by reducing separation distress and emotional bonding (Hopster *et al.*, 1995; Beaver *et al.*, 2019). Other arguments include better management of calf nutrition by controlling calves' colostrum or milk intake while feeding them artificially. For dairy producers, late separation has an economic cost, as the amount of milk available for sale is reduced or when calves drink too much milk, leading to an increased risk of diarrhoea (Ventura *et al.*, 2013). Another important point to consider is that commonly used housing and breeding systems were not designed to raise cow–calf pairs. Nowadays, the practice of separating the calf from the mother after birth has become very controversial when the public became aware of it (Busch *et al.*, 2017). Society's expectations are compassionate when it comes to animal welfare, as cow–calf contact appears natural and beneficial for both the cow and the calf.

In practice, calf feeding uses colostrum often preserved by refrigeration or freezing. It appears, however, that compared with fresh colostrum, frozen colostrum is detrimental to the development and functioning of the innate immune system of calves (Costa *et al.*, 2017). Indeed, colostrum provides a wide range of molecules for the nutrition and immunity of the young, but other cells, including stem cells, which may be beneficial for offspring. However, cells can die if frozen. Stem cells are undifferentiated cells that exhibit specific stemness markers and possess both the ability to self-renew and differentiate into lineages. Although it is well recognized that adult stem cells and their progenitors reside in the mammary gland (Villadsen *et al.*, 2007; Rauner and Barash, 2012), less is known about stem cells recovered in milk secretion. Our team has contributed to demonstrating that in the bovine mammary gland, specific cells called mammary stem cells (MaSC) evolved quantitatively throughout the life of the animal while retaining unique molecular characteristics (Finot *et al.*, 2019), as well as during lactation (Perruchot *et al.*, 2016). These cells were further shown to form mammospheres *in vitro* (Finot *et al.*, 2018).

Several other types of stem cells have been reported in milk, mainly in humans but also in cattle. Using flow cytometry, cell populations in human colostrum and milk were found to exhibit

the expression of mesenchymal, haematopoietic, neural, embryonic and endothelial stem-like markers (Goudarzi *et al.*, 2020). Additionally, the group of Pipino (Pipino *et al.*, 2018) identified in bovine milk a stem-like cell population expressing mesenchymal markers, possessing adherent properties and capable of differentiating *in vitro* into osteogenic, chondrogenic and adipogenic lineages. These properties, previously used to characterize multipotent mesenchymal stromal cells (Dominici *et al.*, 2006), led the authors to conclude that cow's milk contained mesenchymal stem-like cells (Pipino *et al.*, 2018). Following these observations, we wanted to identify these types of stem-like cells, in addition to MaSCs, in cow's milk secretions (colostrum and milk).

As described above, most studies regarding stem cells present in milk secretions and their transfer from mother to offspring have been performed in mouse models (Weiler *et al.*, 1983; Aydin *et al.*, 2018). Studies in cattle are scarce, especially during the neonatal period, a key period for the animal's development. If it were proven that stem cells transferred to offspring through lactation have a positive impact on the immunity and/or development of offspring, this would open perspectives for new breeding practices optimizing agronomic performance in terms of growth, health and well-being. In this context, this study aimed at determining the presence of stem-like cells in the colostrum and milk of dairy cows, focusing on the very beginning of lactation, a period during which the calf could potentially benefit from this supply of important cells, thanks to its immature digestive system, which might allow the passage of these cellular elements through the gastrointestinal barrier.

Materials and methods

Ethical statements

All animal procedures were discussed and approved by the CNREEA No. 07 (Local Ethics Committee in Animal Experiment of Rennes) in compliance with French regulations (Decree No. 2013-118, February 07, 2013). The experiment was conducted at the INRAE experimental dairy farm of Méjusseaume (IE PL, Domaine de Méjusseaume, Le Rheu, France; https://pegase.rennes.hub.inrae.fr/recherche/installation-experimentale; https://doi.org/10.15454/yk9q-pf68).

