

STSM REPORT

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Host Institution: Farm Animal Health and Production Group, Hawkshead Campus, Royal Veterinary College, University of London.

I started work at the RVC on 28th January 2016, with my supervisor Professor Claire Wathes and her research group, in the Department of Production and Population Health, in the Royal Veterinary College, University of London.

Introduction

My research in the Host Institution was under an ongoing EU project “GplusE” (Genotype plus Environment). GplusE aims to contribute to the sustainability of dairy cow production systems through the optimal integration of genomic selection and novel management protocols based on the development and exploitation of genomic data and supporting novel phenotyping approaches. The end result of GplusE will be a comprehensive, integrated identification of genomic – phenotypic associations relevant to dairy production across Europe. The overall objectives are improvement in productivity, efficiency, animal health, welfare and fertility in an environmentally sustainable way. Results from the project will also increase biological understanding of the mechanisms by which genotype, environment and their interaction influence performance.

One component of GplusE is to undertake a Genome Wide Association Study (GWAS) to link key physiological markers of cow health with genetic makeup. This will involve recruitment of between 10-15,000 Holstein cows from commercial dairy herds, of which about 1,000 have been sourced from the UK.

The RVC group and others have previously shown that the circulating concentration of the hormone Insulin-like Growth Factor-1 (IGF1) collected within the first month after calving is a good indicator of metabolic health. IGF1 plays several important roles in controlling metabolism, reproduction and milk production (Taylor et al. 2004). The IGF1 concentration generally declines after calving, and the level reached at this time could be predictive of future fertility (Wathes 2012). Low IGF-1 concentrations were associated with failure to conceive whereas high IGF-1 concentration were associated with conception to first service. However, the concentration of IGF1 varies with age, breed and stage of lactation (Taylor et al. 2004). IGF1 concentration is also associated with energy balance and immunity around calving. Circulating concentrations of IGF-I remained lower in the cows with negative energy balance (NEB) after calving. Immune responses of the postpartum uterus are altered compared to other periods of time e.g. pregnancy, anoestrous or cyclicity. Immune function is mainly suppressed over the periparturient period (Mallard et al. 1998) and poor energy balance i.e. NEB and fatty liver have been shown to impair peripheral blood neutrophil function (Hammon et al. 2006; Leslie 1983; Zerbe et al. 2000). After calving uterine

immune function may become compromised, resulting in the development of inflammatory disease (Oguejiofor et al. 2015).

Postpartum milk production and the requisite nutritional adaptations induce a physiological state of NEB. Dairy cows also mobilise body energy storage in different tissues to support milk production and, because glucose supplies are limited, lipids are used preferentially for energy production. When these internal reserves are mobilised, this will lead to a loss of body condition (Wathes et al. 2013).

Aim of my research

The aim of my research is to measure the IGF1 concentration in blood samples collected from UK cows as part of the GWAS study. These results will be used to select subsets of cows with either high or low IGF1 concentrations after calving. The immune status of these two groups will be compared by measuring expression levels of candidate genes involved in immunity in white blood cells. These results will then be related to the health and fertility data recorded for each animal.

Materials and Methods

Animals

Blood samples were collected before my arrival from dairy cows from 4 UK herds. These were taken from the coccygeal vein under Home Office license. For IGF1 assay, 10 ml samples were taken into heparinized vacutainers, the plasma was separated by centrifugation and stored at -20 °C in the RVC freezer bank. For the PCR analysis, 3ml samples were taken into Tempus tubes (Ambion™ Applied Biosystems, USA) and stored -80 °C in the RVC freezer bank. The Tempus tubes contain 6 mL of Stabilizing Reagent, which effectively lyses blood cells, inactivates cellular RNases and selectively precipitates RNA; genomic DNA and proteins remain in solution. Using the Tempus tube and associated extraction kit is possible to purify high quality RNA without sample pretreatments such as leukocyte isolation or selective red blood cell lysis. Data about calving date, blood collection time (days post-partum) and parity were recorded for each animal. Further information on health and fertility of each animal during that lactation are being obtained from herd records.

