The 3rd CZU Prague hybrid seminar "Biotechnology in small ruminant reproduction: an international experience"

UNIVERSIDAD DE MURCIA

Molecular and biochemical factors contributing to ewe breed differences in cervical sperm transport

Dr. Laura Abril Parreño

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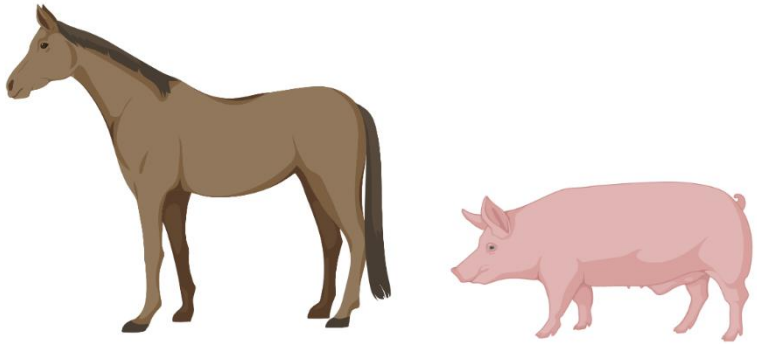
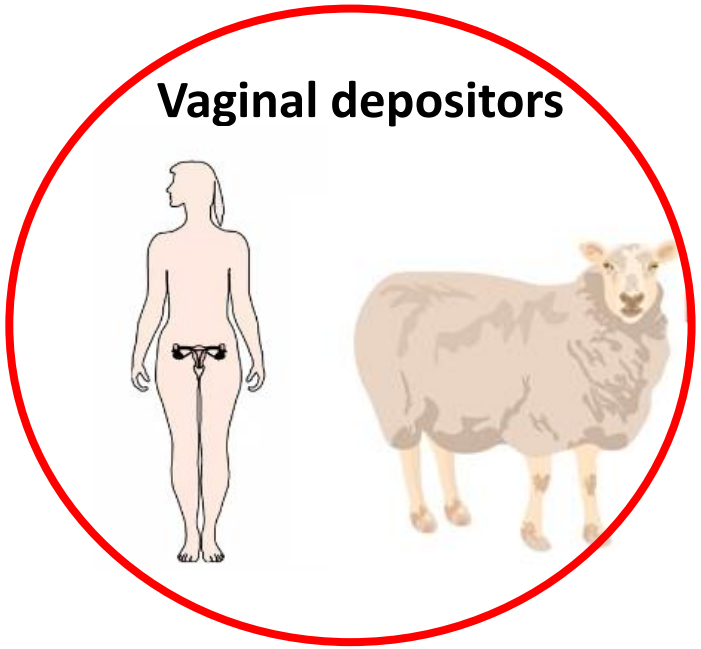
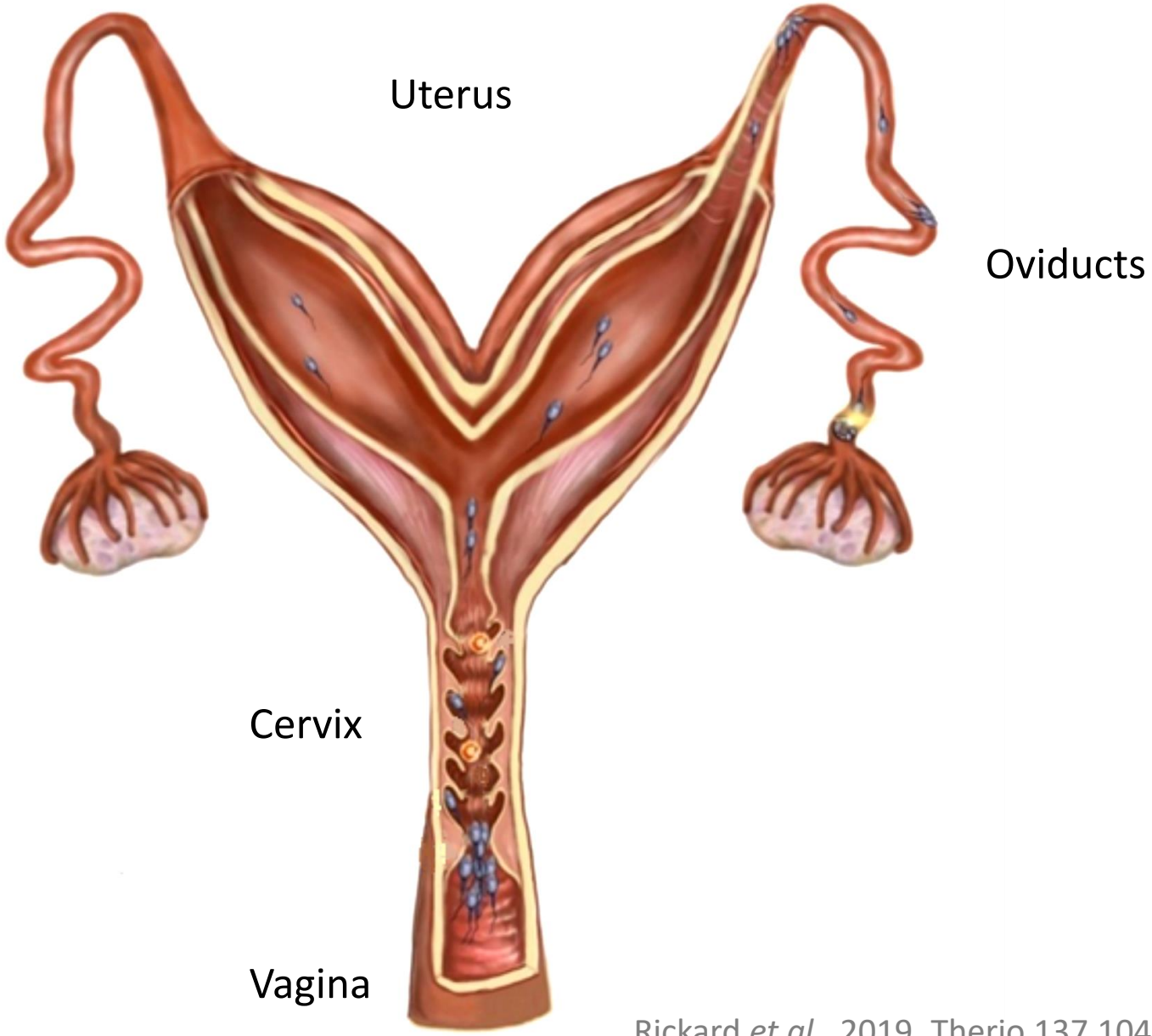
Semen deposition in the female reproductive tract

Uterine depositors



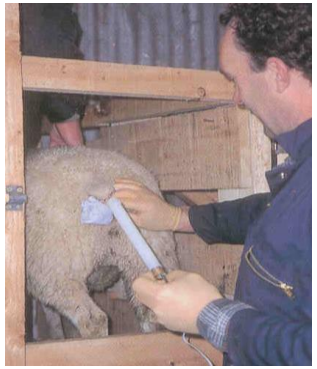
Vaginal depositors





Rickard *et al.*, 2019. Therio 137,104-137

Limitations of sheep artificial breeding



The problem!

Cervical Artificial Insemination using frozen-thawed semen → low pregnancy rates

Norway is the exception → ~60 -70% Pregnancy Rates with frozen-thawed semen by the farmers themselves

(Paulenz *et al.*, 2010, Paulenz *et al.*, 2007, Paulenz *et al.*, 2004)

SHOT-IN-THE-DARK AI

Norwegian White Sheep ~60 **%**

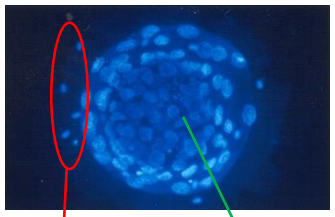
Why the success in Norway?