Animals and experimental design

Five second parity Holstein dairy cows were used in this study. The selected animals had not received antibiotic treatment at first dry off and from calving to the last day (d) of milk collection. A kinetic of milk secretion (colostrum and milk) collection from calving to the seventh day of lactation was performed. The calf was separated from the cow immediately after birth and the first colostrum (time 0, d 0) was collected. Cows were then milked each 3 h (3, 6, 9 and 12 post-calving, d 0) and 24 h after calf delivery (d 1) using an automatic individual milking machine (vacuum pump MGD TT1, 200 L/m at 50 KPa). Milk was then further collected for analysis at morning milking on d 2, 4 and 7 post-calving, while cows were milked twice daily at 06:30 and 16:30 in the rotary milking parlour, at 45 KPa. Amount of colostrum (d 0 and d 1) and milk collected at milking were weighed and milk yields were used to calculate the whole quantity of cells (in colostrum or milk) per milking. After collection, complete milk secretions, or aliquots (2 L), were immediately conveyed at room temperature to the laboratory for sampling and cell isolation.

Flow cytometry analysis

Cells were isolated from fresh colostrum or milk samples, as described previously by Boutinaud *et al.* (2008) with some modifications (see Supplementary File). The concentration of cellular elements (live cells, dead cells, milk fat globules, etc.) potentially detectable by flow cytometry (events) was measured using the ViCell® cell counter (Beckman Coulter). Cells were distributed into 3.0×10^5 cells per vial and labelled with relevant antibodies (see Table S1), as described in the Supplementary File. Analysis was performed with a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec), which was calibrated daily (calibration beads, 130-093-607, Miltenyi Biotec). We stopped event acquisition at 20,000 events per sample. The flow cytometry analysis, based on the gating strategy illustrated in Fig. 1A, was performed using the appropriate negative control isotype and the Flow Minus One control proceeding. Data were processed using the FlowLogic software (Inivai, Mentone, Victoria, Australia).

Our approach to identify and quantify the various types of stem-like cells in milk secretions by flow cytometry was based on reported phenotypes of cell-surface markers, or clusters of differentiation (CD) expressions, previously validated in our group for the MaSC (Finot *et al.*, 2018), or reported in the literature for mesenchymal stem cells (MSC) (Rossi *et al.*, 2014; Somal *et al.*, 2016) and haematopoietic stem cells (HSC) (Doi *et al.*, 1997; Shin *et al.*, 2014; Anjos-Afonso *et al.*, 2022). Antigenic profiles intended to identify MSC were characterized by the absence of the haematopoietic differentiation cluster CD34 and the expression of CD105, CD90 and CD29. We defined the HSC profile by the expression of all surface markers CD34, CD90 and CD117 (c-kit) in the absence of CD45 expression. We measured the percentage of each stem-like cell population within the viable cell population. We then calculated the concentration of each stem-like cell populations in milk secretions (see below data processing and statistical analysis).

Measurement of immunoglobulins, Na^+ and K^+ concentrations

Immunoglobulins (Ig) G and A in mammary secretions were measured by Iodolab (Grézieu-La-Varenne, France). Their concentration was evaluated by ELISA (Cow IGG ELISA kit, IGG-11 and Cow IGA ELISA kit, IGA-11, Life Diagnostics Ltd, United Kingdom).

Concentrations of Na^+ and K^+ ions were measured in whole mammary secretions using the ICP-OES method (5110 Agilent Technology, Les Ulis, France), as previously reported (Herve *et al.*, 2023).

Data processing and statistical analyses

Data produced in this study and the file describing the variables were versed in two separate files available at the entrepot.recherche.data.gouv.fr/dataverse (https://doi.org/10.57745/OXMPJJ).

Total and viable cell concentrations (cells/kg) in milk secretion samples were calculated from their proportions in the samples analysed by flow cytometry (relevant labelled cells among the 20,000 events recorded by flow cytometry). For the calculation of the concentration of each stem-like cell population (MaSC, MSC and HSC), the percentage of relevant labelled cells among the 20,000 events recorded by flow cytometry was multiplied by the calculated concentration of viable cells. To calculate the whole

