Research

Characterization of Extracellular Particles from Equine Milk and Colostrum

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Abstract:
Extracellular particles (EPs) from mare (equine) milk and colostrum were investigated. The samples were taken from a Posavje mare on days 1, 3 and 7 after the parturition. Number density n and hydrodynamic diameter D_h of EPs were measured by interferometric light microscopy (ILM). Higher number density of nano - sized EPs were found in skimmed milk when compared to the whole milk on days 1, 3 and 7 after the parturition, however the difference was statistically significant only on the day 7 after the parturition. The average D_h was statistically significantly lower in the whole milk than in the skimmed milk at day 7 after the parturition.

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

1. Introduction

1.1. Mare udder, lactation, colostrum and milk

Colostrum (the first milk secretion after the parturition) and milk are being produced in the mammary glands of a female mammals (Reiter and Reed, 2023). There are two halves of the mare udder. In each half, there are two separate mammary gland complexes one in front and the other behind. Each of the two separate mammary complexes has its own separate teat cistern and teat canal. Each of both teats has therefore two openings, one in front and the other behind (Dzidic, 2003).

Lactation is energetically very demanding for a mare. It may last for over a year in the wild. It is highest 30 to 60 days after foaling, when daily milk production can range from 12 to 15 liters (Morresey, 2012). Both, colostrum and milk are essential for the foal's survival. The foal's early growth, development, and immune function depend on colostrum. Colostrum provides the newborn foal with essential nutrients as amino acids, bioactive proteins, immunological factors, and antioxidants (Reiter and Reed, 2023).

The transfer of passive immunity occurs via colostrum which is critical for the proper development of the immune system in the foal (Reiter and Reed, 2023). After the parturition, the immune system of the foal is very immature because the equine placenta does not enable the permeability to proteins like immunoglobulins (Reiter and Reed, 2023). Foals 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. Approximately 60% of the protein in the colostrum are immunoglobulins (Reiter and Reed, 2023). The predominant immunoglobulin in equine colostrum is immunoglobulin G (IgG), followed by IgA occurring at a lower concentration (Reiter and Reed, 2023). Approximately 12 hours after the parturition the colostrum immunoglobulin concentration decreases (Pasquini et al., 2005). The foal's intestine enables the absorption of the immunoglobulins only first 6 to 12 hours of life (Reiter and Redde, 2023).

Colostrum turns to milk in approximately 2 days after the parturition, but the transition from colostrum to mature milk is gradual and takes several weeks (Reiter and Reed, 2023). The mature milk contains nutrients for the foal and non-nutritive bioactive factors (Reiter and Reed, 2023). Due to limited fat and carbohydrate content, equine milk contains less energy in comparison to bovine or human milk (Reiter and Reed, 2023). However, mature equine milk contains more crude proteins than human milk but less than bovine milk (Malacarne et al., 2002).

Figure 1. Posavje mare with the foal.

2. Material and Methods

2.1. Milk Sampling

Colostrum (milk collected on the day 1 after the parturition) or milk was milked by hand from a Posavje (breed) mare (**Figure 1**) on days 1, 3 and 7 of the lactation. Colostrum or 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, the whole milk was centrifuged at 300 g for 15 min. The centrifugation was after that repeated once again. 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**.