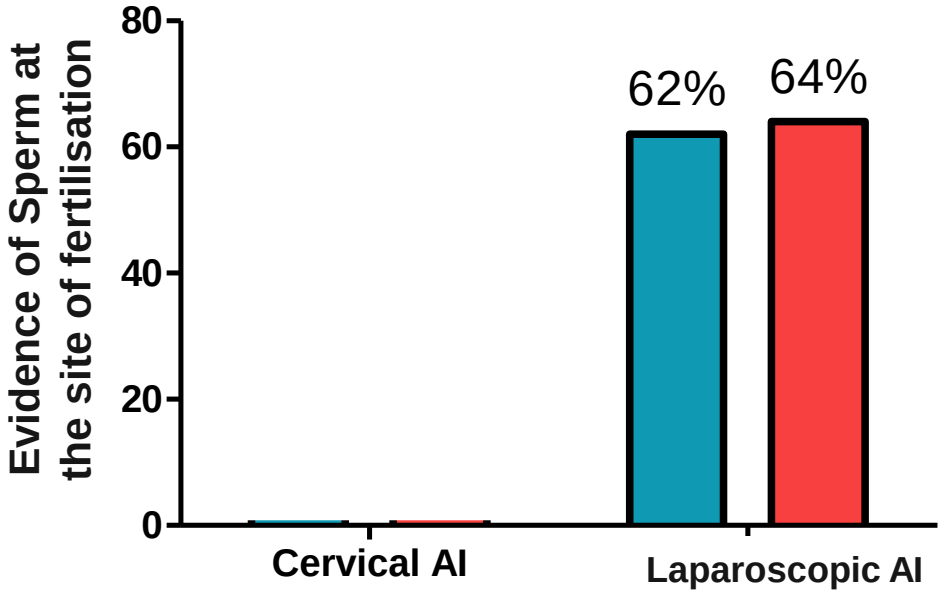
Ewe breed differences in pregnancy rates


Prof. Sean Fair

- No differences in sperm sources, quality and preservation
- Ewes with evidence of sperm at the site of fertilisation:

Blastocyst stained with Hoechst





Belclare

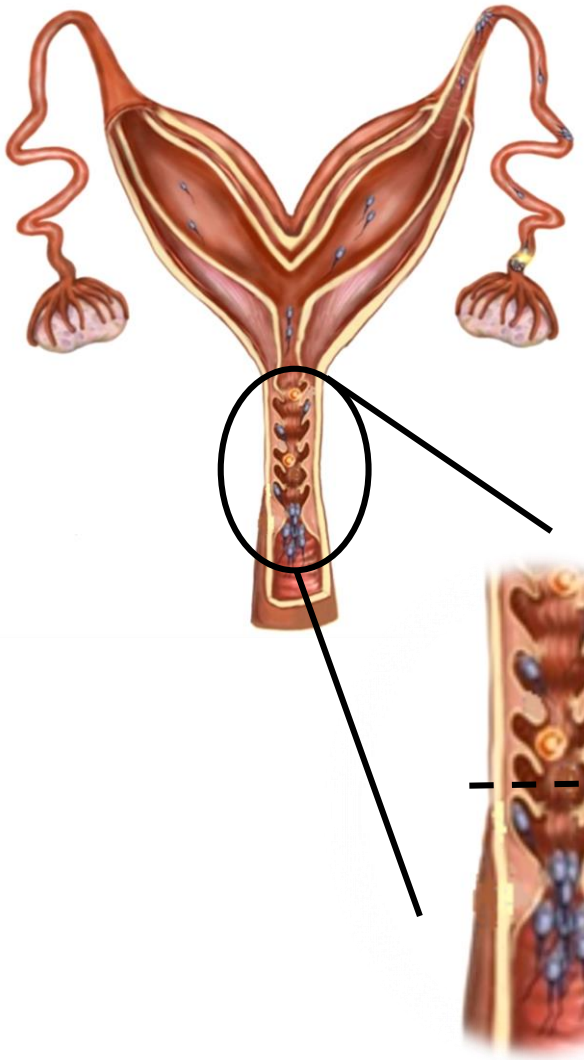
Suffolk

(Fair *et al.*, 2005)

Hypothesis

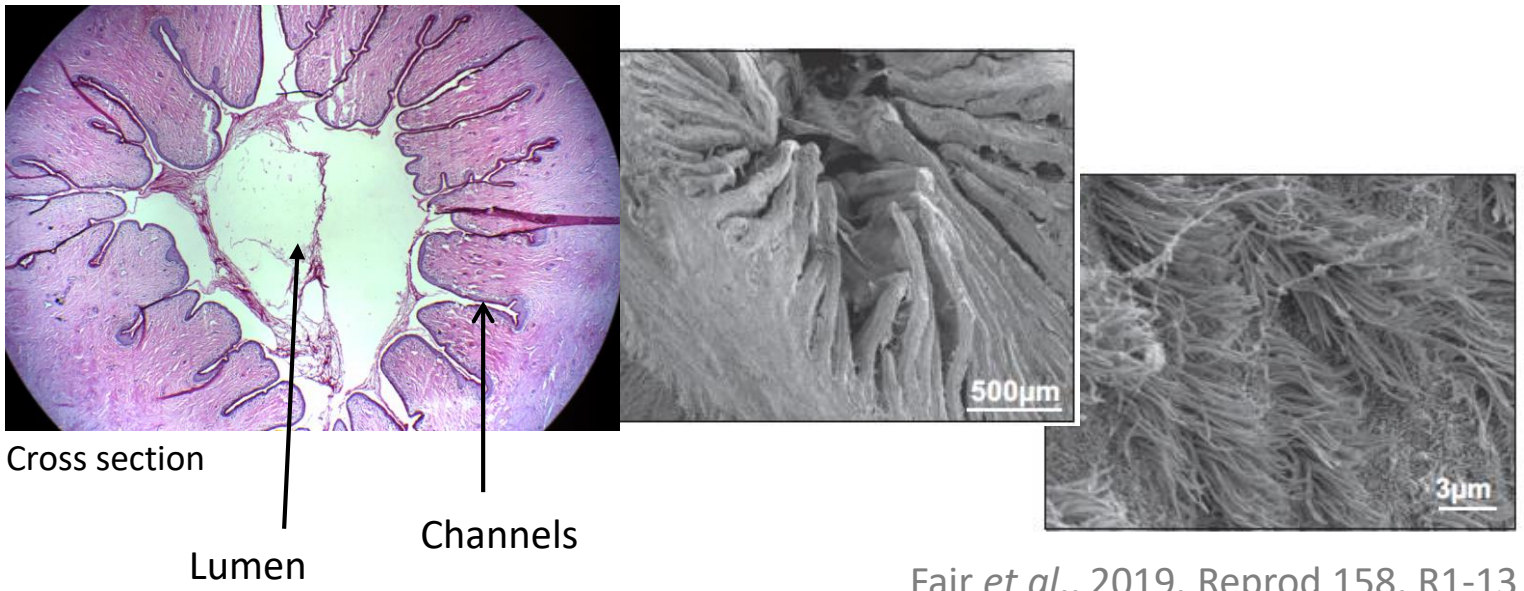
Differences between ewe breeds in the cervix and cervical mucus are the principal reasons why cervical AI with frozen-thawed semen works in Norway but not elsewhere

Sperm selection in the cervix



Physical Barrier

- Complex cervical anatomy
- Outward flow of mucus in the lumen is faster than within the channels
- Sperm move along the longitudinal channels, called “Privileged pathways”
- Cilia