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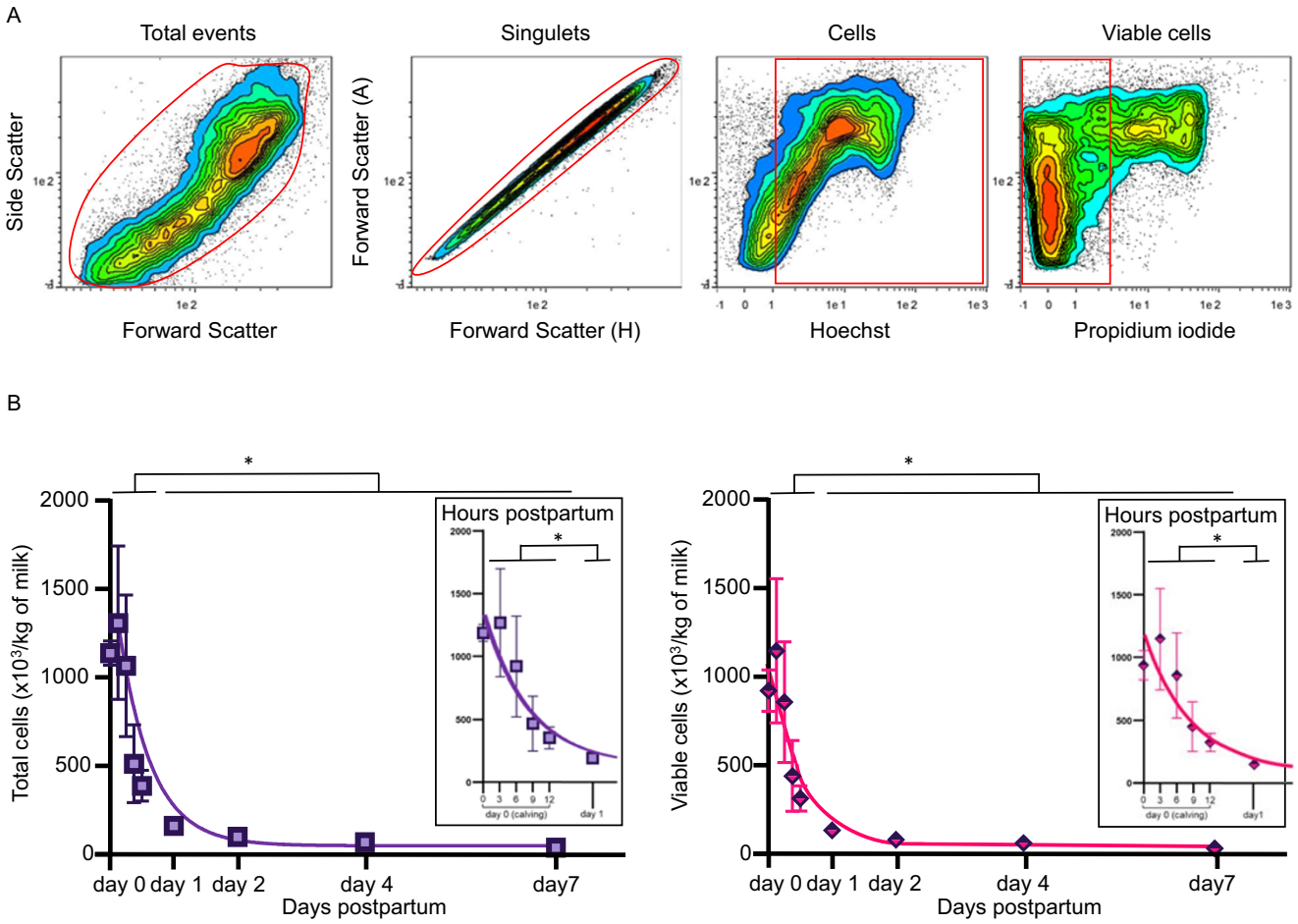

Figure 1. Kinetics of the total and viable cell concentrations in the colostrum and milk of cows after parturition.
Colostrum and milk were collected from Holstein cows following a kinetics from calving (d 0) to the seventh day after parturition (d 7). During d 0, colostrum was collected each 3 h after calving until the 12th-h postpartum. Cells were isolated and quantified by flow cytometry. A) Representative plots of flow cytometry illustrating the gating strategy used to determine the total cells (Cells) and viable cells (Viable cells) using Hoechst and propidium iodide labelling, respectively, after selecting (within the red lines) the single events (Singulets) from the total events (Total events). B) Concentration of total and viable cells per kg of colostrum or milk at the indicated times. Significance levels are indicated by $^*P < 0.05$.

quantity of cells recovered in the colostrum or milk produced at a given time, the concentration of cells per kg was multiplied by the quantity of colostrum or milk recovered during that milking.

Milk secretion yields and concentrations of Ig, ions and cells (total, viable, stem-like cells and other labelled cells) were expressed as means ± SEM, and subjected to variance analysis (ANOVA) with the RStudio software.

