

Welcome to



RESEARCH
ADVANCED BREEDING
FOOD FUTURES
TRAINING



The 75th EAAP Annual Meeting

1/5 September 2024 - Florence, Italy



SemenRate® Canada/UK: Transforming Germplasm and Genetic Quality to Drive Livestock Productivity

EAAP Florence, Italy – 2nd September 2024

‘Seminal Cytokine Profiles as Markers of Bovine Semen Quality’

Jonathan.M.E. Statham, N. Gahir, K. Burton, C. Freer, G. Mappa, C. Smalley, A. Lancaster, N. Orsi



UK-Canada: enhancing
agricultural productivity and
sustainability
Application 60115



UK-Canada Study:

“Seminal cytokine profiles as markers of bovine sperm quality”

This study aimed to:

1. determine if variations in multimodal spermatozoa (spz) quality parameters were associated with seminal plasma cytokine concentrations.
2. investigate semen cytokine interactions
3. investigate cytokine associations with field breeding outcomes



RAFT, BVG, HKVS & InSHAW

Research

Advanced breeding

Food futures

Training

“Innovation in Sustainable Livestock Food”

RAFT established in October 2010

Developed by Bishopton Vet Group



**Innovation in
sustainable
livestock food
production**



RESEARCH



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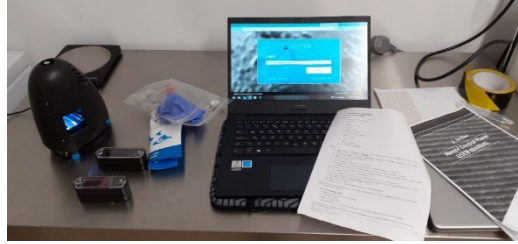


TRAINING

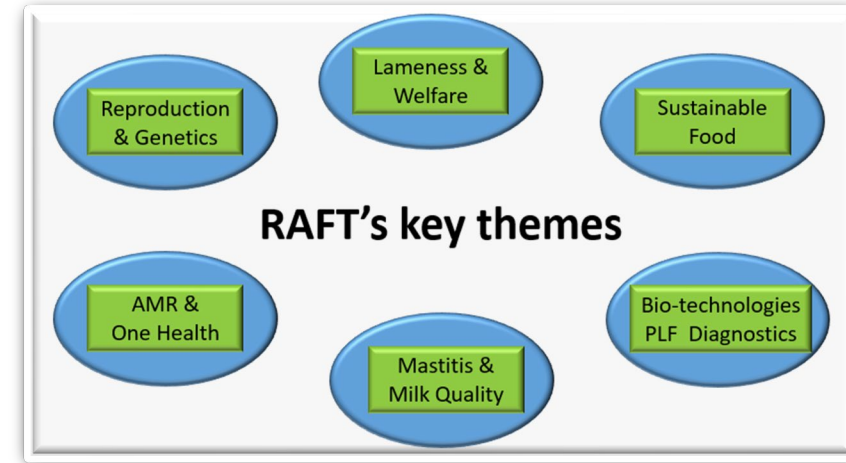
Innovation in Sustainable Livestock Production

Innovation pipeline incubator ➡ Market entry exploitation “spin-out” generator

Research

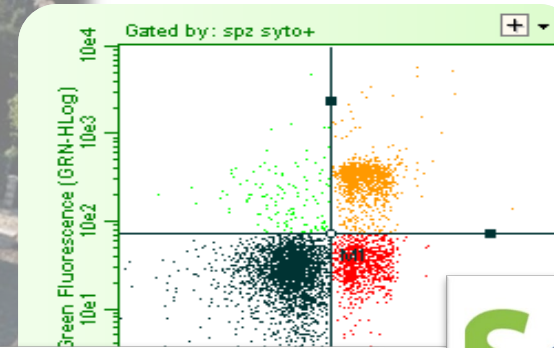
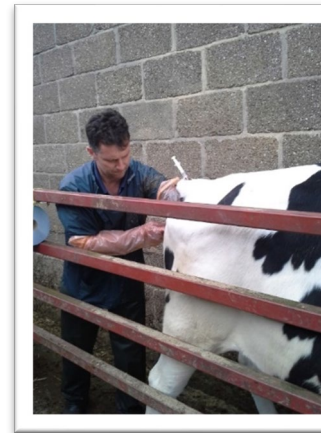


- We deliver:
 - UKRI, IUK BBSRC, NRC IRAP & Horizon Europe
 - Clinical field trials to GCP standards,
 - Collaborative translation of technologies into Ag-tech to deliver appropriate solutions to vets, farmers and industry,
 - Dedicated and experienced project management team including Recognised PRINCE2 Practitioners.
- Joint service with Fera Science 'Lab to Livestock', VetDx and InSHAW
- Developing publications and linking to our advanced breeding, consultancy and training divisions to facilitate application in the field