Fair *et al*., 2019. Reprod 158, R1-13

Sperm selection in the cervix

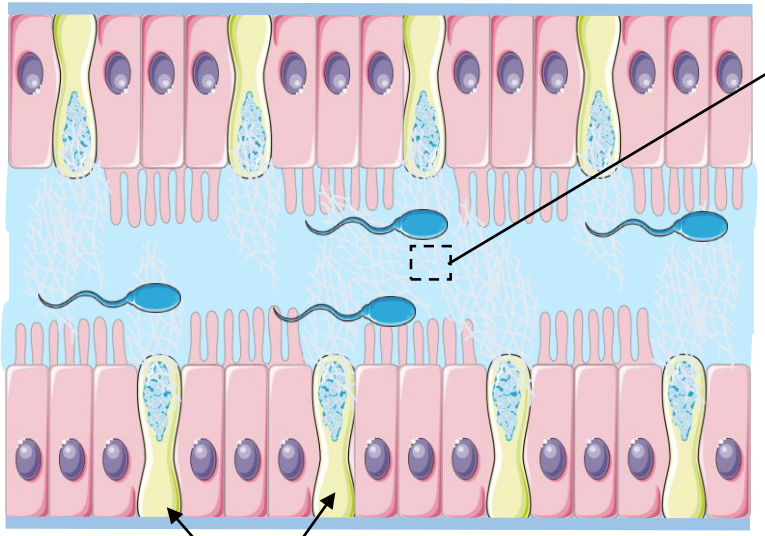
Biochemical Barrier

- **Cervical mucus** is produced by non-ciliated secretory cells in the cervical epithelium.
- Composition: water (~94%)

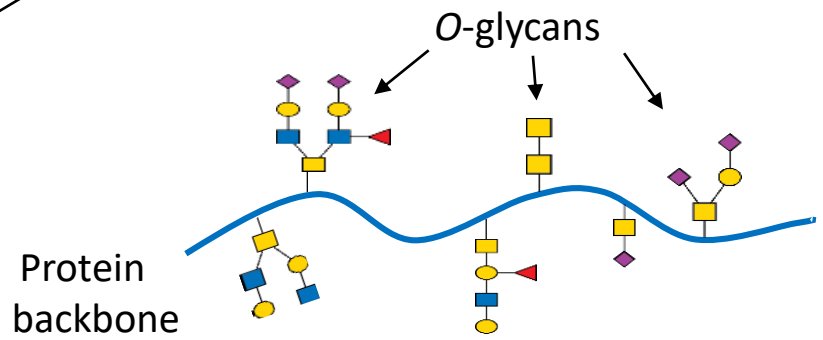
  mixture of various cell molecules and electrolytes (~1%)

  glycoproteins → mucins (~ 5%)

Cervical canal



Mucins = *O*-glycans attached to a protein backbone



Monosaccharaides

- N-acetylglucosamine (GlcNac)
- N-acetylgalactosamine (GalNac)
- Galactose (Gal)
- Fucose (Fuc)
- Sialic acid (Neu5Ac)
- Sialic acid (Neu5Gc)

A novel European sheep model

Ireland — **Low** and **medium** fertility

Suffolk

Belclare

France — **Medium** fertility

Ile de France

Romanov

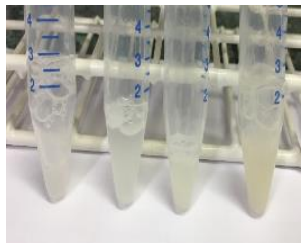
Norway — Both **high** fertility

Fur

NWS

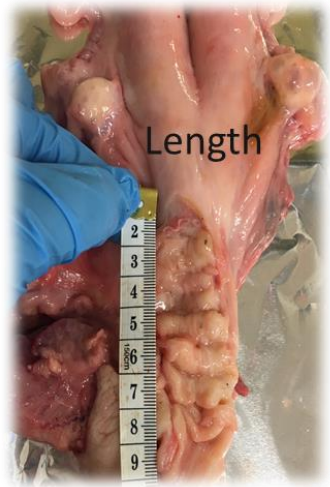
Mucus collection (3 replicates)

1. **Cervical mucus properties**
2. ***O*-glycan characterisation**
3. **Sialic acid analysis**
4. **Proteomics**
5. **Metabolomics**
6. **Microbiome analysis**

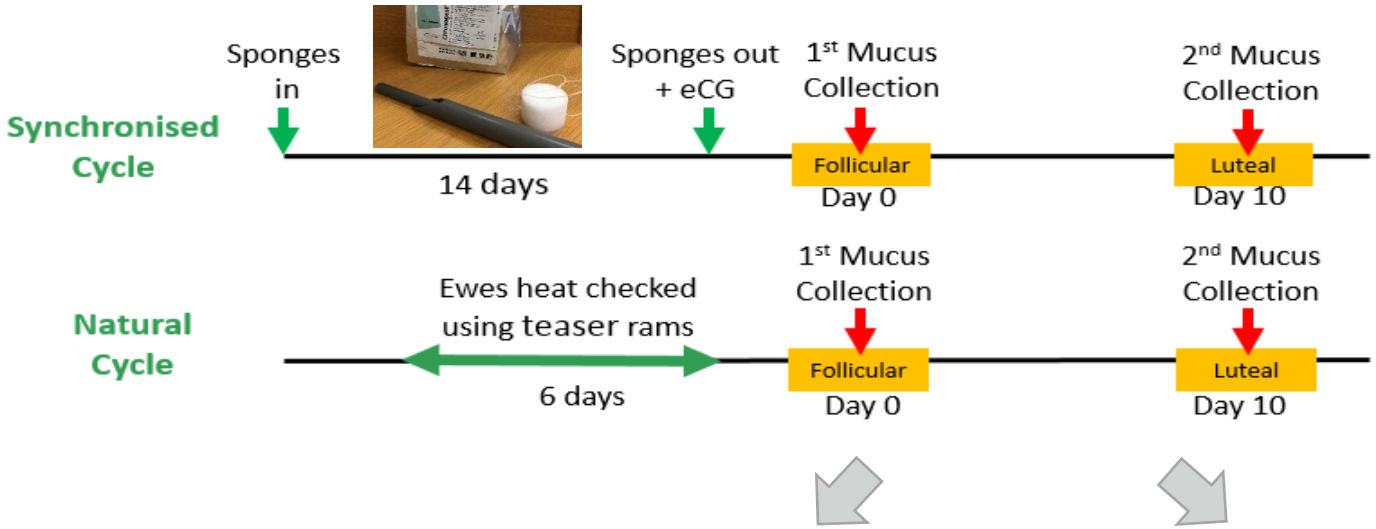


Post-mortem cervices collection

1. **Anatomy of the cervix**
   - Length, cervical rings and appearance of the external *opening*
2. **Cervical gene expression by RNAseq**
3. **Histology analysis**



Cervicovaginal mucus properties and cervical anatomy



X 3 replicates over a period of ~ 6 months

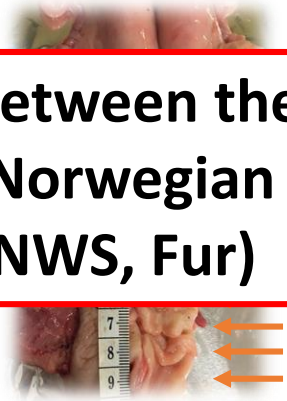
6 breeds x 28-30 ewes/breed

Mucus collection

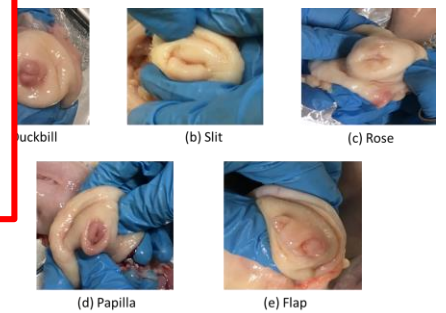
1. Mucus weight (grams)
2. Mucus viscosity (Tim
   chamber)
3. Mucus colour (Scorin
   from 1 to 7)

No significant differences between the low fertility Suffolk breed and Norwegian high fertility ewe breeds (NWS, Fur)