To test the differences due to changes over time, the following linear mixed model was used:

$$y_{ij} = \mu + time_i + cow_j + e_{ij}$$

where y_{ij} is the observed value for each dependent variable (colostrum or milk yield, amount of colostrum or milk, number of cells, IgG, IgA, Na^+, K^+ and the Na^+:K^+ ratio), μ is the mean, $time_i$ is the fixed effect of the time *post-partum* (i = d 0:0, d 0:3, d 0:6, d 0:9, d 0:12, d 1, d 2, d 4 and d 7), cow_j is the random effect of the jth cow involved in the experiment to account for repeated recordings and e_{ij} are the residuals. The models were performed in R (R Core Team, 2024) using the *lmer* function from the *lme4* package (Bates *et al.*, 2015).

Results

Longitudinal evolution of total cells in early stages of lactation

As shown in Table 1, a mean volume of almost 10 kg was collected just after calving. Colostrum volumes collected latter on the day of calving were low due to short time intervals between milkings. Then, the volume of newly made milk secretions increased sharply and appeared to plateau as from d 4 after calving ($P < 0.05$; see also colostrum or milk yield per day or hour). Note, however, that the volumes of colostrum showed greater dispersion during the first day of calving compared with those collected at 24 h and the volumes of milk from morning milking on the following days.

Concerning the concentrations of IgG and IgA in mammary secretions, they were at the highest levels at the time of calving and rapidly dropped by two-thirds already 6 h after calving (Table 1). As to ion measurements, we observed that the concentration of Na^+ slowly decreased and became significantly different at d 4 post-calving compared with d 0 to d 2 ($P < 0.05$), whereas the concentration of K^+ slowly increased between 9 and 12 h post-calving, compared with 3 and 6 h. Thus, the Na^+:K^+ ratio averaged 0.33 ± 0.03 at calving and remained unchanged during the day of calving. It became significantly lower at d 4 to d 7 ($P < 0.05$).

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Table 1. Change in the composition of colostrum and milk from second-lactation Holstein cows after calving

Day after parturition	d 0					d 1	d 2	d 4	d 7	*P*-value
Hours after parturition	0	3	6	9	12	24	48	96	168	
Amount of colostrum or milk (kg)	9.8^{b} ± *2.3*	1.6^{a} ± *0.6*	1.0^{a} ± *0.2*	1.1^{a} ± *0.2*	2.0^{a} ± *0.3*	11.7^{b} ± *1.2*	16.2^{b} ± *1.5*	18.5^{b} ± *1.1*	18.6^{b} ± *1.6*	<0.001
Colostrum or milk yield per day (kg)	(9.8^{ab} ± *2.3*)				5.6^{a} ± *0.11*	15.5^{bc} ± *2.1*	22.0^{cd} ± *1.2*	30.3^{d} ± *1.8*	32.5^{d} ± *1.7*	<0.001
Colostrum or milk yield per hour (kg)		0.53^{ab} ± *0.19*	0.32^{a} ± *0.08*	0.37^{ab} ± *0.07*	0.66^{abc} ± *0.10*	0.64^{abc} ± *0.09*	0.92^{bc} ± *0.05*	1.26^{c} ± *0.08*	1.36^{c} ± *0.07*	<0.001
Whole number of cells in colostrum or milk ($\times 10^{6}$)	$10\ 623^{b}$ ± *1839*	2980^{ab} ± *1659*	1317^{a} ± *543*	714^{a} ± *394*	846^{a} ± *298*	1794^{ab} ± *263*	1623^{ab} ± *330*	1294^{ab} ± *398*	672^{a} ± *118*	<0.01
Cell viability (%)	0.80 ± *0.07*	0.81 ± *0.08*	0.82 ± *0.07*	0.77 ± *0.10*	0.81 ± *0.04*	0.81 ± *0.08*	0.79 ± *0.08*	0.83 ± *0.06*	0.82 ± *0.05*	0.674
IgG (g/L)*	62.2^{b} ± *92*	43.9^{ab} ± *5.9*	25.5^{a} ± *5.9*	29.8^{a} ± *12.5*	<17	<17	<17	<17	<17	<0.01
IgA (g/L)	12.3^{f} ± *2.2*	8.3^{ef} ± *1.3*	4.5^{de} ± *0.1*	4.8^{cd} ± *1.6*	3.0^{c} ± *0.7*	1.5^{b} ± *0.5*	1.4^{ab} ± *0.7*	0.8^{ab} ± *0.1*	0.7^{a} ± *0.1*	<0.001
Na^{+} (mg/kg)	591^{c} ± *7*	580^{c} ± *38*	584^{c} ± *37*	620^{c} ± *43*	600^{c} ± *42*	529^{bc} ± *21*	504^{bc} ± *18*	443^{ab} ± *27*	395^{a} ± *24*	<0.001
K^{+} (mg/kg)	1876^{ab} ± *194*	1686^{ab} ± *54*	1641^{a} ± *97*	1939^{ab} ± *201*	2055^{b} ± *284*	2033^{b} ± *160*	2056^{b} ± *175*	1970^{ab} ± *178*	1977^{ab} ± *185*	<0.05
Na^{+}:K^{+} ratio	0.33^{de} ± *0.03*	0.35^{de} ± *0.03*	0.37^{e} ± *0.04*	0.33^{de} ± *0.03*	0.30^{cde} ± *0.02*	0.26^{bcd} ± *0.02*	0.25^{abc} ± *0.01*	0.23^{ab} ± *0.01*	0.20^{a} ± *0.01*	<0.001