Advanced Breeding

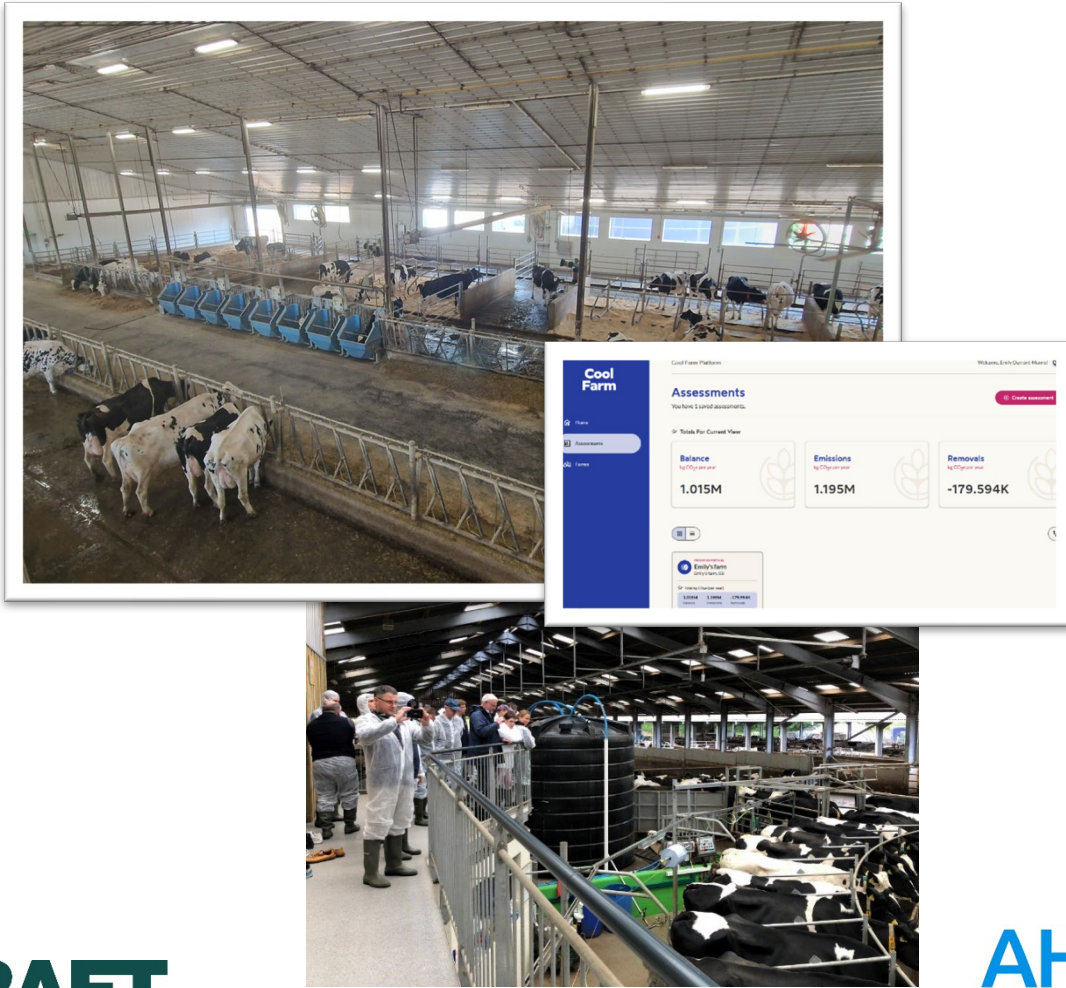
- Semen quality analysis 'SemenRate' *an independent lab service*
- Fertility assessment
- Embryo transfer
- Ovum Pick Up/In-Vitro Fertilisation
- Bull semen collection
- Residential centre
- Genomics



SemenRate



Food Futures

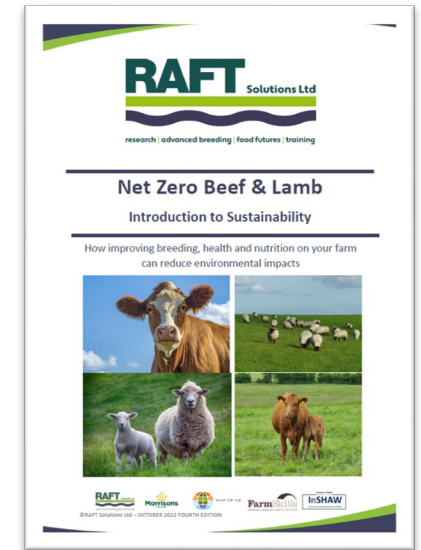


- Bespoke consultancy programmes for processors, retailers, farmers, Government and others
- Linking livestock health, nutrition and fertility with resource use efficiency to understand the foundations to sustainable livestock production
- Management of industry wide livestock sustainability, health and disease control programmes – The National Johne's Management Plan and AHDB Health Schemes, BVD Free ...

Training



- **Practical, small group, veterinary-led training** in key livestock management delivered via VetSkills and FarmSkills brands
- Knowledge exchange to support exploitation/commercialisation of solutions
- Sustainability in Livestock Production
- Precision Livestock Farming – adding value ‘in the field’
- OneHealth
- InSHAW



Networks



RAFT Solutions Ltd

research | advanced breeding | food futures | training



UK, Ireland,
Canada,
New Zealand



Harper & Keele

VETERINARY SCHOOL

InSHAW

Institute for sustainable livestock health and welfare

ision Our Approach Key Areas Get Involved

Institute for Sustainable livestock Health and Welfare

IN ASSOCIATION WITH



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VETERINARY SCHOOL



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MISSION STATEMENT

We aim to promote and develop sustainable livestock health and welfare and the central role of vets in delivering these goals to promote a balance across four Key Areas:



Food
Security



Animal Health
and Welfare



Environmental
Management

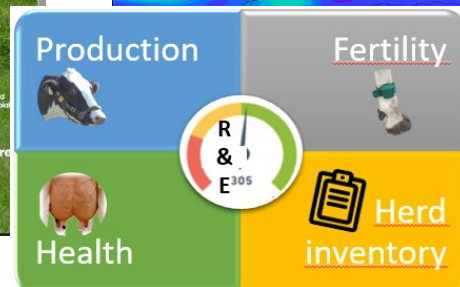
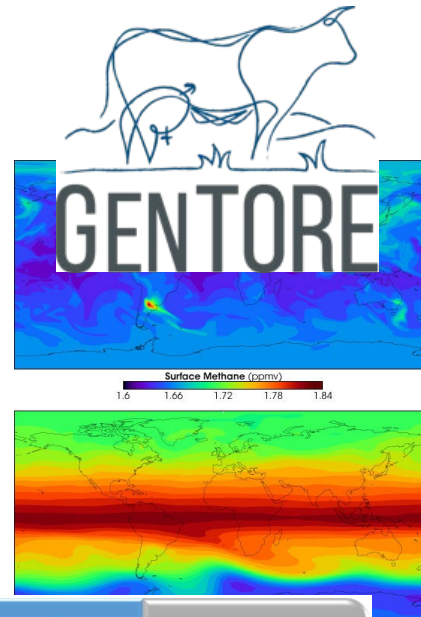


One Health and
Antimicrobial
Resistance (AMR)

Global Organisations & Networks



EAAP Porto 2022-Reproductive PLF strategies for Resilience & Efficiency ...and sustainable food...