Post-mortem cervices collection



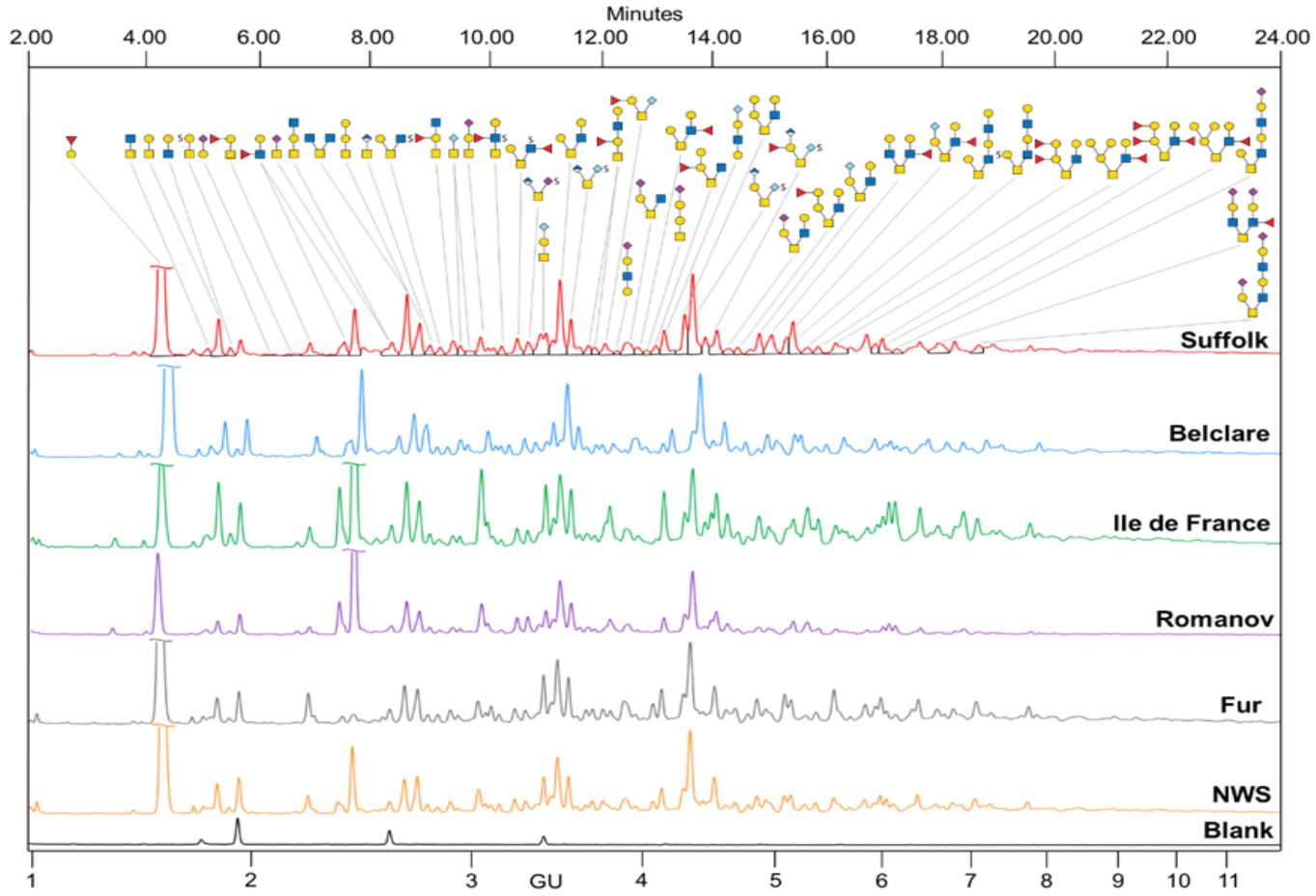
Appearance of Cervical Opening by Kershaw et al., 2005



Abril-Parreño L *et al.* 2021. Ewe breed differences in cervical anatomy and cervicovaginal mucus properties: An international study. Theriogenology.

Biochemical composition of cervical mucus

- Total of 124 *O*-glycans identified from which 51 were the major *O*-glycans



L. Abril-Parreño *et al*. 2021. *Identification and characterisation of O-linked glycans in cervical mucus as biomarkers of sperm transport: A novel sheep model*. Glycobiology