Amount of colostrum or milk for d 2 to d 7 corresponds to morning milking. Colostrum or milk yield per day or hour includes evening milking. Cells were analysed for all time points on d 0 but only in morning milking samples for d 1 to d 7. Values are means ± *SEM* ($n = 5$ cows). Levels of statistical significance ($P < 0.05$) between time points are indicated with letters (a to f) and the global time effect by the *P*-value.
*The limit of quantification for IgG was 17 g/L.

We next focused on the cells contained in milk secretions using flow cytometry. With this aim, cells were labelled with the Hoechst DNA intercalating dye (Fig. 1A, third dot plot), allowing the calculation of the total cell concentration in mammary secretion samples (whole number of or total cells/kg of milk). Similarly, cells were also labelled with propidium iodide to determine the percentage of viable cells and to calculate their concentration (Viable cells/kg of milk, Fig. 1A, fourth dot plot). Of note, the percentage of viable cells (≈ 80%) was high and constant throughout the kinetics (Table 1). As shown in Fig. 1B, both total (Fig. 1B, left) and viable (Fig. 1B, right) cell concentrations were at the highest level during the day of calving. Then, total and viable cell concentrations decreased in samples collected from 9 h on the day of calving (Fig. 1B, insets). Thus, the cell concentrations observed from 24 h post-calving (d 1) were significantly different from those measured in samples at d 0 ($P < 0.05$). From d 1 to d 7, both total and viable cell concentrations slowly decreased, with the kinetic curves plateauing at a basal level. Also, the colostrum at calving was 7 times richer in total and viable cells than milk secretions collected on d 1, and more than 10 times richer (11 times for total and 13 times for viable cells) than the milk secretions collected at d 2 (Table 1).

Stem-like cells with mesenchymal and epithelial phenotypes are more abundant in post-calving colostrum than in milk

Flow cytometry showed that cells expressing the mesenchymal cell marker CD105 represented the largest proportion (≈47–62%) of colostrum and milk cells throughout these kinetics (Table S2). In contrast, the proportion of cells expressing the immune cell marker CD45 steadily decreased from ≈25% to ≈4% between the first milking and d 7. In agreement with these data, the majority of the cells do not express CD49f and are therefore not epithelial-oriented (Supplementary Figure S1). Indeed, epithelial cells ($CD49f^{pos}$ cells, Table S2) represented on average 1.84 ± 0.23% of the viable cells in colostrum and milk, their proportion being fairly constant over time. While analysing stem-like cell populations, we found that the proportion of $CD49f^{pos}CD24^{pos}$ cells, i.e. expressing markers identifying the MaSC population, was maximal at time of calving (Table S2). These cells were previously shown to form mammospheres *in vitro* (Finot *et al.*, 2018). Furthermore, we recently found that transplantation of both $CD49f^{high}CD24^{pos}$ and $CD49f^{high}CD24^{neg}$ cells from 17-month-old Holstein heifers into mice cleared the mammary fat pad resulted in the development of outgrowths (Finot *et al.*, 2024). Since the number of viable cells was at its highest level at calving (Fig. 1B, right panel), the MaSC concentration reached its highest value in this very first colostrum with 7.86×10^3 cells/kg ± 2.77 (Fig. 2, top graph). Then the concentration of MaSC decreased rapidly and became significantly lower from 6 h post-calving with 0.72×10^3 cells/kg ± 0.32 ($P < 0.05$). Subsequent MaSC concentrations slowly decreased from 0.37×10^3 cells/kg ± 0.15 at d 1 to 0.04×10^3 cells/kg ± 0.01 at d 7.

