Research

Extracellular Vesicles in Bovine Colostrum and Milk

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Abstract:
Cow (bovine) colostrum (milk on the day after the calf birth) and milk (milked on the days 3 and 6 after the parturition) extracellular particles (EPs) were investigated by interferometric light microscopy (ILM). Colostrum and milk samples were taken from a Limousin cow on days 1, 3 and 6 after the parturition. Number density n and hydrodynamic diameter D_h of EPs were measured. Higher n was found in skimmed samples compared to the whole samples on days 1, 3 and 6 after the parturition, however the differences were statistically significant only on days 1 and 3 after the parturition. The sizes of the particles (D_h) were within the error of the method, and the differences between the skimmed and whole samples were statistically significant only on the day 3.

Keywords: Extracellular vesicles; Light scattering; Vesicle characterization; Cow; Colostrum; Milk; Nanovesicles

1. Introduction

1.1. About cow udder, lactation, colostrum and milk

Colostrum (first milk) and milk are being produced in the mammary glands of female mammals. Colostrum is the milk produced in the cow's udder during the first days after the birth (Playford et al., 2021). Colostrum is rich in nutrients, immunoglobulins, peptides with antimicrobial activity and growth factors.

There are four quarters of the cow udder. In each quarter, there is a separate mammary gland complex. Each of the four separate mammary complexes has its own separate teat cistern and teat canal. Every one of four teats therefore has one opening (Nickerson et al., 2020).

Lactation is energetically very demanding for a cow. The transition from a dry cow to lactation requires a big amount of energy from the dairy cow (Santos et al., 2012). Standard lactation in diary cattle lasts for approximately 305 days. The lactation curve increases rapidly after calving, peaks a few weeks later and gradually declines until the animal gets dry in approximately 10 months (Wood, 1967).

Both colostrum and milk are necessary for the calf to survive. The first milk secretion after the parturition is known as colostrum. The early growth, development and immune function in the calf depend on colostrum. It is essential that maternal colostrum is fed to the newborn calf during the first hours of life. Colostrum provides the calf with essential nutrients as proteins, lipids and carbohydrates. There are essential vitamins and minerals present in bovine colostrum, bioactive proteins and immunological factors (Lopez et al., 2022).

The transfer of passive immunity occurs via colostrum which is critical for the proper development of the immune system in the calf. After the parturition, the immune system of the calf is very immature because the bovine placenta does not enable the permeability to proteins like immunoglobulins. Calves are born without immunoglobulins in circulation. Therefore, immunoglobulins from the colostrum are critical for the protection of the neonate against the pathogens from the environment and therefore help the development of the immune system (Sutter et al., 2023).

The colostrum turns to milk in approximately 2 or 3 days after the parturition, but the transition from colostrum to mature milk is gradual and takes several weeks. (Mcgrath et al., 2016.)

Figure 1. Limousin cow with the calf (Image from: https://cattleinternationalseries.weebly.com/limousin.html

2. Material and Methods

2.1. Milk Sampling

For interferometric light microscopy, the samples were milked by hand from a Limousin (breed) cow (**Figure 1**) on 1st, 3rd and 6th day of lactation. Milk was collected into tubes VACUETTE® TUBE 3 ml Z No Additive 13x75 white cap-black ring, non-ridged (Greiner AG, Kremsmünster, Austria). To obtain skimmed milk, whole milk was centrifuged at 300g for 15 min. This procedure was repeated twice. The cream was removed from the top using a pipette with the tip shortened for 2 mm by scissors. The tubes with colostrum and milk are shown in **Figure 2**.

Figure 2. From left to right: cow colostrum milked on the day of parturition, cow milk from the third day after the parturition and cow milk milked from the seventh day after the parturition after the skimming procedure. On the top, the cream layer can be distinguished. Colostrum has a yellow hue unlike the white milk.

