



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.
- investigate semen cytokine interactions
- 3. investigate cytokine associations with field breeding outcomes









RAFT, BVG, HKVS & InSHAW

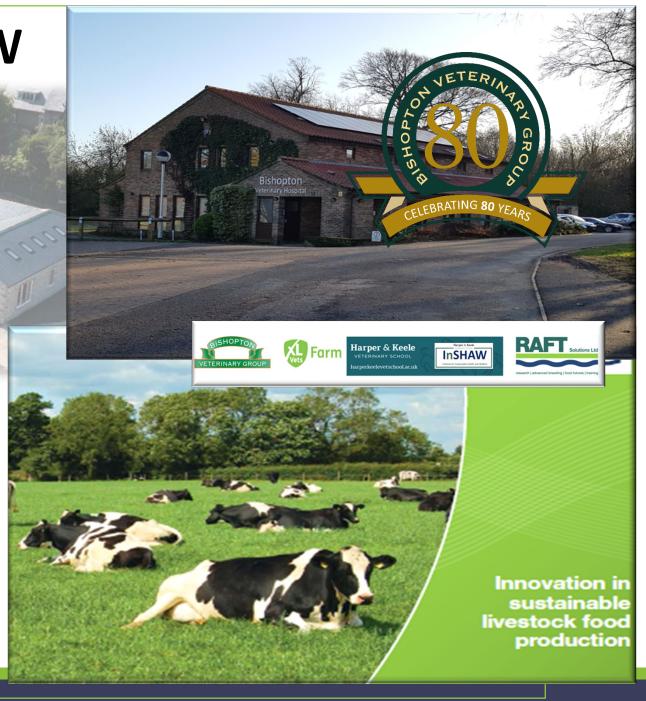
Research
Advanced breeding
Food futures
Training

"Innovation in Sustainable Livestock Food"

RAFT established in October 2010 Developed by Bishopton Vet Group



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RESEARCH

ADVANCED BREEDING FOOD FUTURES

TRAINING

Innovation in Sustainable Livestock Production

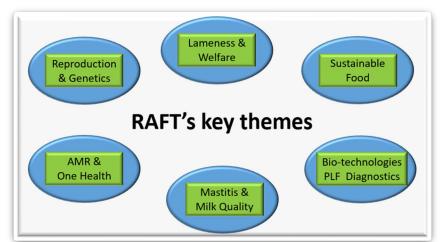
Innovation pipeline incubator Market entry exploitation "spin-out" generator



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 Practioners.
- 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













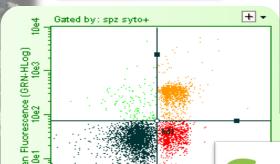
Advanced Breeding

 Semen quality analysis 'SemenRate' an independent lab service

- Fertility assessment
- Embryo transfer
- Ovum Pick Up/In-Vitro Fertilisation
- Bull semen collection
- Residential centre
- Genomics







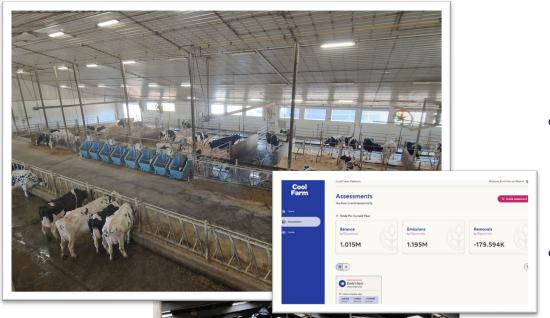








Food Futures



- 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

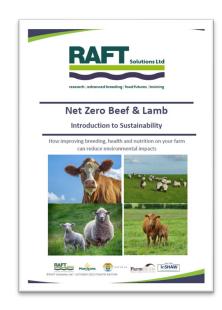




- **Practical, small group, veterinary-led training** in key livestock management delivered via VetSkills and FarmSkills brands
- Knowledge exchange to support exploitation/commercialisation of solutions
- Sustainability in Livestock Production
- Precision Livestock Farming adding value 'in the field'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











Networks

Farm







UK, Ireland, Canada, **New Zealand**





Farm Network and Operational Group



Harper & Keele

InSHAW







IN ASSOCIATION WITH



Harper & Keele
VETERINARY SCHOOL





IN ASSOCIATION WITH









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

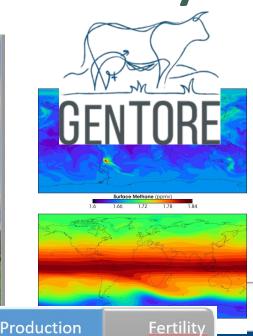






EAAP Porto 2022-Reproductive PLF strategies for Resilience & Efficiency ...and sustainable food...







