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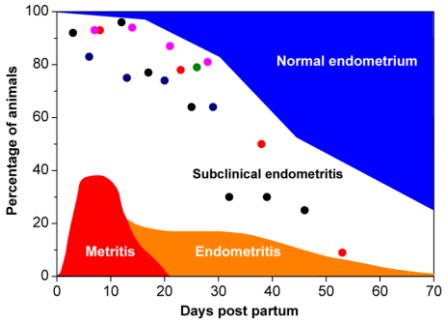
Early diagnosis of postpartum uterine disease for enhancement of reproduction and improved cow health



Key external stakeholders:
Pharmaceutical companies, DAFM, Teagasc commercial partners, veterinary surgeons, Animal Health Ireland, dairy industry

Practical implications for stakeholders:

- A subset of cows are susceptible to developing post-partum endometritis but diagnosing this disease on the basis of clinical signs (purulent vaginal discharge) is inefficient as disease has progressed, treatment outcomes are less effective and fertility may be compromised.
- Endometritis is an inflammatory disease, and the detection of inflammatory markers in uterine fluid at 7 day post-partum (DPP) identifies which cows subsequently develop endometritis.
- High milk yield is an important pre-disposing factor to developing endometritis in the subsequent production year.

Main results:

1. From our analysis of 440 cows, high-yielding pasture-based Irish dairy cows were 1.6 times more likely to present with PVD in the subsequent lactation. Cows with PVD were 4.9 times more likely not to conceive, 2.7 times more likely to require multiple services to conceive, 2.1 times more likely to remain not pregnant by 100 d postpartum, and 4.4 times more likely to remain not pregnant by 150 d postpartum. The calving to conception period (CCP) was also significantly longer in cows with PVD than their healthy counterparts (115.9 ± 4.9 and 104 ± 7.4 d, respectively). In conclusion, PVD significantly increased the CCP in all cows, but to a greater extent in cows with a high milk yield in the lactation before disease diagnosis.
2. A comprehensive panel of immunological, metabolic and microbiological markers were profiled both in the blood (systemically) and in the uterus (locally) of cows with and without disease. The most sensitive biomarkers were found to be those that measured local changes in cows with PVD. We developed a novel assay to measure inflammatory markers in the uterine fluid of cows. We now have a panel of informative markers that predict subsequent disease occurrence and these are currently under assessment for commercialization.
3. It is now clear that clinical disease has a negative impact on fertility but we also used proteomic analysis to identify proteins that contribute to the development of sub-clinical disease, known as cytological endometritis.

Opportunity / Benefit:

- This project will benefit initiatives to enhance oral immunity in calves and also add weight to the selection of genes to enhance disease resistance in cattle.

Collaborating Institutions:

University College Dublin, Trinity College Dublin

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Teagasc project team: Amy Brewer
Dr Kieran Meade (PL)
Professor Cliona O' Farrelly, Trinity College Dublin.

External collaborators: Professors Alex Evans, Marijke Beltman and Wim Meijer, University College Dublin.
Dr Erin Williams, University of Edinburgh.

1. Project background:

Postpartum uterine infections are a leading cause of compromised fertility, which is a serious threat to the expanding Irish dairy sector. The consequences of infertility, together with production losses and treatment costs associated with uterine disease, costs farmers an estimated €292/cow/year. This disease also contributes to an overuse of antibiotics and diagnosis on the basis of clinical signs delays resumption of fertility and requires costly treatments. While all cows experience an influx of bacteria into the uterus after calving, it is not known why some cows on the same farm, under identical management conditions develop uterine disease while others do not. The aim of this project was to establish if early changes in the post-partum cow could identify those on a trajectory toward developing disease and to integrate information from multiple levels to identify a reliable prognostic biomarker of disease.

2. Questions addressed by the project:

1. What is the impact of uterine disease on Irish pasture-based dairy cows?
2. Can we identify prognostic biomarkers associated with subsequent disease development?
3. What panel of prognostic biomarkers offers optimal specificity and sensitivity?