“Dairy herd reproduction management strategies for improved efficiency”

J.M.E. Statham, M.W.Spilman & K.L. Burton



73rd ANNUAL MEETING OF THE EUROPEAN FEDERATION OF ANIMAL SCIENCE

THE COEXISTENCE OF WILDLIFE AND LIVESTOCK

PORTO – PORTUGAL

4 SEPTEMBER – 9 SEPTEMBER 2022



research | advanced breeding | food futures | training



This project has received funding from the European Union's Horizon 2020 research and innovation program under Grant Agreement No 727213



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“Dairy herd reproduction management strategies for improved efficiency”

Goals?

- Efficiency & Resilience for:
 - Food security?
 - GHG mitigation..sustainability?
 - Genetic progress?
- *Definitions:*

Reproductive Efficiency (Pregnancy rate) = (CR x SR)

CR=Conception Risk – more difficult to improve

SR=Submission Rate – easier to change...

EFFICIENCY RANKING

= $\frac{\text{dry matter intake (DMI, kg/lactation)}}{\text{milk yield output (TMY, kg/lactation)}}$



Sustainable breeding project 2021-2024

*UK-Canada: enhancing
agricultural productivity and
sustainability*
Application 60115

- Jointly funded project – UK and Canadian partners
- Allowed for mirrored applications of technologies in both countries
- Multiple work packages
 - Semen Analysis
 - Germplasm transportation
 - Semen quality markers
- Combination of UK and Canadian based companies led by RAFT Solutions

UKRI funds for dairy and beef fertility research

UK Research and Innovation (UKRI) has awarded £400,000 for research into higher cattle fertility and breeding productivity in UK and Canadian dairy and beef herds. The project will be delivered by an agritech and biotech consortium led by Ripon-based **RAFT Solutions**.

The project aims to reduce wasted genetic potential, while improving sustainability and cattle farm financial results. It will “transform genetic progress, through the adoption of precision technologies, diagnostics, advanced breeding and big data, leading to more sustainable livestock production and export opportunities”, says RAFT’s Jonathan Statham.

This transatlantic initiative has parallel financial support from Canadian government funding body IRAP for a Canadian partner, Bow Valley Genetics. It also involves XL Vets practices in UK and Canada, and the Universities of Guelph and Saskatchewan.

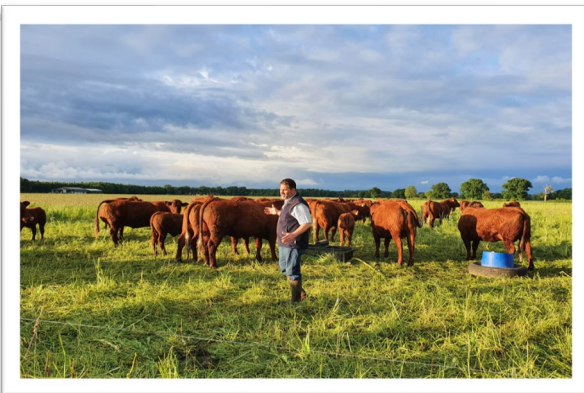
In practical terms, the project’s aims include higher conception rates and thereby fewer breeding cycles, says Mr Statham. He says this will come about through screening for higher quality semen and embryos, thereby delivering the promise of genomics by producing more calves that join the herd with better health and fertility.

The funding is awarded through UKRI’s ‘UK-Canada: enhancing agricultural productivity and sustainability competition’. The competition format brought together UK and Canadian companies, through on-line and in-person events, to identify and build project concepts in sustainable

agriculture. RAFT Solutions’ UK project partners are Atelerix (stem cell



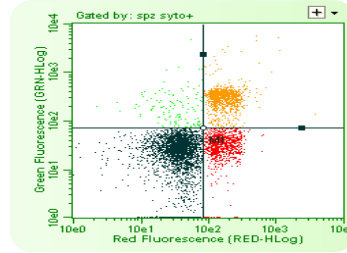
will receive funding support through the National Research Council of Canada Industrial Research Assistance Program (NRC IRAP).



I. Semen Analysis



- Semen quality analysis 'SemenRate' *an independent lab service*
- Fertility assessment
- Embryo transfer
- Ovum Pick Up/In-Vitro Fertilisation
- Bull semen collection
- *Survey cytokines from >90 bulls*



SemenRate

SemenRate

Lab ID: 2024173 Batch: 09 154 Straw size: 0.25 Report Status: Final Location: Low		
1. Normal Morphology [N] The shape of the spermatozoa. A minimum of 70% normal morphology is acceptable.	This Test Result: 76.00%	
2. Motile Initial [N] The percentage of motile spermatozoa in this sample immediately after thawing.	This Test Result: 50.10%	
3. Motile Stress [N] The percentage of motile sperm in this sample after a 2 hour thermoresistance (stress) test.	This Test Result: 40.20%	
4. Progressive Initial [N] The % of progressively motile sperm within sample immediately after thawing.	This Test Result: 34.60%	
5. Progressive Stress [N] The percentage of sperm moving quickly and in a straight line after a 2 hour thermoresistance (stress) test.	This Test Result: 26.00%	
6. Initial Motile Sperm Dose (million / straw) The number of motile spermatozoa in the straw.	This Test Result: 16.16	
7. Straight Line Velocity (µm/sec) Average straight line speed between start and finish of sperm track measured by CASA.	This Test Result: 80.44	
8. Polarised Mitochondria [N] Percentage of spermatozoa containing mitochondria capable of producing energy for motility.	This Test Result: 2.02%	
9. Viable & Intact Acrosome [N] Percentage of sperm that are viable & have an intact acrosome capable of fertilisation.	This Test Result: 39.32%	





Bovine semen quality control in artificial insemination centers

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¹L'Alliance Boviteq, Saint-Hyacinthe, Quebec, Canada.

²The Semex Alliance, Guelph, Ontario, Canada.