- **Most common structures:**

Core 1
Galβ1-3GalNAc

Core 2
Galβ1-3(GlcNAcβ1-6)GalNAc

- **Most common modifications:**

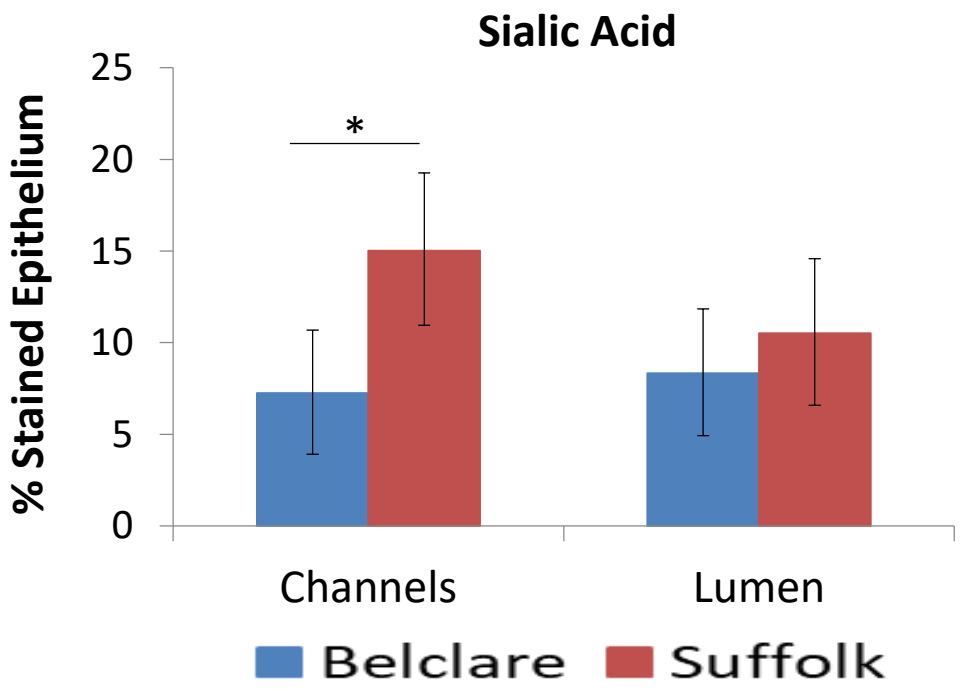
Fucosylated glycans

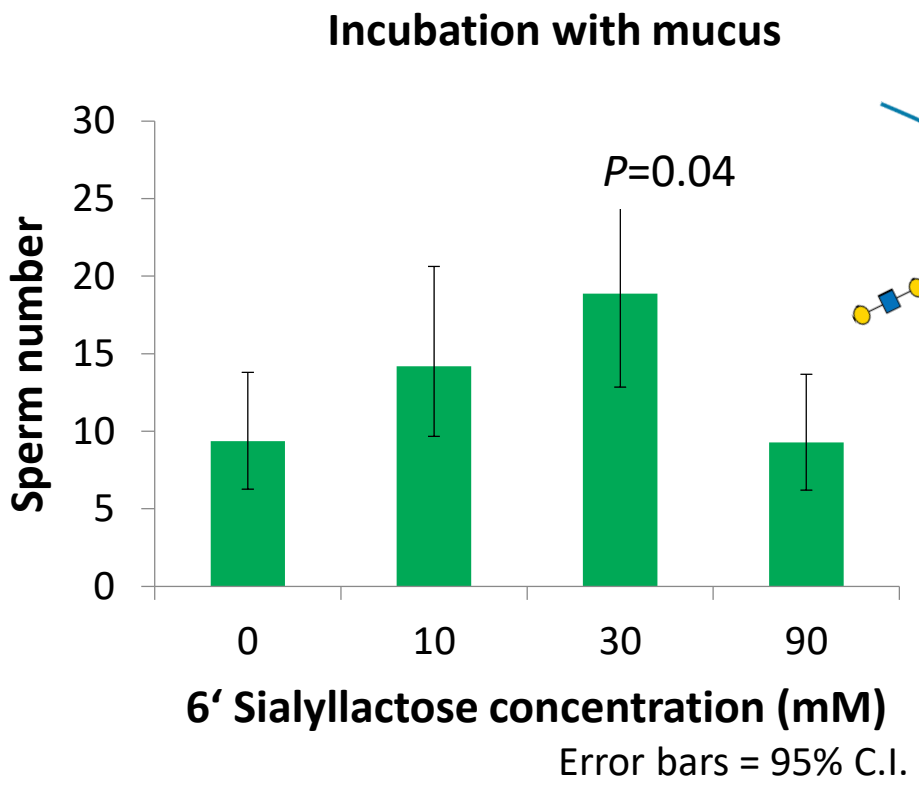
Sialylated glycans

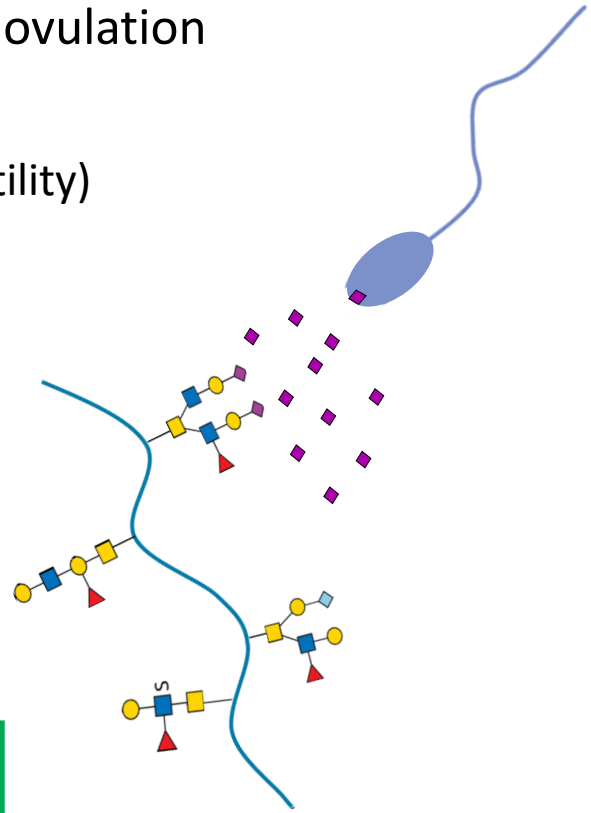
Monosaccharaides

Role of sialic acid in the cervical mucus

- Modulation of mucus viscosity: low levels of sialylated glycans around the time of ovulation (Andersch-Bjorkman *et al.*, 2007)
- Sperm transport: higher sialic acid content in the cervical channels of Suffolk ewes (low fertility)





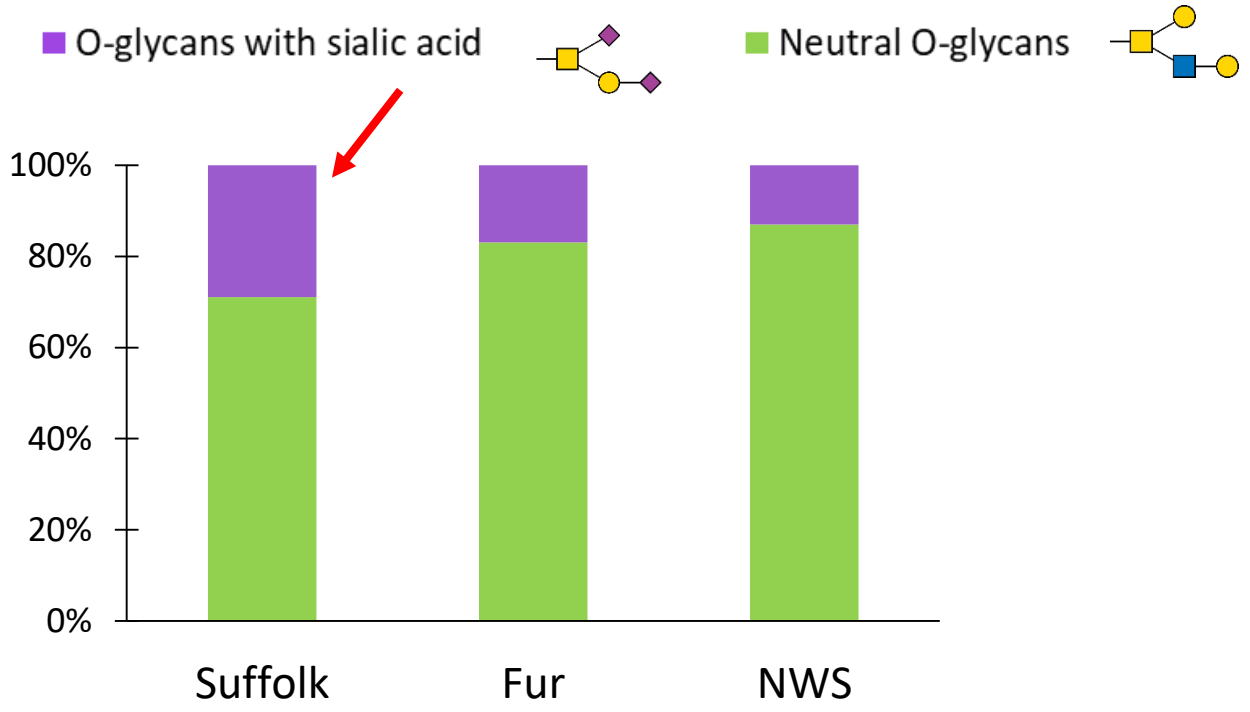


Richardson *et al.*, 2019

Higher levels of sialic acid in the low fertility breed

- Glycans were separated based on the **number of sialic acids** by Weak Anion Exchange Chromatography (WAX) – UPLC

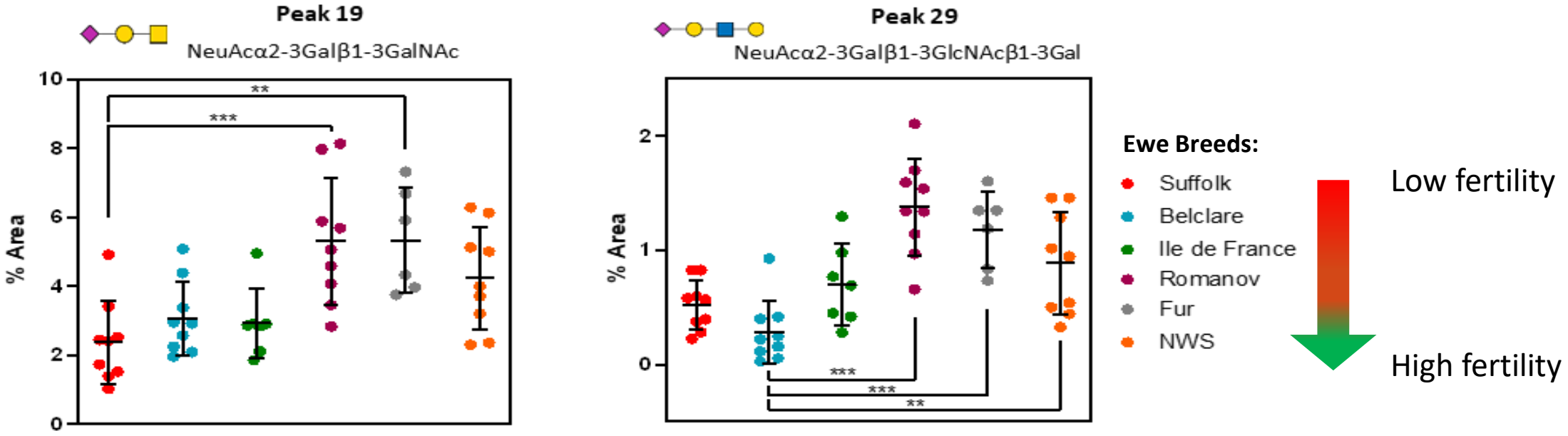
- **Higher levels of sialic acid** in the low fertility Suffolk breed at both the natural and synchronised oestrous cycle



