New phenotypes for milk

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**Objectives**
1) To set up and implement a routine procedure for collecting monthly milk samples from all cows in the RVC’s Boltons Park dairy herd
2) To collect the first sets of samples from the herd
3) To extract RNA and extracellular vesicles (EV) from the collected samples
4) To report the results of these collections in both written and oral form, the latter at an appropriate scientific meeting

**Key techniques**
- 2 complete collections from Bolton’s Park Farm, Hertfordshire, from Holstein Friesian cows at different time points during lactation
- Newly calved samples (under 40 days of lactation) were used for experiments
- Whole milk was centrifuged to remove cream / skimmed
- Cellular material collected for later RNA collection and analysis
- EV from the first collection were counted using flow cytometry
- A protocol to optimise isolation of exosomes was outlined (ultracentrifuge)
- T cells from human blood were isolated for further analysis of effects of EV on human T cell function

Two complete milk collections were carried out at Boltons Park Farm, (Hertfordshire) from 100 Holstein Friesian cows at the same time as the August and September 2015 NMR milk recording. The cattle graze outside during the day but come inside for milking and are in different lactation stages. 50ml of milk was taken from each cow and was brought back to the Camden campus of the Royal Veterinary College immediately for processing and analysis. A 2ml sample of whole milk was taken from each 50ml tube and frozen at -80°C. The rest of the milk was sequentially centrifuged to remove cream i.e. skim it. The full 50mL samples from early lactating cows, defined as cows within 40 days of calving, were stored so that we can follow these cows further. Consecutive centrifugation of skimmed milk allowed for the isolation of RNA from 24 samples including those cows deemed to be in early lactation.

Extracellular vesicles from the first collection have been isolated and quantified using flow cytometry. A protocol to optimise isolation of exosomes was outlined using ultracentrifugation. Naïve CD4+ T cells were isolated from fresh human blood and cultured in vitro. These cells are to be used for further experiments investigating the effects of milk derived EVs on human T cell function.

This project allowed me to visit a small-scale dairy farm and showed me how automated milking is carried out. I learnt the process of EV purification from milk by sequential centrifugation using an ultracentrifuge and gained an insight into their hypothesised role in the body. I have additionally learnt to isolate and expand human T cells in vitro, learning in detail about their structure and function. I look forward to carrying out further experiments to try and identify the role of milk derived EV in both human T cell function and milk production in cows. I hope to present my results at BSAS next year.