Abstract

Quality control (QC) is a fundamental area of management for semen production centers (SPCs) supplying bovine semen to breeders and producers. Semen production centers are moving away from subjective semen assessment, that is largely uncorrelated to field fertility, to objective semen analyses that incorporate computer assisted sperm analysis (CASA) and flow cytometry. A multiparametric approach to semen analysis using a combination of CASA and flow cytometry can provide SPCs with the highest QC for all semen production. In this paper we review probes used for labelling spermatozoa for viability, acrosomal integrity, mitochondrial activity, DNA integrity and calcium release. Limitations of CASA and flow cytometry when analyzing spermatozoa, especially frozen-thawed samples, are discussed. Finally, we described how a multiparametric approach using CASA and flow cytometry could be applied in SPCs to establish QC of production before the release of the product in the field.

Keywords: bovine, CASA, fertility, flow cytometry, quality control.

Introduction

Fertility is a multiparametric phenomenon that relies on the use of semen of sufficient quality and quantity, accurate timing and method of insemination,

post-thaw quality control procedures undertaken by SPCs prior to distribution. QC is the assurance that each batch of straws has undergone semen analysis to verify that the sample is likely to be fertile.

Although semen analysis may seem easy to perform, meticulous attention to detail and technique is essential in order to obtain an accurate and reproducible analysis. Manual semen analysis using a light microscope has been the standard method for analysis in most SPCs. However, manual analyses can be very subjective and prone to within and between technician errors. Similarly, the use of fluorescence microscopy to assess spermatozoa for acrosome, membrane and DNA integrity is markedly slow and limited due to the low number of spermatozoa analyzed from each sample and the incapacity for an extensive multiparametric analysis.

To maximise accuracy in QC, SPCs are realizing the benefit of a multiparametric approach and have increased the rigor of their semen testing, moving from time-consuming basic subjective assessment of a few hundred spermatozoa for concentration, motility and morphology using microscopy, to the use of computer-assisted tracking to assess motility, and flow cytometry to analyse thousands of cells within seconds for characteristics such as viability, mitochondrial activity, acrosome, DNA and capacitation status. The topics for discussion within this review are the various tools and assays in use in cattle SPCs to determine QC values, factors to consider when using these tools and

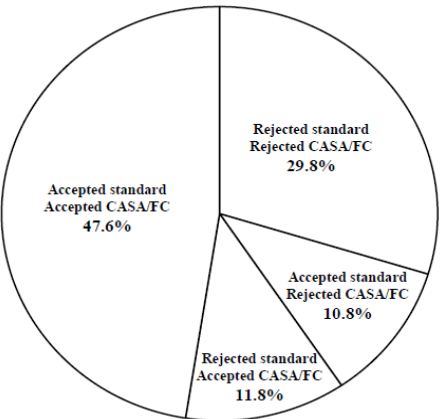


Figure 3. Comparative analysis of pass/fail percentage between standard QC and CASA/flow cytometry during quality control of 660 frozen-thawed semen lots.

Table 1. CASA/flow cytometry cutoff thawed semen immediately after thaw; these cutoffs values passed the evaluation.

Parameter	Post thaw	After 2 h stress
Total motility (%)	40	35
Progressive motility (%)	15	10
Intact acrosome (%)	66	61
Membrane intact cells (%)	40	40
Mitochondrial activity (%)	40	45

of frozen-exceeding

Sellem et al 2015

Table 3
Simple correlations (Spearman, r) between cryopreserved bovine semen quality parameters and fertility value ($n = 114$ ejaculates, $P < 0.05$).

Analyses	Technology	Condition	Parameters	r	r^2
Oxidation	Flow cytometry	Ratio: 40m/10m	Viable oxidized, %	0.494	0.244
Sperm motility (speed)	CASA	T0	VSL, $\mu\text{m/s}$	-0.340	0.116
Acrosome/viability	Flow cytometry	Delta (T4-T0)	Viable with disrupted acrosome, %	0.322	0.104
Sperm morphologic abnormalities	CASA	T0	DMR, %	-0.306	0.094
DNA compaction	Flow cytometry	T4	Sperm with fragmented DNA, %	-0.287	0.082
Mitochondrial activity	Flow cytometry	T0	Polarized mitochondria sperm, %	0.271	0.073

Table 4
Prediction models of the fertility value for the two set of ejaculates.

Models	Technologies	Protocols	r^2_{adj} ($n = 114$)	r^2_{adj} ($n = 39$)
Complete model	Flow cytometry and CASA	Oxidation, acrosomal integrity, DNA compaction, mitochondrial activity, viability, velocity, sperm morphologic abnormalities	0.40	0.39
Simplified model	Flow cytometry and CASA	Oxidation, DNA compaction, velocity, sperm morphologic abnormalities	0.34	0.37
Flow cytometry model	Flow cytometry	Oxidation, acrosomal integrity, DNA compaction, mitochondrial activity	0.33	0.22
CASA model	CASA	Sperm morphologic abnormalities, velocity	0.24	0.23



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Review

Use of combinations of *in vitro* quality assessments to predict fertility of bovine semen

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ABSTRACT

Predicting *in vivo* fertility of bull ejaculates using *in vitro*-assessed semen quality criteria remains challenging for the breeding industry. New technologies such as computer-assisted semen analysis (CASA) and flow cytometry may provide accurate and objective methods to improve semen quality control. The aim of this study was to evaluate the relationship between semen quality parameters and field fertility of bull ejaculates. A total of 153 ejaculates from 19 Holstein bulls have been analyzed using CASA (post-thawing semen motility and morphology) and several flow cytometric tests, including sperm DNA integrity, viability (estimated by membrane integrity), acrosomal integrity, mitochondrial aerobic functionality and oxidation. Samples were analyzed both immediately after thawing and after 4 hours at 37°C. A fertility value (FV), based on nonreturn rate at 56 days after insemination and adjusted for environment factors, was calculated for each ejaculate. Simple and multiple regressions have been used to correlate FV with CASA and flow cytometric parameters. Significant simple correlations have been observed between some parameters and FV (e.g., straight line velocity [$\mu\text{m/s}$], $r^2 = -0.12$; polarized mitochondria sperm [X], $r^2 = 0.07$), but the relation between simple parameter and FV was too weak to predict the fertility. Partial least square procedure identified several mathematical models combining flow cytometer and CASA variables and had better correlations with FV (adjusted r^2 ranging between 0.24 and 0.40 [$P < 0.0001$], depending on the number of included variables). In conclusion, this study suggests that quality assessment of thawed bull sperm using CASA and flow cytometry may provide a reasonable prediction of bovine semen fertility. Additional work will be required to increase the prediction reliability and promote this technology in routine artificial insemination laboratory practice.

