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Proteomic comparison of human milk from term and pre-term mothers



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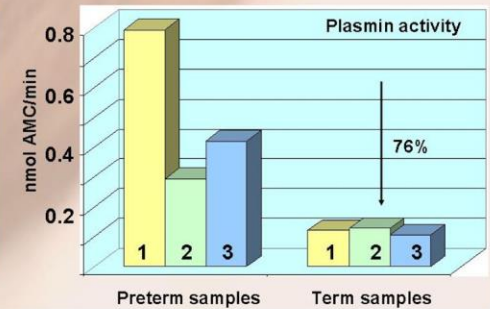
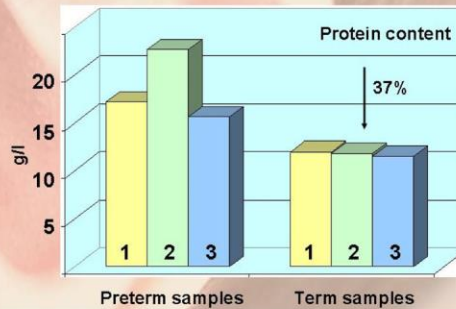
The composition of milk obtained from mothers giving birth prematurely is of interest because of the key role of this milk as a nutrient source for the premature infant. Preterm milk of early lactation is generally found to have higher levels of protein, no-protein nitrogen, fat, energy, sodium, and chloride, but lower levels of lactose and greater inter-individual variation. However, by the end of the first month of lactation the preterm mother's milk is very similar in nutrient composition to term milk. The goal of this work is to investigate the protein composition of breast milk for pre-term babies and to compare it with the "normal" breast milk in terms of protein concentration, plasmin activity and protein variety and abundance.

Compositions of breast milk between samples from mothers of pre-term and term infants

Six breast milk samples (3 samples from mothers giving birth prematurely and 3 samples from mothers giving birth at term) were selected and compared for their protein content and plasmin activity.

According to the kjeldahl method, the mean **protein content** was 18.4 g/l for preterm milk samples and 11.7 g/l for term milk samples (37% decrease).

According to the plasmin activity assay, the mean **plasmin activity** was 0.252 nmol AMC/min for preterm milk samples and 0.059 nmol AMC/min for term milk samples (76% decrease).



Two-dimensional electrophoretic comparison of protein profiles of breast milk samples from mothers of pre-term and term infants

Experimental conditions

Protein solubilisation: 9M urea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT

Analytical gels (7.5 µl of milk / gels)

1st dimension : Precast 7 cm strips pH 3-10 (Bio-Rad)

with the Protean IEF Cell (Bio-Rad)

2nd dimension : 12.5% SDS-polyacrylamide gel, using the Criterion® Dodeca

Cell electrophoresis unit (Bio-Rad)

Preparative gels (20 µl of milk / gels)

1st dimension : Precast 17 cm strips pH 3-10 (Bio-Rad)

with the Protean IEF Cell (Bio-Rad)

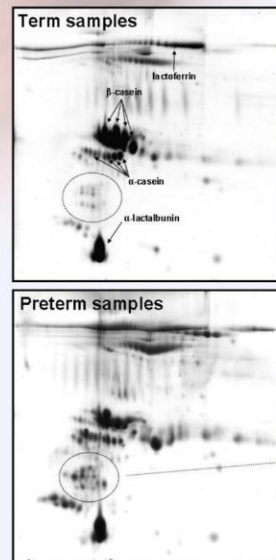
2nd dimension : 12.5% SDS-polyacrylamide gel, using the Protean II XL Cell electrophoresis unit (Bio-Rad)

Staining conditions : Colloidal Coomassie blue

Gel images were digitalized with a GS 800 densitometer (Bio-Rad)

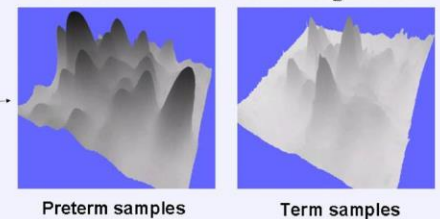
Gel were analyzed using the PDQuest software v.7.3.1.

Mass spectrometry analysis: MALDI-TOF peptide mass fingerprint with a Axima CFR plus mass spectrometer (Shimadzu Biotech) was used to identified proteins labeled on gel.

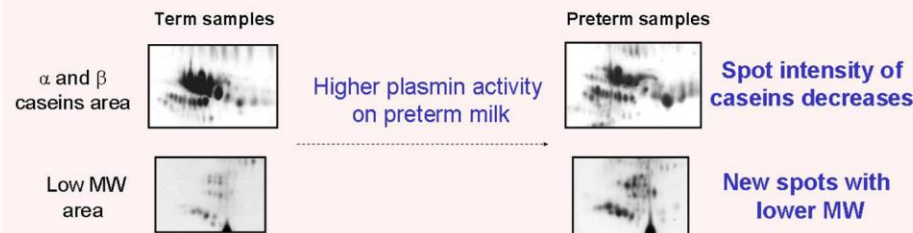


	Preterm	Term
Common spots		84
Specific spots	69	10
Over-expressed spots	48	7
Under-expressed spots	7	48
No differences	29	
Total spots	153	94

3D view of low molecular weight area



Proposed mechanism



With a higher protein content and a higher plasmin activity, preterm milk samples display a higher number of spot, with a decrease intensity of the major native casein spots.

Additionally, new specific low molecular weight spots are observed on preterm milk sample, possibly resulting from the degradation of casein proteins.

The increased variety of "pre-digested" proteins on preterm sample can lead to a easier digestion of breast milk for premature babies.