In contrast to MaSC, quantification of MSC, defined as the $CD34^{neg}CD105^{pos}CD90^{pos}CD29^{pos}$ cell population, showed that their average concentration at 0 and 3 h was roughly constant (3.1 10^3/kg ± 1.4 at d 0, 0 h and 2.1 10^3/kg ± 0.9 at d 0, 3 h Fig. 2, middle graph). Then, their concentration declined during the first day of calving to a basal level close to 0, their concentration becoming significantly lower from 6 h post-calving. The concentration of MSC was therefore the highest during the first hours of lactation, as was for MaSC.

With regards to haematopoietic stem-like cells or HSC, defined as $CD45^{neg}CD34^{pos}CD117^{pos}CD90^{pos}$ cell population, they were only found in the colostrum collected at the time of calving, their concentration being close to zero in all subsequent milk secretion samples (Fig. 2, bottom graph and Table S2).

In conclusion, for all the types of stem-like cells studied here, the highest quantity was observed in the colostrum collected at the time of calving.

Discussion

The presence of cells in mammary gland secretions appears to be a general feature, although only a few mammals have been tested. It is also clear that milk-born cells are heterogeneous and vary both qualitatively and quantitatively throughout lactation, particularly between colostrum and mature milk. Indeed, some studies in humans and livestock have shown that the total number of cells is higher in colostrum than in milk (Wall *et al.*, 2015; Goudarzi *et al.*, 2020). However, to our knowledge, no study presents a kinetic analysis of the detailed content of cell types, particularly stem-like (see comment below about the use of the term stem-like) cells, in mammary gland secretions from parturition to early lactation, as described here in cows.

The variation in first colostrum and milk yield in our kinetic analysis during the first 7 days after parturition is in agreement with previous studies (Kessler *et al.*, 2020; Găvan and Riza, 2023). As has been reported by others (Goudarzi *et al.*, 2020; Mane *et al.*, 2022), we observed that cells were largely more numerous in colostrum than in more mature milk, with total and viable cell concentrations being at their highest levels at the time of calving and in the colostrum collected 3 and 6 h later. Indeed, we counted ≈9 times more cells in the colostrum produced on d 0 (during the 12 h following calving) than in the milk produced during the 12 h preceding milking on d 7. In line with this, Madsen *et al.* (2004) found that the somatic cell count decreased from approximately 1,000,000 cells per mL for the colostral period to less than 100,000 in mature milk. Since the colostrum present in the mammary gland cisternae at the time of calving has accumulated there for days (Thompson, 1988; Kanazawa and Kohmoto, 2002), it cannot be excluded that this is also the case for part of its cellular contents. It should be noted that the vast majority of these cells were alive (the average proportion of viable cells remained constant at about 80% from d 0 to d 7). Interestingly, we even found high concentrations of cells in the colostrum collected 3 and 6 h after calving, showing that maternal cells are continuously transferred at a high rate to mammary secretion during this first period of lactation. As early as 9 h after calving, however, total and viable cell concentrations decreased sharply and reached basal levels in milks collected after the second day of lactation. The cell concentrations we found in colostrum and milk, as well as their relative proportions, are of the same order of magnitude as those previously reported (see McGrath *et al.*, 2016 and references therein). The longitudinal changes observed here in terms of milk cell concentrations also fit well with the notion that colostrum corresponds to the secretion of the mammary gland up to the first 2 days post-partum, although there are significant variations in the accepted duration of the standard colostrum period among reports (Silva *et al.*, 2024). Also in line with the above considerations, we also found a gradual decrease of the proportion of the cell population expressing the immune cell marker CD45 from the first milking to d 7.