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Introduction

In vitro fertility is a multifactorial process, relying on sperm quality, female fertility, appropriate herd management and accurate timing when using artificial insemination (AI). Female fertility is now included in breeding goals in many countries, leading to efficient fertility improvement. In contrast, bull fertility has received much less consideration. Nevertheless, a significant percentage of reproductive failures in dairy cattle has been attributed to bull subfertility [1], and subfertile bulls can lead to significant financial loss. Furthermore, semen quality may vary along the bull career, and even fertile bulls may produce ejaculates with poor fertilizing capacities, depending on their age or environment [2]. Because bull fertility cannot be assumed, it must be monitored by regular examination of breeding records and assessment of semen quality.

Ensuring an optimal quality of semen straws is thus a key concern for AI centers. To ensure optimal fertility after

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E-mail address: elise.sellem@rafft.com (E. Sellem).

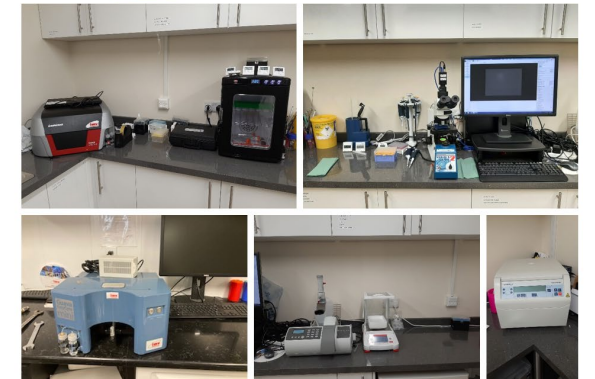
Bow Valley Genetics

Advanced Breeding company based in Alberta & Saskatchewan, Canada

- Established in 2009
- SemenRate™ Laboratory in Western Canada
- IVF laboratory
- Semen production stud
- Team of 19
- Bow Valley Genetics now fully equipped with flow cytometry and a calibrated CASA procedure to the equipment used for SemenRate®
 - SemenRate™ deployed with BVGL.
- Collaboration between BVGL and RAFT
- WCVL & OVC members supporting

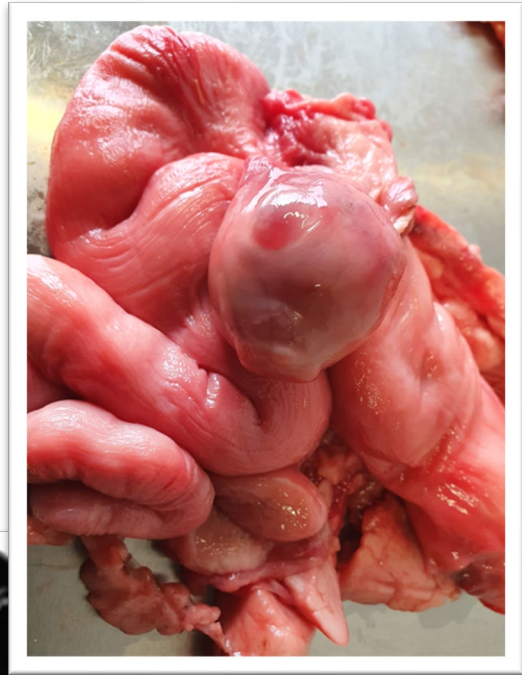


The UK-
Canada
SemenRate
Laboratories
& Data Hubs



II. Immuno-priming cytokines and AI Semen Processing

- Cytokines orchestrate pathophysiological inflammation and priming of the cervix/uterus for embryo implantation.
- Artificial insemination (AI) is one of the key, farm-based reproductive interventions in assisted reproduction technologies.
- Bovine Germplasm Collection - well established
- Routine semen preparation techniques from bull ejaculates involve washing and diluting sperm to target concentrations prior to their cryostorage;
- ***Semen processing consequently can result in seminal fluid immunomodulatory moieties such as cytokines/chemokines being removed.***



Seminal cytokine networks

- **Seminal plasma** governs the development of maternal reproductive tract immunomodulation essential for the establishment of pregnancy and maternal tolerance of the foetal allograft
- immunomodulatory moieties such as **cytokines**, steroid binding proteins and prostaglandins result in the relocation of immune effector cells to implantation sites and other mucosal surfaces
- **genital tract immune defences are inhibited**, resulting in reduced cell-mediated responses and immunosurveillance
- individual cytokines studied- but **cytokines operate as networks**, exhibiting synergy, antagonism and functional redundancy –we consider the putative interactions with other mediators in governing their own concentrations
- What is the extent to which these **seminal cytokines profiles are conserved across species??**

[Johnson et al 2017]



RESEARCH ARTICLE

A Bayesian view of murine seminal cytokine networks

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¹ Department of Pathology and Tumour Biology, Leeds Institute of Cancer and Pathology, The University of Leeds, Leeds, United Kingdom, ² Sierker, Brookline, Massachusetts, United States of America, ³ Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, United States of America, ⁴ Ostara Biomedical Ltd, Liverpool, United Kingdom

