



# MilkEV

**TEAGASC** research is examining extracellular vesicles in milk and how they can be incorporated into infant formula.

The 'Milking extracellular vesicles for improved infant formula' (MilkEV) project is a new collaboration between Teagasc Moorepark and Trinity College Dublin, which aims to quantify and characterise extracellular vesicles in Irish cow's milk.

### Extracellular vesicles

Extracellular vesicles (EVs) are small cellular particles composed of a lipid membrane encasing proteins and nucleic acids. They are released from cells and can be found in all body fluids (blood, milk, sperm). Initially scientists viewed them as cell debris or as playing a sinister role in cancer. We now know that EVs are secreted in every physiological condition and are a form of cell-to-cell communication. This communication can be a health benefit; for example, milk EVs carry immune signals from mother to baby.

### Research question

We will track EVs during milk processing and the production of infant milk formula. The project will also seek to answer the following questions:

- what is the composition of milk EVs?;
- can EVs survive processing?;
- can EVs survive digestion?; and,
- are they transported across the gut barrier?

To understand the effect of processing on EVs, commercial first-stage infant milk formula has been produced at pilot plant scale.

### Studies in Teagasc and Trinity College Dublin

Bulk milk from the current Irish dairy herd, as well as colostrum, first milk and mature milk from individual cows (representing the next-generation dairy herd) was collected from Teagasc dairy farms from February to December 2019. In total, 159 milk samples have been collected and EV extraction is currently underway using ultracentrifugation density gradients. In short, EVs are purified and collected from milk according to their density (1.1-1.2 g/mL), following the International Society of Extracellular Vesicles guidelines (Théry *et al.*, 2018).

To understand the effect of processing, commercial first-stage infant milk formula has been produced at pilot plant scale using two different processing methods: standard thermal processing; and, membrane cascade processing. The membrane



This research aims to create a next-generation infant formula enriched with EVs.

cascade process incorporates low heat, so we will be able to understand if reducing the thermal load during processing protects EVs, and ensures the presence of intact, bioactive EVs in the final product.

Teagasc scientists are global leaders in modelling food digestion in the laboratory. Using the INFOGEST protocol, infant milk formula has been subjected to semi-dynamic gastrointestinal digestion. We are currently attempting to extract EVs from both gastric and intestinal samples. The effect of EVs on the health of the intestinal barrier is estimated by monitoring transepithelial electrical resistance, and quantifying tight junction proteins (i.e., occludin and claudin-1) in 21-day-old Caco2-HT29MTX cell monolayers. Cellular bioassays have been developed in house to test the benefits of consumed EVs for immune cells based on THP-1 macrophages treated with bioavailable milk digesta.

### Benefits of milk EVs

This project will be of benefit to the Irish dairy industry as it determines the quantity and composition of EVs present in Irish milk over the lactation curve. It will help infant formula manufacturers by tracking the fate of EVs during processing. Whether or not bovine EVs can play a positive role in maturing the gut barrier and boosting the immune system will also be investigated.

### New knowledge gained

This collaboration brings together experts in EVs, gastrointestinal digestion and dairy processing. Ultimately this research activity aims to create a next-generation infant formula enriched with EVs.

### Reference

Théry, C., Witwer, K., Aikawa, A., *et al.* (2018). 'Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines'. *Journal of Extracellular Vesicles*, 7: 1.

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