"Dairy herd reproduction management strategies for improved efficiency"

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

7310 ANNUAL MEETING OF THE EUROPEAN FEDERATION OF ANIMAL SCIENCE

THE COEXISTENCE OF WILDLIFE AND LIVESTOCK

PORTO - PORTUGAL

4 SEPTEMBER - 9 SEPTEMBER 2022







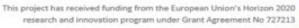
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inventory









"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

= dry matter intake (DMI, kg/lactation) 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"w, 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



National Research Council of Canada Industrial Research Assistance Program (NRC IRAP).







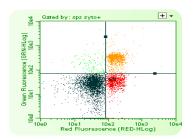






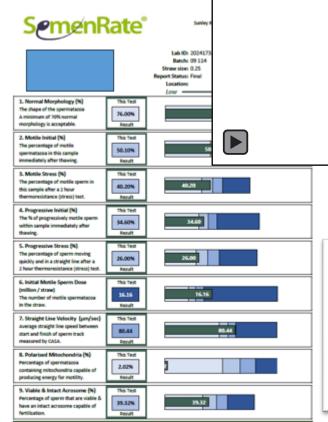


I. Semen Analysis



- 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



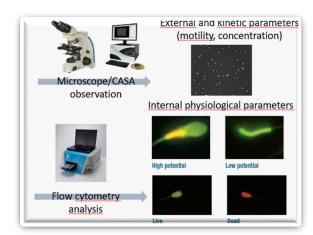








Predicting field outcomes from multimodal andrology with **SemenRate®**







qualified from the University of Cambridge in 1996. He is a cattle vet and partner at Bishopton Veterinary Group and CEO of RAFT Solutions, an innovative veterinary research and consultancy company. He holds the RCVS diploma in cattle health and production, is an RCVS-recognised Specialist in cattle health and production.



her MRes and PhD in at the University of Liverpool, following her undergraduate degree in bioveterinary science. She is the reproduction at RAFT Solutions



from the University of Liverpool in 2006. He is a partner at Bishopton Veterinary Group and also an advanced breeding director at RAFT Solutions, where he performs embryowork. bull breeding soundness assessments and semen collection and analysis.

Looking after the bull: guide to management and assessment of fertility

Jonathan Statham, Katie Burton, Mark Spilman

Despite commanding such an important role in both achieving reproductive success and introducing new genetics, the bull remains an often overlooked part of the cattle herd. Fertility performance remains central to profitable beef and dairy production. Although artificial insemination (AI) is used extensively in the UK dairy industry, natural service with 'sweeper bulls' is still common, either exclusively or as an adjunct to AI. The UK beef suckler industry remains dominated by natural service, although the use of AI is rising. Bull performance is highly significant in both sectors, but with very few suckler herds actually in profit (EBLEX 2009), the beef sector, in particular, can ill-afford poor reproductive results. This is of particular significance when considering the uncertainty in this sector associated with exit from the European Union (EU) and thus the Common Agricultural Policy (CAP). This article discusses the role of the bull and provides a guide to managing the different aspects of bull production, including assessing a bull's fertility.

How long is the average working life of a bull?

Of 18.658 beef bulls, born between January 2000 and December 2003, that had progeny assigned to them from the British Cattle Movement Service database, 20 per cent died on farm and approximately 80 per cent went to a slaughterhouse. Of the bulls given a date of death, the Bull selection average lifespan was 6.29 years; that is, a typical bull had a working life of just over four seasons. However, 23 per cent of bulls worked for two seasons or less and 34 per cent of bulls worked for three seasons or less, indicating that a large proportion of bulls did not reach their potential. Poor mobility was the main cause for culling (Laws

Calves produced per bull

The number of calves a bull produces in its lifetime largely determines the return on its purchase cost. The number of years worked and number of cows mated determines the number of calves produced in a bull's lifetime. Increasing the cow:bull ratio requires careful management and a physically fit and fertile bull; this approach may justify a greater level of investment in the genetic merit of a bull, but it should be remembered that increased returns may only be seen if the cows are also cycling normally, in good body condition and are free of disease.

health management sections:

- Bull selection and genetics, including use of estimated breeding values (FRVs).
- Management of nutrition, including rearing phases and
- Management of environment and routine tasks, including health and safety:

■ Fertility, including both semen evaluation and physical breeding capability.

Each of these five themes offers key opportunities for veterinary herd health input.

Bull selection represents a critical management decisions for both commercial and pedigree dairy and beef breeders. A bull's genetics will not only potentially influence the long-term composition of a breeding herd and beef and dairy production of the progeny it sires, but also its reproductive capability can impact the economic performance of the immediate breeding season.