Abril-Parreño L, Morgan J, Krogenæs A, Druart X, Cormican P, Gallagher ME, Reid C, Meade K, Saldova R, Fair S. Biochemical and molecular characterisation of sialylated cervical mucins in sheep. Biol Reprod. 2022 Apr 26:ioac077

Specific differences in sialic acid composition

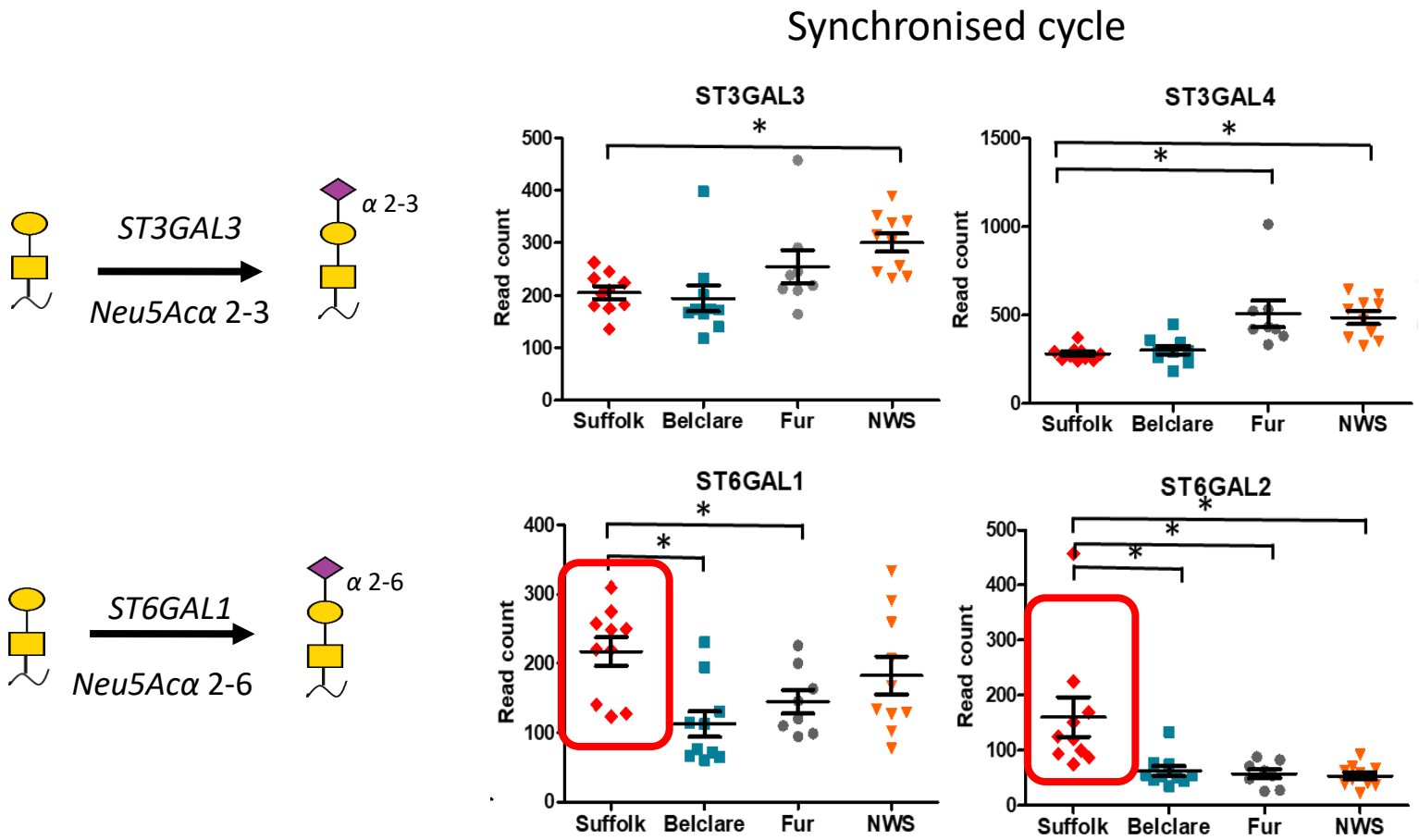
- High fertility ewe breeds had higher proportion of α 2-3 sialylated glycans



*($P < 0.05$), **($P < 0.01$), ***($P < 0.001$)

Up-regulation of sialic acid related genes

- Cervical gene expression analysis using RNA-sequencing
- Genes encoding enzymes that transfer sialic acid terminals to *O*-glycans



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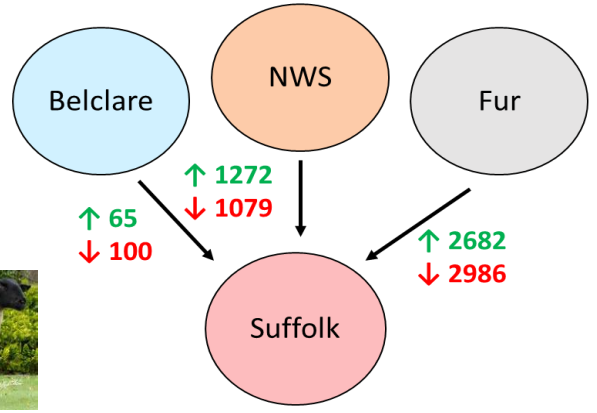
Cervical gene expression

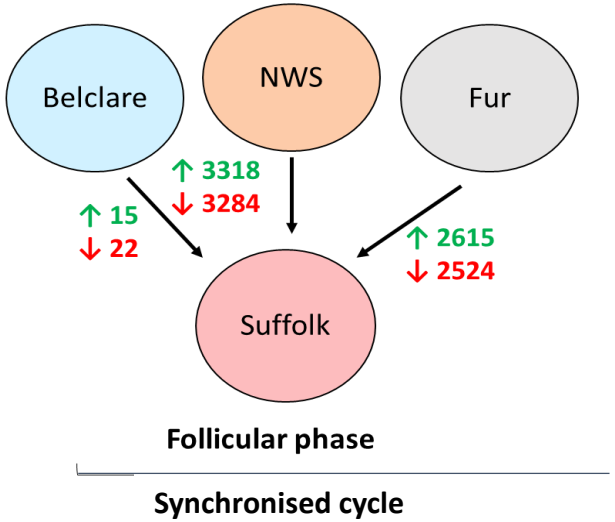
- Significant **differences in gene expression** between **high** and **low fertility ewe breeds**

Prof Kieran Meade

Dr Paul Cormican

Suffolk
(**low** fertility)





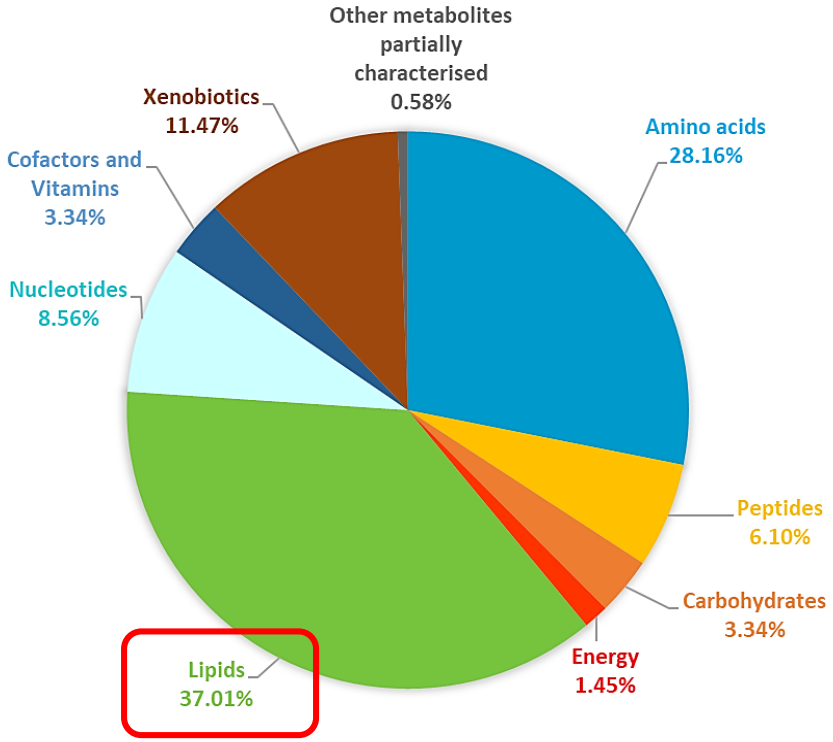
- **Inflammatory environment** and **compromised immune protection** in the cervix of the low fertility