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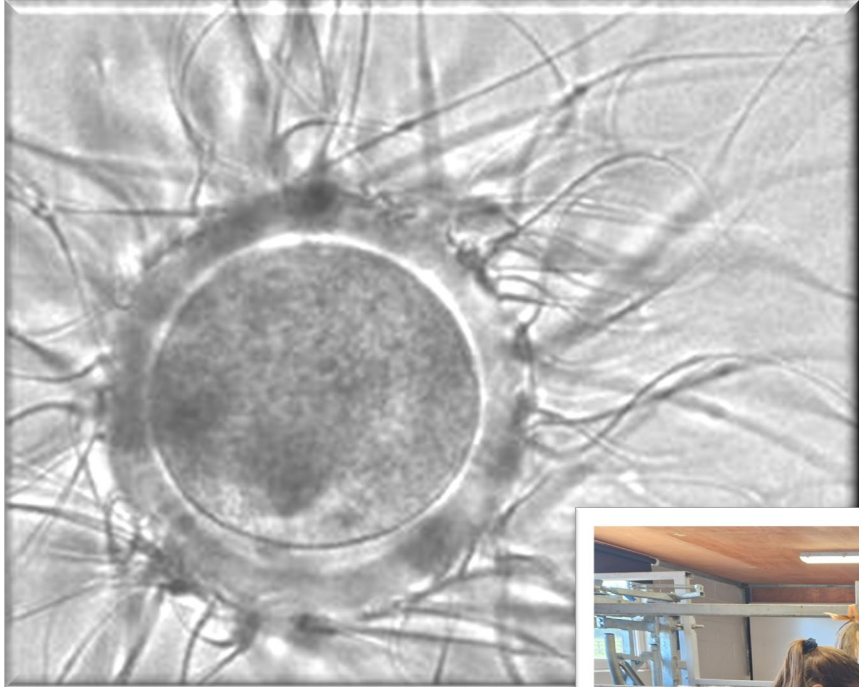
Abstract

It has long been established that active agents in seminal fluid are key to initiating and coordinating mating-induced immunomodulation. This is in part governed by the actions of a network of cytokine interactions which, to date, remain largely undefined, and whose inter-specific evolutionary conservation is unknown. This study applied Bayesian methods to illustrate the interrelationships between seminal profiles of interleukin (IL)-1alpha, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p70), IL-13, IL-17, eotaxin, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)-gamma, keratinocyte-derived chemokine (KC), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1) alpha, MIP-1beta, regulated on activation normal T cell expressed and secreted (RANTES), tumour necrosis factor (TNF)-alpha, leptin, inducible protein (IP)-10 and vascular endothelial growth factor (VEGF) in a rat model. IL-2, IL-9, IL-12 (p70), IL-13, IL-18, eotaxin, IFN-gamma, IP-10, KC, leptin, MCP-1, MIP-1alpha and TNF-alpha were significantly higher in serum, whilst IL-1beta, IL-5, IL-6, IL-10, IL-17, G-CSF and GM-CSF were significantly higher in seminal fluid. When compared to mouse profiles, only G-CSF was present at significantly higher levels in the seminal fluid in both species. Bayesian modelling highlighted key shared features across mouse and rat networks, namely TNF-alpha as the terminal node in both serum and seminal plasma, and MCP-1 as a central coordinator of seminal cytokine networks through the intermediary of KC and RANTES. These findings reveal a marked interspecific conservation of seminal cytokine networks.

Introduction

It is well established that seminal plasma governs the development of maternal reproductive tract immunomodulation essential for the establishment of pregnancy and maternal tolerance of the foetal allograft [1–5]. This process is driven by immunomodulatory moieties such as cytokines, steroid binding proteins and prostaglandins, which results in the relocation of

Seminal cytokines and fertilisation



The aim of this study was therefore to analyse bull semen from a range of breeds with a view to:

- (i) identify the presence and concentration of multiple seminal fluid cytokines,
- (ii) relate these profiles to lab semen quality parameters, and
- (iii) to identify which of these would be putative biomarkers of semen quality and key to uterine priming.