2.2. Interferometric Light Microscopy (ILM)

The average hydrodynamic diameter (D_h) and the number density of small particles n were determined by ILM using Videodrop (Myriade, Paris, France) as described previously (Romolo et al., 2022). Before measurement, the milk was diluted 100 × by saline for injections (Braun, Melsungen, Germany) to obtain a measurable dilution. Signals of the saline were under the detection limit. The threshold value 4.2 was used. 7 μL of sample was placed between cover glasses and illuminated by 2W blue LED light. The light scattered on the particle was imaged by a bright-field microscope objective and allowed to interfere with the incoming light. The image was recorded by a complementary metal–oxide–semiconductor high resolution speed camera. The obtained pattern that includes contrasting black and white spots was recognized as a particle and its position in the sample was assessed. Number density of the particles is the number of the detected particles within the detected volume (e.g. 15 pL). D_h was determined by tracking the position of the imaged particle within the recorded movie. It was assumed that particles undergo Brownian motion due to collisions with surrounding particles. The diffusion coefficient D of the motion of the particle is taken to be proportional to the mean square displacement d of the particle between two consecutive frames taken in the time interval Δt, $\langle d^2(\Delta t)\rangle = \langle 4D\ \Delta t\rangle$ while D_h was estimated by assuming that the particles were spherical and using the Stokes-Einstein relation $D_h = kT/3\pi\eta D$. Each particle that was included in the analysis was tracked and processed individually and the respective incident light signal was subtracted from each image. Processing of the images and of the movies was performed by using the associated software QVIR 2.6.0 (Myriade, Paris, France).

2.5. *Statistical analysis*

All measurements were performed in triplicates and presented by the average values and standard deviations. The differences were evaluated by the t-test using the Excel sofware. The value $p = 0.05$ was taken as a threshold for statistical significance.

3. Results

Higher number density n of nano size particles was found in skimmed colostrum or milk when compared to the whole milk on days 1, 3 and 6 after the parturition, however differences were statistically significant only on day 1 and 3 **(Figure 3)**.

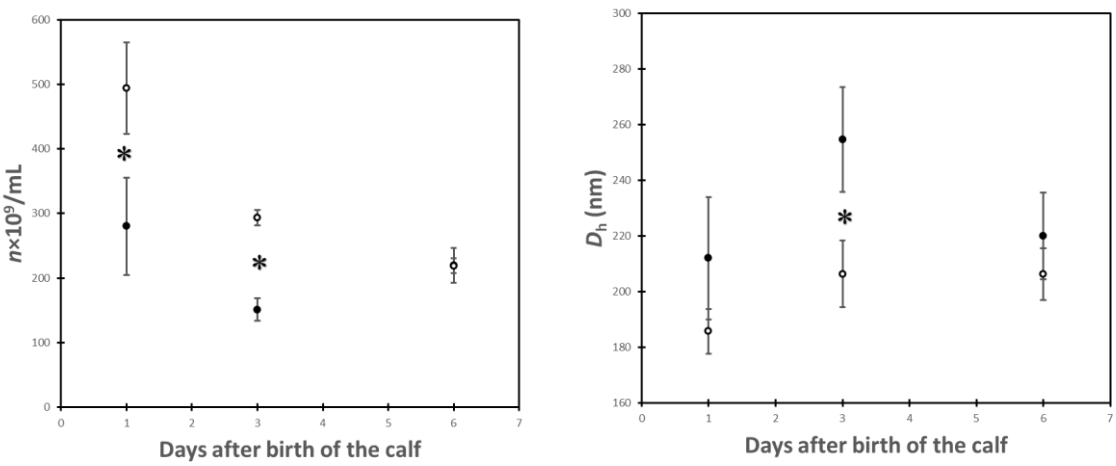

Figure 3. A: average number density n and B: average hydrodynamic diameter D_h of EPs in the cow whole samples (full circles) and skimmed (empty circles) samples. The error bars represent standard deviations. Asterisk (*) represents statistically significant difference between the whole and the skimmed samples.

The differences in hydrodynamic diameters of the particles D_h were within the error of the method. Larger particles were found in the whole samples, however differences were statistically significant only on day 3 **(Figure 3)**.

4. Discussion

Higher n and lower D_h in skimmed samples might be explained by removal of larger particles with the cream during the preparation of skimed milk. Previously, higher n of micro and nano-sized particles was found in skimmed mature equine milk (measured by flow cytometry and interferometric light microscopy, respectively) than in the whole mature equine milk, and the difference was statistically significant (Arko et al. 2024a). Furthermore, higher n was found in the skimmed samples than in the whole samples of equine colostrum and milk when measured by interferometric light microscopy on days 1, 3 and 7 after parturition, but the difference was statistically significant only on day 7 after the parturition (Arko et al. 2024b).

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Conflicts of Interest: The authors declare no conflict of interest.

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