Most pedigree breeding bulls are sold to commercial beef producers as terminal sires, rather than to other pedigree breeders. These commercial breeders represent the biggest target market; yet too often the best bull is not purchased for their business. A bull is a significant financial investment and needs to remain fit and fertile to pass on its genetic traits and produce healthy, viable calves.

A fertile bull has the ability to impregnate (by natural service) at least 90 per cent of 50 normal cycling disease-free females within nine weeks, achieving pregnancy in 65 per cent of cows within the first 21 days of the breeding period. Recent UK studies suggest that 20 to 30 per cent of fertil-Looking after the bull may be divided into five main herd ity tested bulls are subfertile (Eppink 2005, Walters and

Looking after the bull begins with initial selection. What Management of health and welfare, both single-agent is the appropriate specification of a bull for the needs of infectious diseases and multifactorial or management a particular herd? Over half of UK bull buyers now use EBVs as part of their bull selection process (Laws 2014). However, farmers at bull sales still tend to focus, inappropriately, on size or weight, which in turn encourages breeders to overfeed bulls to achieve bigger bulls at sale. But, an overfed bull may only last a few seasons and pro-

In Practice March 2019 | Volume 41 | 69-83 69



CASA and flow-cytometric parameters of semen for use in bovine in vitro embryo production (IVP)

PC Dodd12, KL Burton3, JME Statham3, D Tutt1 and KD Sinclain

Department 1: SemenRate Analysis Pre- and Post-Swim-Up

% pytilone head was corrected by swim-up in Money VSL increased more for Bold than Money

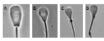
normal levels, except for DCDs. Size x Swim-up interaction for % puriform heads and VS

Swim-up improved all motility parameters (Pail 2002) and % pyriforn heads (P-6.001)



- abre embryo production; NP) for generation of progeny of high genetic value
- Combined computer-a existed semen analysis (CASA) and flow-oyeanetry (FACS) cost it sip screen semen for use in bovine firP 344.
 - Value for screening/selecting usprepared frozen/haved semen uncertain ciestial to ecreen prepared 5 e. post-swim up, density gradient) may be greate

- CASA/FACS assessment of post-but not pre-swim up sperm can be used to acree
- Optimising began concentration for individual sites and batches of seman within six



- Analyses of traces-thought bovine spermittages, both pre- and post-swim-up
- was undertaken according to ³. CASA assessed morphological and motility parameters.
- FACS assessed acrosome and mitochondrial physiology.

Experiment 2: Optimum Reparls Concentration for NT (3 replicates)

- irsee 1 and 2 LULUs greatly assistant to 10, as and 166 jg/mi septimen we since A and 8) compared.

 Suad Bay 2 disease check of aygotes.

 Classed (e=470) further cultured to Day 8.

 Unclassed ceytes (e=172) PV stalend to check for polyspermy.
- Mosphological stage(grade of Day 8 blastocysts (n=137) illustly assessed (ETS) IETS stage 6-6 blastocysts (n=123) stained with DAPI for stal-cell count. Sententiate data analysed by ANOVA.
- Proportions final yeard by analysis of deviance using GLM assuming binomial error









- Swimup did not improve the % DCDs. % morphologically normal and % viable with inter-
- VSL, DCDs and acrossome Vability could all be potential predictors of IVF success. Optimize Repails concentration is 50 µg/ml for both buils.
- Money favoured the highest 100 gird hepsels dose, but this led to decreased developme

- Equipment Market K. Walton K. W. Market J. (2011). "B Secretal Scient The gase of corruption

- Singer Tel, Tarri F, Marson B, Canadralin C S, Pierri F (1977). The construction of binetic and inches and comment of the control of the comment of the com University of Wilcomein (2012). Marghalogy of Buff Systematores. [Chilos]

- Further investigations into the effects of hepseln on polyspenn
- inclusion of morpholisesic (time-lapse) assessment

DEDCRICANK (DEROFFNEST) In vitre embryo production in enimal breeding. Enhancing ecoyae quality from per-pulseral stoners to promote biosecure and sustainable food

Vincent et al 2012

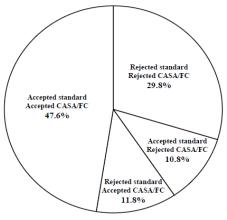


Table 1. CASA/flow cytometry cutoff thawed semen immediately after thawi these cutoffs values passed the evaluation.