3. The experimental studies:

Comprehensive sampling was undertaken in a cohort of 440 post-partum dairy cows from commercial farms across multiple years at two critical time-points after calving. At Day 7 and Day 21 post-partum, blood samples, uterine swabs and uterine cytobrushes were collected. Disease diagnosis was performed at 21 DPP. Extensive microbiological analysis was done by the team led by Wim Meijer in UCD. Haematological, proteomic and transcriptomic (RNA-seq) analysis was performed by the Teagasc team. Immunological analysis was performed in partnership with TCD. Multiple putative markers were assessed in collected samples and the best panel were validated in a new panel of samples collected in 2019. As an inflammatory disease, inflammatory markers in uterine fluid showed significant promise and accurate measurement required the optimisation of a new assay to process uterine fluid for quantitative assessment of cytokines and acute phase proteins. Vitamin D analysis was also performed using a newly optimised ELISA.

4. Main results:

- From our analysis of 440 cows, high-yielding pasture-based Irish dairy cows were 1.6 times more likely to present with PVD in the subsequent lactation. Cows with PVD were 4.9 times more likely not to conceive, 2.7 times more likely to require multiple services to conceive, 2.1 times more likely to remain not pregnant by 100 d postpartum, and 4.4 times more likely to remain not pregnant by 150 d postpartum. The calving to conception period (CCP) was also significantly longer in cows with PVD than their healthy counterparts (115.9 ± 4.9 and 104 ± 7.4 d, respectively). In conclusion, PVD significantly increased the CCP in all cows, but to a greater extent in cows with a high milk yield in the lactation before disease diagnosis.
- A comprehensive panel of immunological, metabolic and microbiological markers were profiled both in the blood (systemically) and in the uterus (locally) of cows with and without disease. The most sensitive biomarkers were found to be those that measured local changes in cows with PVD. We developed a novel assay to measure inflammatory markers in the uterine fluid of cows. We now have a panel of informative markers that predict subsequent disease occurrence and these are currently under assessment for commercialization.
- It is now clear that clinical disease has a negative impact on fertility but we also used proteomic analysis to identify proteins that contribute to the development of sub-clinical disease, known as cytological endometritis.

5. Opportunity/Benefit:

This multidisciplinary project has provided a true systems level insight into the development of uterine disease and shown that early diagnosis fi this disease is possible and feasible in a non-invasive manner.

6. Dissemination:

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Main publications:

- Adnane M, Kelly P, Chapwanya A, Meade KG, O'Farrelly C. Improved detection of biomarkers in cervico-vaginal mucus (CVM) from postpartum cattle. BMC Vet Res. 2018 Sep 29;14(1):297. doi: 10.1186/s12917-018-1619-5. PMID: 30268128
- Kelly P, Meade KG, O'Farrelly C. Non-canonical Inflammasome-Mediated IL-1β Production by Primary Endometrial Epithelial and Stromal Fibroblast Cells Is NLRP3 and Caspase-4 Dependent. Front Immunol. 2019 Feb 5;10:102. doi: 10.3389/fimmu.2019.00102. eCollection 2019. PMID: 30804935
- Ryan NJ, Meade KG, Williams EJ, O'Farrelly C, Grant J, Evans ACO, Beltman ME. Purulent vaginal discharge diagnosed in pasture-based Holstein-Friesian cows at 21 days postpartum is influenced by previous lactation milk yield and results in diminished fertility. J Dairy Sci. 2020 Jan;103(1):666-675. doi: 10.3168/jds.2019-17116. Epub 2019 Nov 14. PMID: 31733846

Presentations:

Research was presented at multiple national and international conferences including: Association of Veterinary Teaching and Research Work (AVTRW; 2016, 2017), British Society for Animal Science Conference (BSAS; 2018, 2019), Endometritis (2017), European Society for Domestic Animal Reproduction (ESDAR; 2016, 2018), Irish Society of Immunology (ISI, 2018) and various local research seminars.

7. Compiled by: Dr Kieran Meade

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