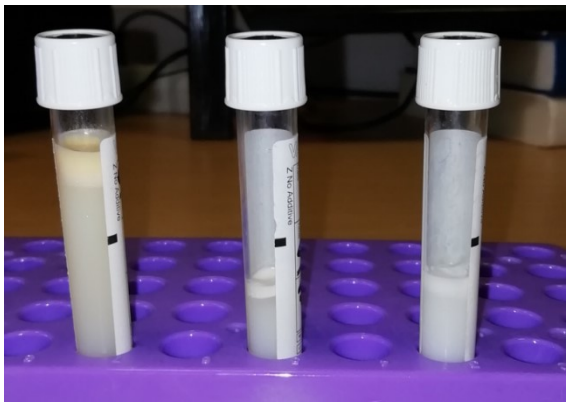

Figure 2. The tubes with mare colostrum and milk. From left to right: The tube on the left contains mare colostrum milked on the day of parturition, the middle tube contains mare milk from the third day after the parturition and the tube on the right contains mare milk milked on the seventh day after the parturition. On the top of the samples the cream layer is clearly visible. 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 in milk (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). 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. Briefly, the interference pattern between incident and scattered light forms contrasting black and white spots which were recognized as particles and their respective positions were determined. Counting the number of particles in a defined volume (of the order of 15 pL) yields the number density of EPs. D_h was determined by following the position of EPs with time and assuming Brownian motion of EPs. Assuming that the diffusion coefficient D of EPs is proportional to the mean square displacement d of the EP between two consecutive frames taken in the time interval Δt, $\langle d^2(\Delta t)\rangle = \langle 4D\,\Delta t\rangle$, D_h was estimated by using the Stokes-Einstein relation $D_h = kT/3\pi\eta D$, where k is the Boltzmann constant and T is temperature. 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 concentration of nano - sized particles was found in skimmed milk when compared to the whole milk on days 1, 3 and 7 after the parturition, however differences were statistically significant only on day 7 **(Figure 3. A)**. The differences between average D_h of the particles were within the error of the method **(Figure 3. B)**.

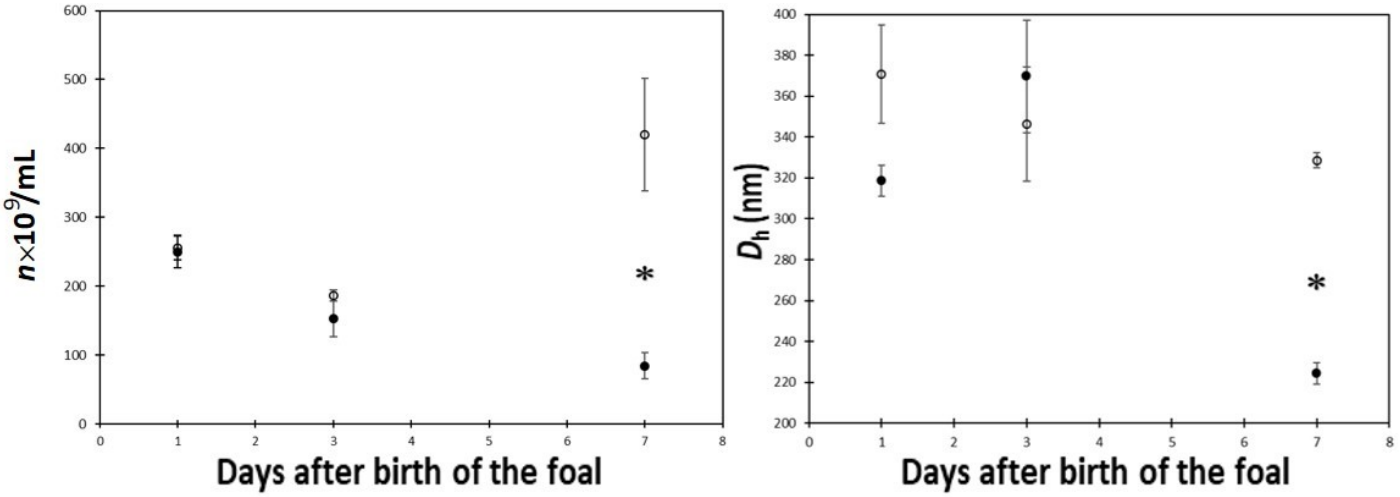

Figure 3. A: average number density n and B: average hydrodynamic diameter D_h of EPs in the mare whole (full circles) and skimmed (empty circles) milk. The error bars represent standard deviations. Asterisk (*) represents statistically significant difference between the whole and the skimmed milk results on day 7.

4. Discussion

Higher concentration of nano - sized particles was found in skimmed milk when compared to the whole milk on days 1, 3 and 7 after the parturition, however differences were statistically significant only on day 7. D_h was smaller on day 7 than in days 1 and 3, the differences were statistically significant.

In our previous work, higher concentration of micro and nano-sized particles was found in skimmed mature equine and bovine milk measured by interferometric light microscopy and flow cytometry when compared to the mature whole milk and the differences were statistically significant (Arko et al., 2024), which is in agreement with our present results.

Higher number density of EPs in skimmed milk could be explained by removal of larger particles with the cream.

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

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