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

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	



Bovine semen quality control in artificial insemination centers

P. Vincent¹, S.L. Underwood¹, C. Dolbec¹, N. Bouchard¹, T. Kroetsch², P. Blondin^{1,3}

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. multiparametric approach to semen analysis using a combination of CASA and flow cytometry can provide SPCs with the highest OC 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



Sellem et al 2015



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journal homepage: www.theriojournal.com



Review

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



E. Sellem 4.*, M.L.W.J. Broekhuijse b, L. Chevrier c, S. Camuglic, E. Schmitt c,

L, Schibler *, E.P.C, Koenen b

* RSO Department, ALUCE, Paris, France

ger, Digit, Prono

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	y 2015
Sperm motility (speed)	CASA	TO	VSL, μm/s	-0.340	0.116	
Acrosome/viability	Flow cytometry	Delta (T4-T0)	Viable with disrupted acrosome, %	0.322	0.104	
Sperm morphologic abnormalities	CASA	TO	DMR, %	-0.306	0.094	
DNA compaction	Flow cytometry	T4	Sperm with fragmented DNA, %	-0.287	0.082	decyrem
Mitochondrial activity	Flow cytometry	TO	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

Predicting it vivo fertility of bull ejaculates using it vitro-assessed semen quality criteria remains challenging for the breeding industry. New technologies such as computerassisted wmen 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 bulk have been analyzed using CASA (postthawing semen motility and morphology) and several flow cytometric tests, including sperm DNA integrity, viability (estimated by membrane integrity), acrosomal integrity, mitochondria aerobic functionality and exidation. 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 insemitation and adjusted for environment factors, was calculated for each ejaculate. Simple and multiple regressions have been used to correlate PV with CASA and flow cytometric parameters. Significant simple correlations have been observed between some parameters and FV (e.g., straight line velocity [µm/s], r² = -0.12; polarized mitochondria spenn (X), r2 = 0.07), but the relation between simple parameter and FV was too week to predict the fertility. Partial least square procedure identified several mathematical models combining flow cytometer and CASA variables and had better correlations with IV (adjusted r² 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 manunable prediction of bovine semen fertility. Additional work will be required to increase the prediction reliability and promote this technology in mutine artificial insemination laboratory practice.

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roduction

vine fertility is a multifactorial process, relying on quality, female fertility, appropriate herd mangeand accurate timing when using artificial insemina-NJ. Female fertility is now included in breeding goals ny countries, leading to efficient fertility improvement, in contrast, buil fertility has received much less

 Corresponding author, Tel: +33 134 6525 TJ, E-mod address: disselectfullicate (E Sellem). reproductive failures in dairy cattle has been attributed to bull subjectify [1], and subjectife bulls on had 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.

consideration. Nevertheless, a significant percentage of

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



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ABSTRACT

Bow Valley Genetics

Advanced Breeding company based in Alberta & Saskatchewan, Canada

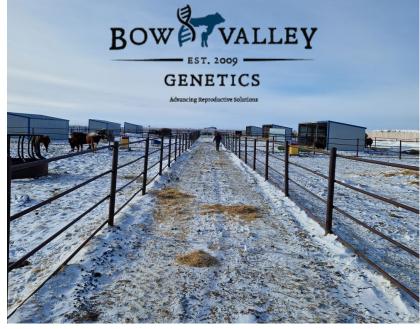
- Established in 2009
- SemenRate[™] Laboratory in Western Canada
- IVF laboratory
- Semen production stud
- Team of 19
- Bow Valley Genetics now fully equipped with flow cytometry and a calibrated CASA procedure to the equipment used for SemenRate®
 - SemenRate ™ deployed with BVGL.
- Collaboration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







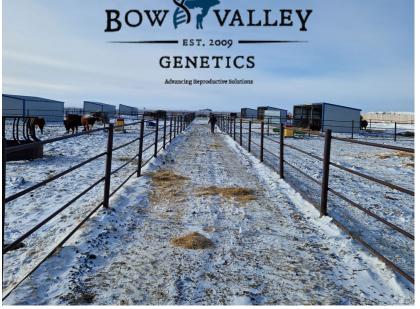




The UK-Canada SemenRate Laboratories & Data Hubs







II. Immuno-priming cytokines and Al 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]





RESEARCHARTICLE

A Bayesian view of murine seminal cytokine networks

Michelle L. Johnson¹, Tathagata Dasgupta^{2,3}, Nadia Gopichandran⁴, Sarah L. Field⁴, Nicolas M. Orsi¹*

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Data Availability Statement: All raw data can be found within the paper and in the Supporting Information files, sufficient to replicate the study findings.