  Suffolk breed → decreased levels of CD receptors, TLRs, chemokines, antimicrobial peptides

Abril-Parreño L, *et al.* 2021. Conserved and breed-specific differences in the cervical transcriptome of sheep with divergent fertility at the follicular phase of a natural oestrus cycle. BMC Genomics.

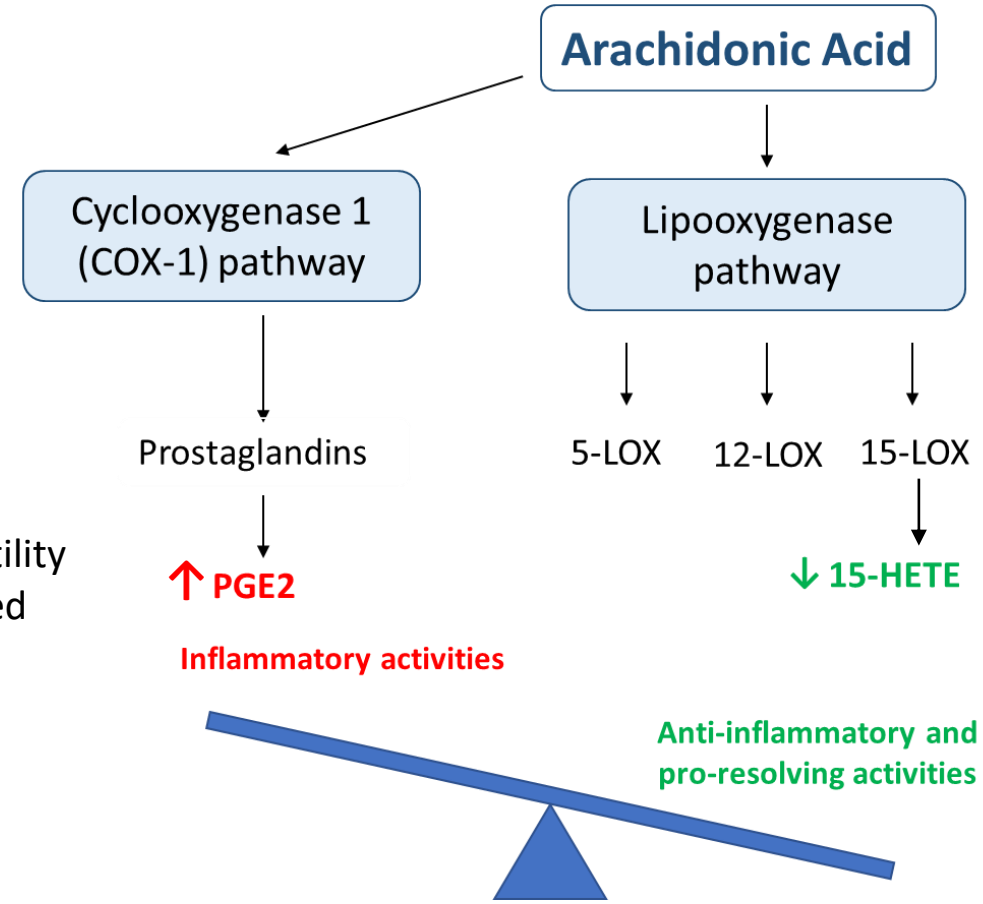
Abril-Parreño L, *et al.* 2022. Cervical immune activation during the luteal phase may compromise subsequent trans-cervical ram sperm transport. Biol Reprod

Cervical metabolome



Metabolon
SMALL MOLECULES, BIG INSIGHTS

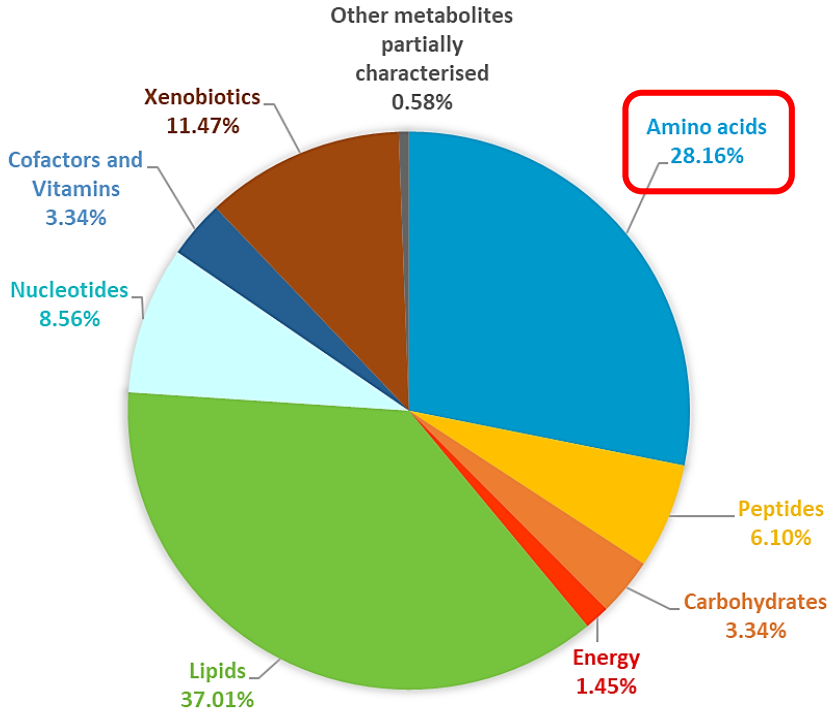
In the low fertility Suffolk breed

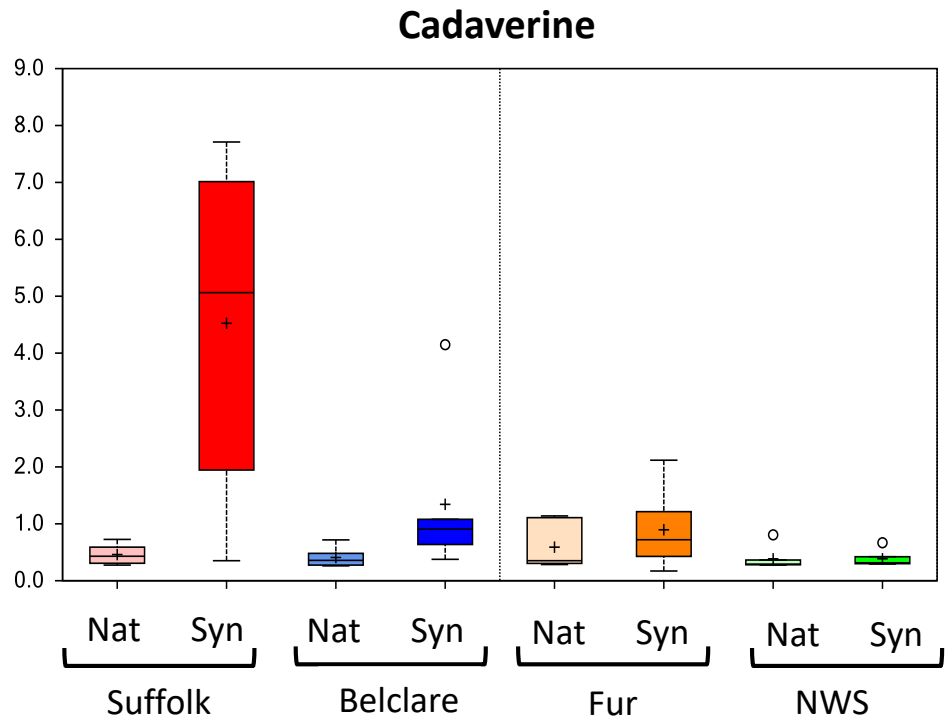


Abril-Parreño L, Krogenæs A, Fair S. Lipidomic profiling of cervical mucus reveals the potential role of pro-inflammatory derived metabolites on sperm transport across the ovine cervix. Animal. 2024 Mar 21;18(5):101136.