The stem cell analysis described here relies entirely on a flow cytometry approach using specific cell-surface markers. As

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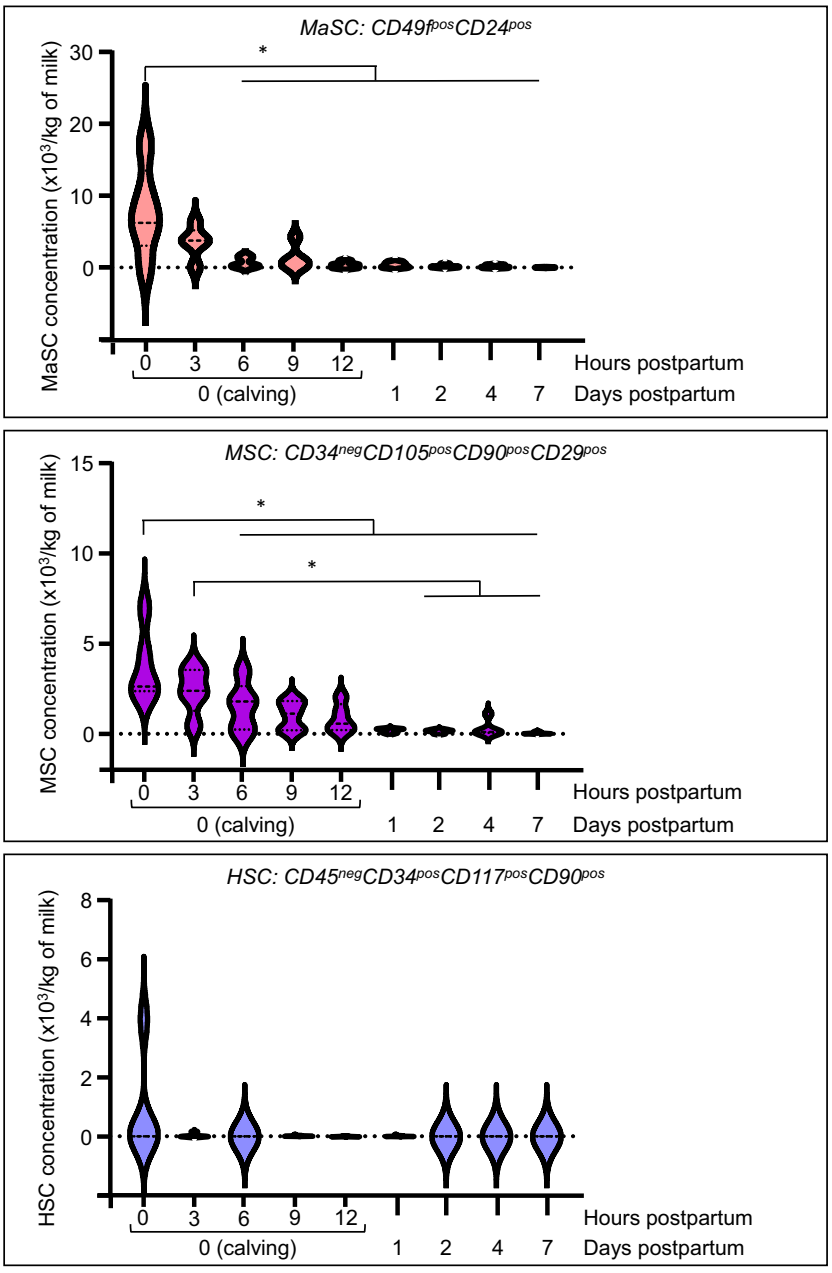

Figure 2. Kinetics of stem-like cell population concentrations in the colostrum and milk of cows after parturition.
Cells isolated from colostrum and milk were labelled using the indicated panels of antibodies and analysed using flow cytometry to either quantify mammary epithelial (MaSC), mesenchymal (MSC) or haematopoietic (HSC) stem-like cell populations. Concentration of stem-like cells was expressed per kg of colostrum or milk through the kinetics. Significance levels are indicated by $^*P < 0.05$.

mentioned earlier, our team has previously used some of these markers to sort stem cells from the bovine mammary gland. However, as we did not fully characterize the cells sorted from colostrum and milk in the current study, we decided to refer to them as stem-like cells. Concerning such specific types of stem-like cells, we observed the substantial amount of both MaSC and MSC in colostrum. However, their concentration decreased rapidly after the day of calving, being very few from d 1 postpartum. On the other hand, HSC were very few in colostrum and milk. Therefore, only colostrum seems to contain a substantial quantity of stem-like cells.