IGF1 measurement

The IGF1 concentration has been measured using an enzyme-linked immunosorbent assay (DRG IGF-1 6000 ELISA Kits, DRG Instruments GmbH, Marburg, Germany). Briefly the assay steps were as follows. 1) Acidification and neutralization of 50 µl of samples, standards and controls. 2) Dispense 20 µl samples, standards and controls into appropriate wells in the plate. 3) Dispense 100 µl Enzyme Conjugate. 4) Incubate for 120 minutes at room temperature. 5) Shake out the contents of the wells and rinse the wells with diluted Wash Solution. 6) Dispense 150 µl Enzyme Complex into each well. 7) Incubate for 120 minutes at room temperature. 8) Shake out the contents of the wells and rinse the wells with diluted Wash Solution. 9) Add 100 µl Substrate Solution to each well. 10) Incubate for 15 minutes at room temperature. 11) Determine the absorbance of each well at 450 +/- 10 nm with a microtiter plate reader. Results from each assay are recorded in Excel.

RNA extraction

RNA extraction will be carried out using Tempus Spin RNA Isolation Kits (Ambion™ Applied Biosystems, USA) according to the manufacturer's instructions. Briefly the RNA extraction procedure is as follows. 1) Defrost blood samples previously collected in Tempus Isolation tubes (stored in -80 °C freezer) at room temperature. 2) Transfer the 3 ml of stabilized blood to 50 ml conical tubes and dilute with 3ml 1X PBS (provided in the kit). 3) Vortex vigorously for at least 30 seconds. 4) Centrifuge at 4°C at 3000 x g for 30 minutes, then carefully discard supernatant. 5) Re-suspend the pellet in 500 µl RNA resuspension buffer. 6) Transfer re-suspended RNA into purification filter, then wash the column filter via RNA purification wash solution1 (500 µl) immediately followed by micro-centrifuge (16,000x g 30 Sec). 7) Wash the column containing RNA with wash solution 2 (500 µl) followed by micro-centrifuge (16,000x g 30 Sec) twice. 8) Upon elution of RNA in Nucleic Acid Purification Elution Solution (90 µl) samples will be stored at (-20 °C or -80 °C for long-term storage). Prior to storage the quantity and quality of the RNA will be assessed using NanoDrop ND-1000 spectrophotometer (ThermoFischer, UK).

qPCR.

Fifteen candidate genes involved in immunity have been selected, including chemokines, chemokine receptors, interleukins and other cytokines. I have designed the Primer sequence for the 15 genes of interest and 3 housekeeping genes using a "Primer 3" web based programme (<http://frodo.wi.mit.edu/primer3>) and DNA sequences from GenBank at NCBI (<http://www.ncbi.nlm.nih.gov/Database/index.html>). Concentrations of the selected target and reference genes will be quantified using qPCR via an absolute quantification approach following the method reported in this group (Cheng et al. 2013). The DNA amplified from cDNA used for standards in the qPCR assay will be purified using a QIAquick PCR purification kit (Qiagen) and their quality and concentrations will be determined with the NanoDrop ND-1000 spectrophotometer. Annealing and amplicon-specific melting temperatures of the primers will be determined using a gradient function of the qPCR machine (Bio-Rad). Absolute qPCR will contain a standard curve, no template control (NTC) and unknown samples using the reagents and protocol described in this group (Cheng et al. 2016) with the above optimized conditions. The results will be analysed using the CFX Manager Software package (Bio-Rad).

Work during the STSM

- I started work in the Host Institution by reviewing the relevant literature about IGF-1, fertility in dairy cows, the IGF system and fertility, milk production in cows and immunology.
- I have learnt the following laboratory techniques: ELISA, RNA extraction, quantitative RT-PCR including primer design.
- I have analyzed the IGF1 concentration in 539 plasma samples selected from cows between 5-21 days after calving.

Next steps

- Select about 50 cows based on the IGF-1 concentration and other parameters (herd, parity).
- Extract RNA from the white blood cells obtained from the Tempus tubes.
- Measure expression of around 15 genes (depending on time available) involved in

immunity using Quantitative RT-PCR.

- Connect IGF1 concentrations with expression of immunity genes, fertility and health in the dairy cows.

Other comments

I think that my experience in the Host Institution has been very important for me. I achieved many objectives such as to become familiar with new laboratory techniques, expanding my knowledge, doing a cultural exchange and improving my written and spoken English.

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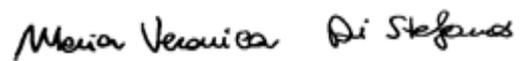
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