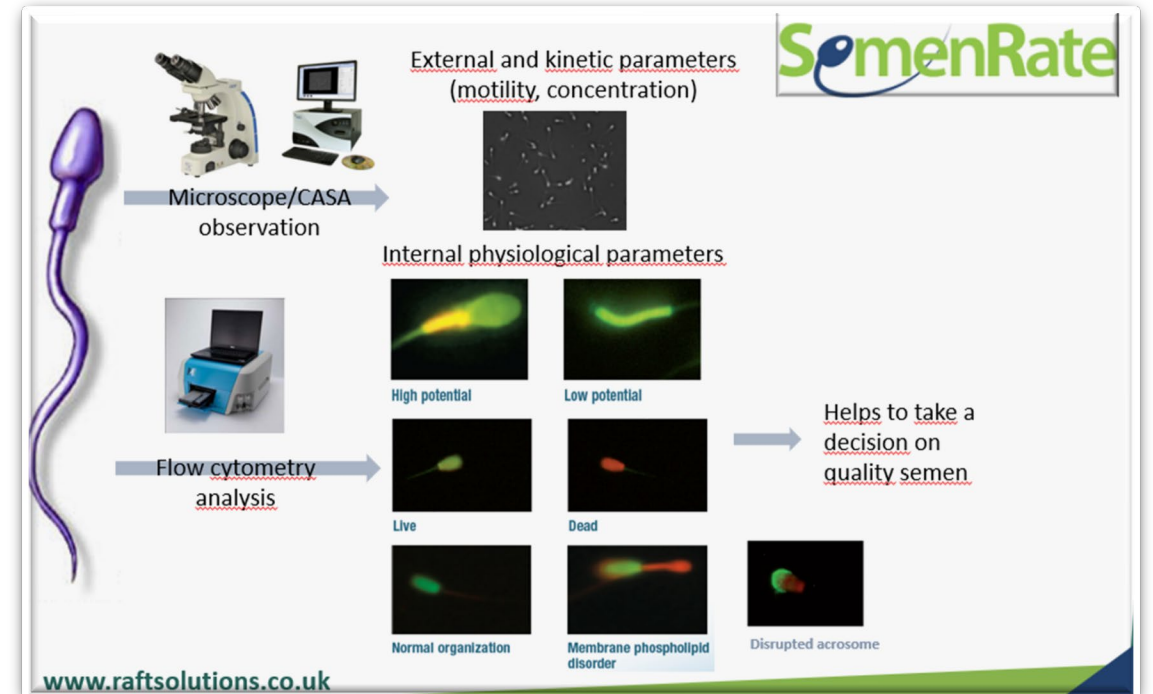


Study Method



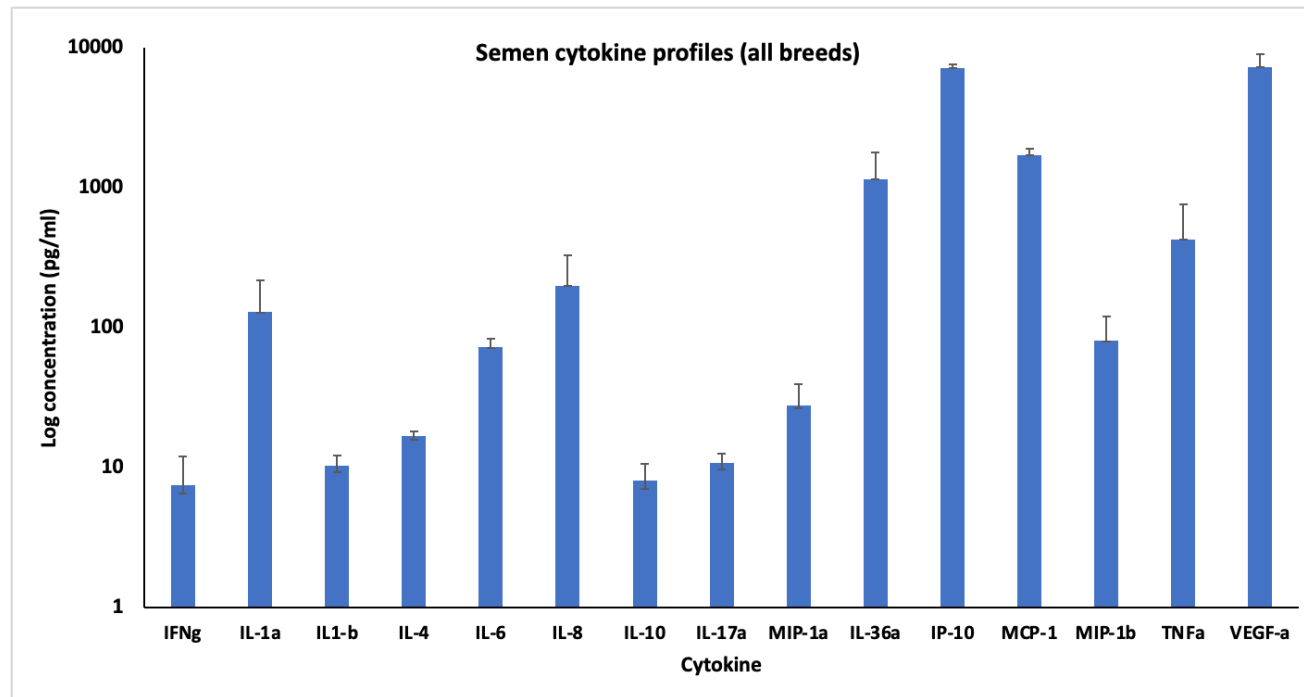
Individual bull semen ejaculates (c.90) were collected on-farm and split;

- (i) diluted 1:1 in PBS with 0.5% polyvinyl alcohol and protease inhibitor and snap frozen for cytokine analysis, and
- (ii) profiled for spz concentration, normal Semenrate[®] morphology, motility parameters, mitochondrial activity and acrosome integrity



Survey Results: immuno-priming cytokines

Measurable seminal plasma cytokine profiles identified



- Samples were analysed for interleukin (IL)-1 α , IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-17a, IL-36a, inflammatory protein (IP)-10, interferon-inducible T cell α chemoattractant (I-TAC), macrophage chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , MIP-1 β , thymus-expressed chemokine (TECK), tumour necrosis factor (TNF)- α and vascular endothelial growth factor (VEGF)-A
- a combination of enzyme-linked immunosorbent assay (IP-10, ITAC, TECK) and fluid-phase multiplex immunoassay (other analytes).
- All analytes were detectable except IL-4 and I-TAC

Analyse interactions between immuno-priming cytokines and SemenRate®

- Random forest-based analysis with 5-fold cross validation and feature ranking for each of the objective sperm parameters suggested that morphology and motility parameters from SemenRate® in particular could be identified using only 5 cytokines (features).
- Initial data showed positive correlations between a subset of cytokines and spz concentration, motility parameters and acrosome integrity. Statistically significant ($P<0.05$) negative correlations were noted between acrosome integrity and certain cytokines.

Sperm parameter	Normal Morphology
Mean CV accuracy	87.6
Mean CV F1	93.3
Top 5 features (Gini)	IL-36a
	MCP-1
	IL-6
	IFN- γ
	IL-17a
Top 5 features (permutation)	IL-36a
	IL-6
	IL-17a
	IL-10
	IFN- γ

Analyse *interactions* between immuno-priming cytokines

- It is well recognised that cytokines operate as part of complex biological networks characterised by synergy, antagonism and functional redundancy.
- Seminal plasma cytokine interactions were modelled *in silico* using Bayesian networks to identify - in conjunction with the random forest analysis output – the key drivers of these cytokine networks.
- *This approach will potentially enable a reduced agent formulation to use in AI treatment at the time of sperm delivery to replace missing immunomodulatory moieties.*

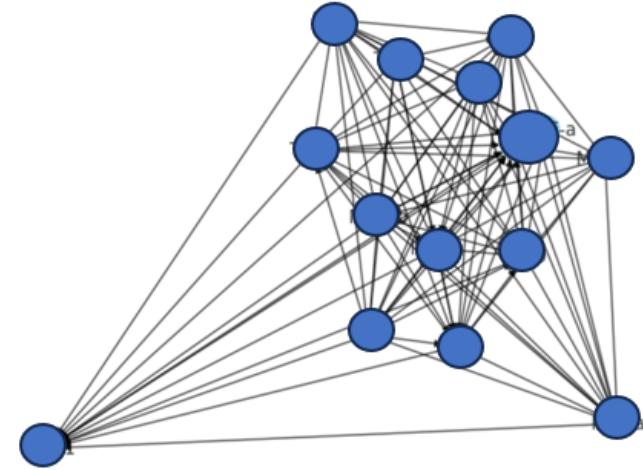
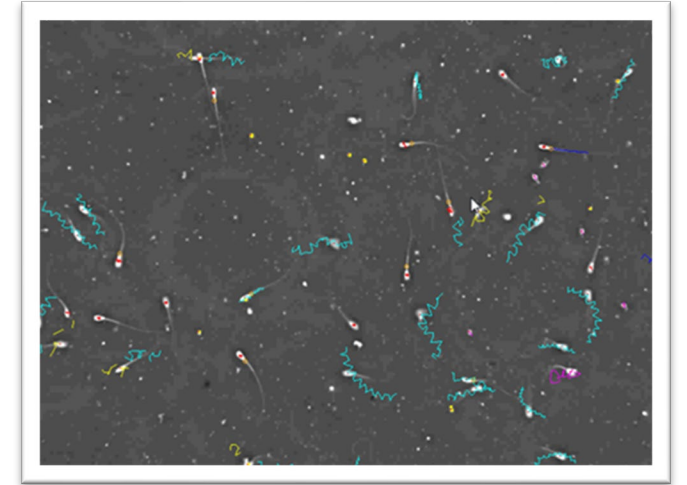