Funding: In relation to our commercial afficiation with Ostara Biomedical and Sisker, we declare that neither of these bodies played a role in the study design, data collection and arralysis, decision to publish, or preparation of the manuscript and only provided financial support in the form of authors' salaries and/or research materials. All named authors contributed to both the work and the

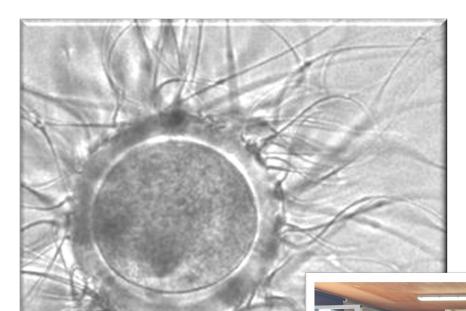
Abstract

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 interspecific 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

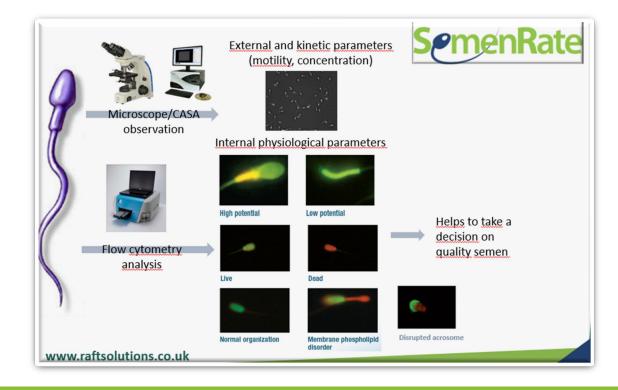


Individual bull semen ejaculates (c.90) were collected on-farm and split;

- (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

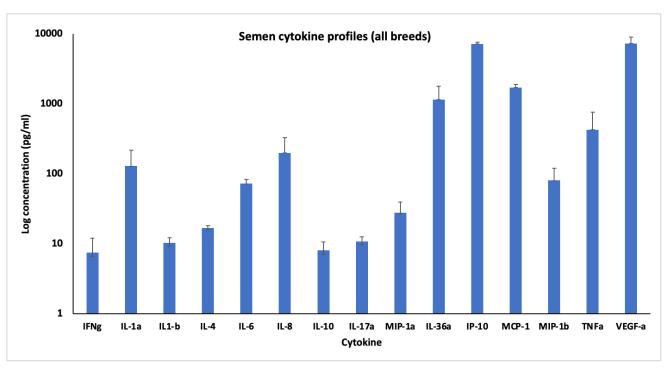






Survey Results: immuno-priming cytokines

Measurable seminal plasma cytokine profiles identified



- Samples were analysed for interleukin (IL)- 1α , IL- 1β , IL-4, IL-6, IL-8, IL-10, IL-17a, IL-36a, inflammatory protein (IP)-10, interferoninducible T cell α chemoattractant (I-TAC), macrophage chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)- 1α , MIP- 1β , 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 5fold 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.

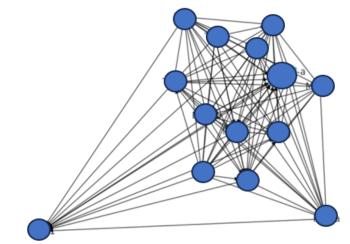


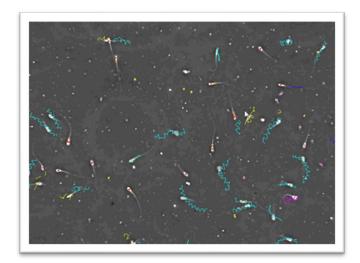
Figure 17: Bayesian network of target cytokine interactions

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:
 - 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

- 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.



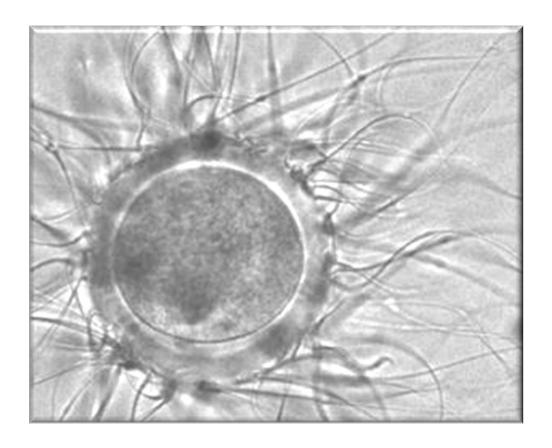






Conclusions and further work

- 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













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

EAAP Florence – 2nd September 2024

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UK-Canada: enhancing agricultural productivity and sustainability
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Thank you Any questions?

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