Cervical metabolome

- **194 amino acids** of which 133 were affected by ewe breed
- **Amino acids produced by anaerobic bacteria**→ higher in the low fertility Suffolk





Amino acids are strong predictors of cervicovaginal microbiota (Bokulich, et al. 2022)

Abril-Parreño L, Druart X, Fair S, Krogenaes A. Metabolic signature of cervical mucus in ewe breeds with divergent cervical sperm transport: a focus on metabolites involved in amino acid metabolism. Metabolomics. 2023 Jun 20;19(7):59.

Cervical microbiome

Suffolk *(n = 27)*
Low fertility

Belclare *(n = 27)*
Medium fertility

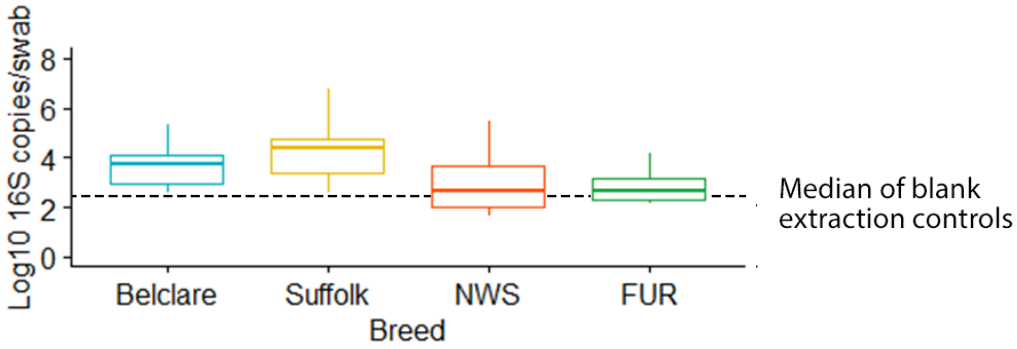
Norwegian Fur Sheep
High fertility *(n = 28)*

Norwegian White Sheep
High fertility *(n = 28)*

- DNA extraction using Indical IndiSpin Pathogen kit
- 16S (V3-V4) amplicon sequencing (Illumina MiSeq)
- Bacterial quantification by qPCR
- Contaminant identification using blank extraction controls

- **Bacterial richness**:

Follicular phase *Synchronised oestrous*



Prof Anette Krogenaes

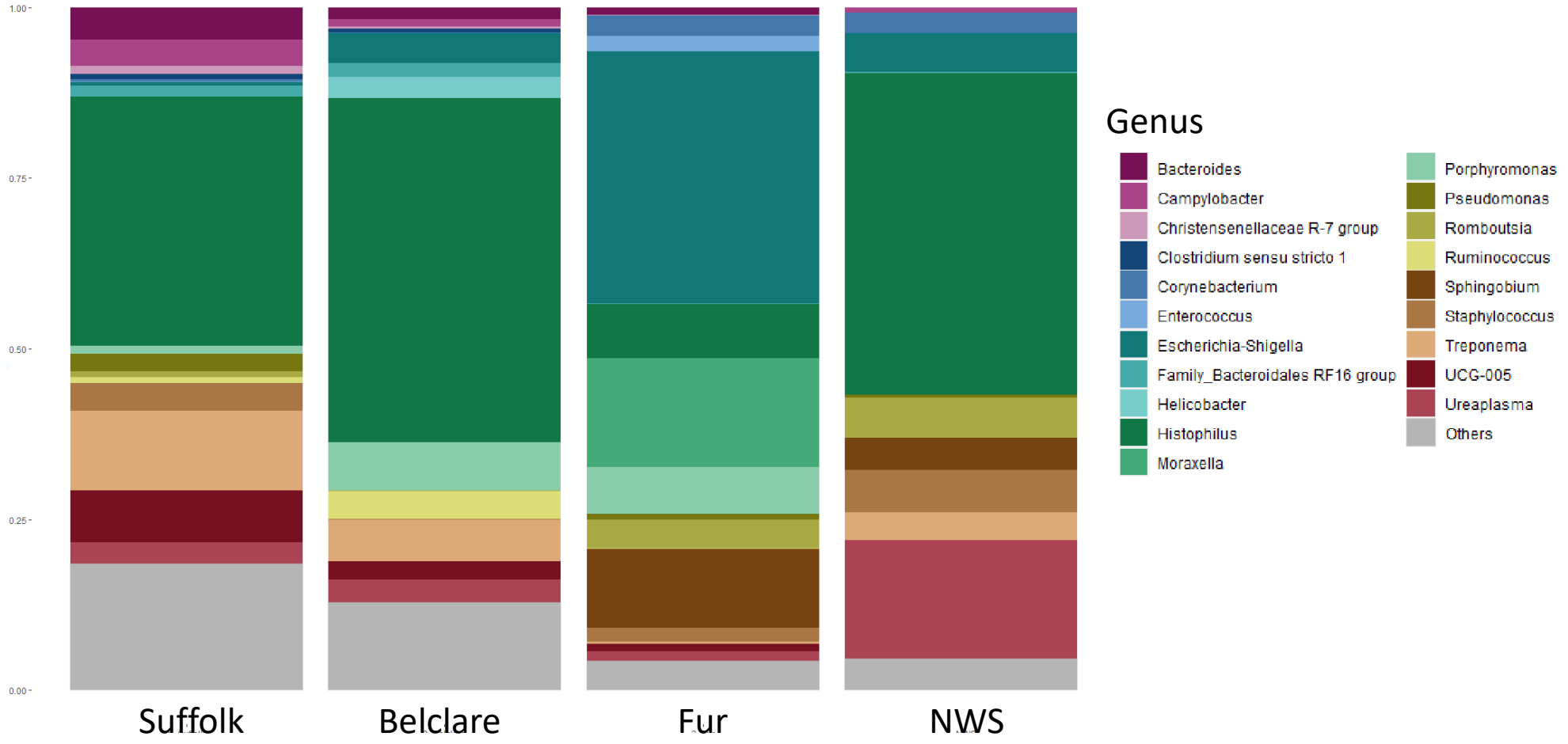
Norwegian University of Life Sciences

NORWEGIAN SEQUENCING CENTRE

Paper in preparation: The cervical microbiome of ewe breeds with known divergent fertility following artificial insemination

Cervical microbiome

- More variation in the cervical microbiome composition of the low fertility breed
  - *Histophilus* (formerly: *Haemophilus*) is the dominating genus in Suffolk, Belclare and NWS
  - *Ruminococcaceae UCG-005, Treponema* and *Campylobacter* seem to be more abundant in the lowest performing breed (Suffolk)



A complex journey for sperm

Summary

- Sialic acid as a regulator of sperm transport across the cervical mucus
- A suboptimal environment in the cervix of some ewe breeds is likely to have negative consequences for sperm transport
- “Omics” technologies allow to reveal molecular mechanisms underlying the optimal environment for cervical sperm transport
- **Future perspectives**: an *ex vivo* model to study sperm-cervix interactions and physiological functions of these identified biomarkers

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Thanks for your attention!

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