Figure 17: Bayesian network of target cytokine interactions

Bayesian network of target cytokine interactions

III. In-field validation – SemenRate® Outcomes Project

- The SemenRate “outcomes” project has been progressing and is gathering a substantial data set. The aim of this is to investigate the links between semen quality and real-world breeding and fertility outcomes.
- These data are divided into three areas:
 1. Individual Cow Data which has pregnancy diagnosis (PD) data
 2. Conception Risk data from the SemenRate analysis database against the conception rates recording on farms
 3. A national data set of all calf births (c.40% with sire data)



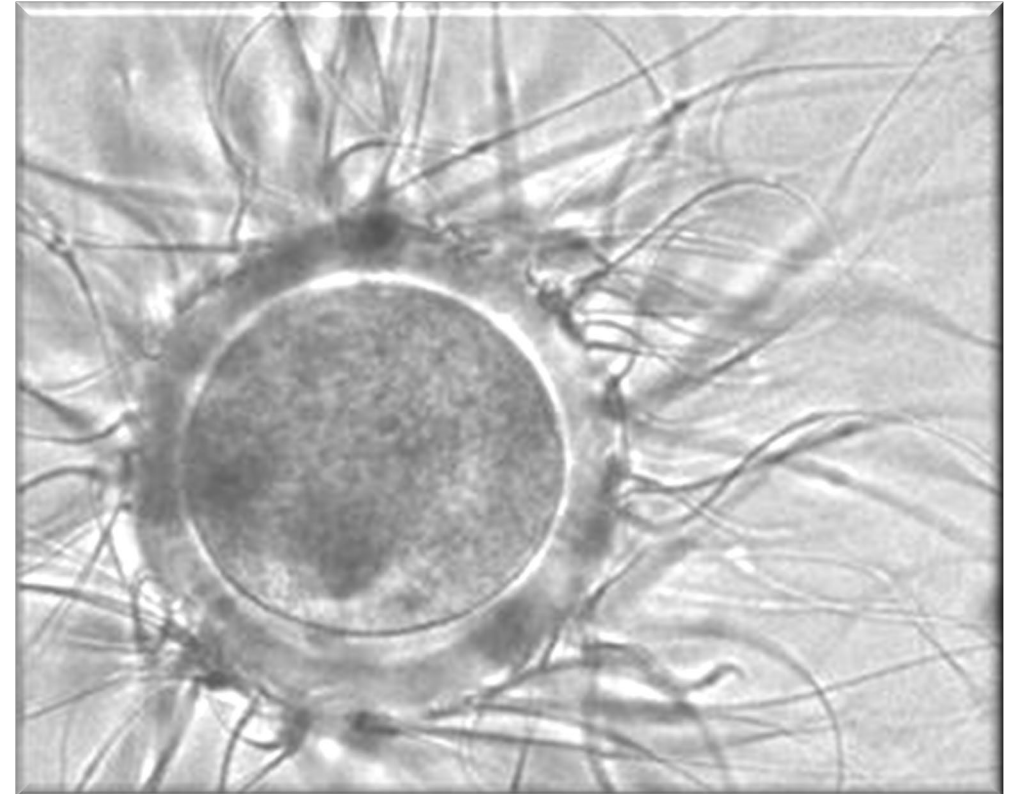
In-field validation –SemenRate® Outcomes Project

1. Individual Cow Data with proactive breeding pregnancy diagnosis (PD) data
 2. Conception Risk data from the SemenRate analysis database
-
- XLVet practices, subcontracted to the project, throughout England submitted c. 200 batches of commercial bull AI semen used for breeding cows and heifers on their dairy and beef herds
 - Working in communication with RAFT, the practices are providing PD breeding outcome results from their herds bred with the same batches of semen that have been analysed by RAFT in our lab using **SemenRate®**
 - The analysis of these results is providing an outcome study for these farms at this time and also adds to the existing outcomes data base.
 - *This work and analysis is ongoing in 2024.*



Conclusions and further work

- These preliminary findings suggest that seminal plasma cytokines show potential for use as surrogate indicators for conventional spz parameters and reflect the semen quality of bull ejaculates.
- Development of an additive/supplement including important cytokines for fertilisation offers a prospect for improved field fertilisation outcomes
- Further work is underway to investigate these laboratory and field fertility relationships in more detail in UK and Canada



Acknowledgements



- Jonathan Statham^{1,3}, Narinder Gahir², Katie Burton¹, Clare Freer², Georgia Mappa², Caitlin Smalley², Alister Lancaster¹, Nicolas Orsi²
- ¹RAFT Solutions, Ripon; ²University of Leeds, Leeds; ³InSHAW at Harper & Keele Veterinary School; UK

SemenRate Canada/UK: Transforming Germplasm and Genetic Quality to Drive Livestock Productivity

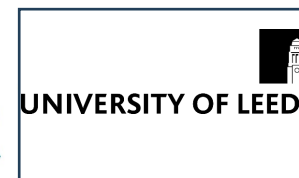
EAAP Florence – 2nd September 2024

‘Seminal Cytokine Profiles as Markers of Bovine Semen Quality’

J.M.E. Statham, N. Gahir, K. Burton, C. Freer, G. Mappa, C. Smalley, A. Lancaster, N. Orsi



*UK-Canada: enhancing
agricultural productivity and
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Application 60115*





**Thank you
Any questions?**

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