Overall, these observations demonstrate that maternal cells are more likely to be transferred into mammary secretions, either by shedding from the expanding mammary epithelium or by transfer from the stroma, before parturition or during the first hours after parturition. This would be possible since it is well known that the mammary epithelial barrier is permeable at the start of lactation, with loose intercellular junctions (Nguyen and Neville, 1998). In fact, the Na^+:K^+ ratio in milk secretions, which reflects the permeability of the mammary epithelium, has been shown to be elevated in colostrum and decline steadily in the days following parturition (Stelwagen *et al.*, 1999). In the present study, we also observed a parallel evolution in the establishment of tight junction closure of the mammary epithelium, evidenced by the decrease of the Na^+:K^+ ratio, and the reduction in the number of cells in milk. The observed decrease in the concentrations of IgG and especially IgA in milk secretions might also reflect the progressive establishment of the cohesive forces between mammary epithelial cells. Indeed, although they are mainly transferred into colostrum via receptor-mediated transcytosis, IgG and IgA can also be partially transferred passively via the paracellular pathway (Delouis *et al.*, 2001). Based on these latter data, one can tentatively conclude that tight junction

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closure occurs from d 1 postpartum. It is therefore conceivable that during the short period when the mammary epithelium is permissive, cells of the mother are able to transfer from the mammary tissue, or from blood, into the lumen of the alveoli. This transfer is clearly greater, and lasts longer, for these stem cells residing in the mammary tissue, namely MaSC and MSC, as compared with HSC. However, the cellular mechanisms underlying the passage of stem-like cells into mammary secretions have yet to be established.

The fact that colostrum contains stem cells is of great potential interest for the newborns. Although in low percentage, thousands of such cells would be ingested during the first days of life. Obviously, knowing their fate and role in the body of the recipient animal is of utmost interest. In order to act in the newborn's body, the major challenge for these cells is to survive the harsh conditions of the offspring's gastrointestinal tract and get through the intestinal barrier to reach the bloodstream. During the neonatal period, however, the gastrointestinal tract conditions are weaker and the permeability of the intestines is higher, both potentially allowing survival and transfer of milk-born cells into the offspring's body (Cabinian *et al.*, 2016). Flow cytometry analysis of stomach milk cells collected from mouse pups foster-nursed by green fluorescent protein (GFP) transgenic dams showed that more than 80% of the GFP-labelled cells were viable, even in two-week-old pups (Ma *et al.*, 2008). These authors also observed the integration of GFP-labelled cells into the small intestinal epithelium and, later on, in the spleen and thymus. Another study in mice found a correlation between milk feeding and the number of maternal cells in the liver of the offspring mice, strongly suggesting the existence of a transfer of maternal cells to the liver via the mother's milk (Dutta and Burlingham, 2010). In sheep, it was demonstrated that fluorescein isothiocyanate-labelled leucocytes were able to cross the neonatal intestinal barrier to migrate into the bloodstream of newborn lambs, and this occurred 6 to 12 h after ingestion (Schnorr and Pearson, 1984). Other studies in mice (Weiler *et al.*, 1983; Zhou *et al.*, 2000; Aydin *et al.*, 2018) and rabbits (Abd Allah *et al.*, 2016) have provided strong evidence that maternal cells in milk secretions are transferred in the offspring's blood circulation and reside in various organs including liver, muscle, heart and duodenum, but also, and more surprisingly, the brain tissue. Of note, it was shown in pig that lymphoid cells present in the colostrum were absorbed from the digestive tract only when the colostrum came from the piglet's own mother (Tuboly *et al.*, 1988). In baboons, leucocytes from maternal milk were found to cross the intestinal wall and enter the bloodstream, subsequently localizing in various organs (Jain *et al.*, 1989). Aydin and colleagues tentatively defined this phenomenon as 'breastfeeding-induced maternal microchimerism' (Aydin *et al.*, 2018), maternal microchimerism being a process known to occur naturally during pregnancy through exchange of maternal stem cells with the embryo via the placenta (Zhou *et al.*, 2000). Finally, strong arguments in favour of the persistence of mother's cells in the offspring's body for decades have been found (Barinaga, 2002). These observations support a new concept according to which maternal material can promote newborn development early in life but might also participate in the development and homeostasis of tissues throughout the life. If, likewise, nursing by the mother proves to be such a major issue for the growth performance and health of farm animals, a hypothesis which remains to be demonstrated in this case, breeding practices will have to be rethought to ensure the optimal development and well-being of livestock.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029925101222.

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