An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

16 RD SusAn ERA NET: SusSheP - Sustainable Sheep Production

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The objectives of SusSheP were to (i) provide new genetic tools for farmers to increase the longevity of ewes (ii) quantify labour input and carbon hoofprint in contrasting sheep systems (iii) develop more socially acceptable methods of artificial insemination (AI) by investigating ewe breed effects in cervical function and (iv) maximise knowledge transfer and uptake of innovation by farmers.

The main findings were that age at last lambing was identified as the optimum ewe longevity trait for inclusion in a sheep breeding index. It is mainly controlled by health traits such as mastitis and tooth loss. Using performance recording on sheep farms, achieved higher genetic merit, increased economic margins, and reduced carbon emissions per kg of meat produced but incurred 10% extra labour. Highly prolific breeds did not significantly increase labour input, at key handling events, and resulted by higher economic margins, without a change in terms of kg CO2e/kg meat produced. Introducing precision livestock farming (PLF) technology on extensive farms reduced labour by 20% and increased economic margins. We identified 124 O-glycans, 1425 proteins and 689 metabolites in cervical mucus across six ewe breeds with known differences in fertility following cervical insemination with frozen-thawed semen. There were a number of key O-glycans, sialic acids and proteins that were differentially abundant between the high and low fertility breeds. Gene expression and protein analysis revealed significantly enriched pathways involved in cervical immune response and muscle contraction. Access to information and farmer age influenced farmer's attitude to change. A life cycle assessment analysis of a lowland pasture-based sheep system varying in stocking rate and ewe prolificacy demonstrated that increasing lambs weaned per ha, through the increase in stocking rate had a negative effect on the environment. In contrast, increasing lambs weaned per ha through improved ewe prolificacy reduced the environmental burden.

KEYWORDS

Methane, cattle, rumen

ACRONYM	16/RD/SusAn/ERA-NET SusSheP
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COLLABORATORS, INSTITUTION	Institut National de Recherche Agronomique, France. Sheep Ireland and Teagasc, Ireland. The Norwegian Association of Sheep and Goat Breeders and Norwegian University of Life Science, Norway. Scotland's Rural College and Maternal Sheep Group, United Kingdom.
PUBLICATION DATE	April 2022

Section 1 - Research Approach & Results

Start Date

01 May 2017

End Date

30 April 2021

Select Research Programme

Research Stimulus Fund

Please select the appropriate TRL Scale

TRL 4: Technology validated in lab

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€244,999.00

Total Project Expenditure

€181,888.38

Rationale for undertaking the Research

Sheep are unproductive (but carbon productive) until they produce their first lamb crop, normally at 2 years of age and, on average, ewes only produce 4 crops of lambs in their lifetime. Despite its importance both from an economic and environmental perspective ewe longevity is not included in sheep breeding indexes across Europe. SusSheP established the genetic factors controlling ewe longevity, under different Sheep Production Systems (SPSs) which has resulted in Sheep Ireland now capturing this phenotypic information. Parallel to this, SusSheP identified the most carbon and labour efficient SPSs under different management systems (eg prolific vs non-prolific sheep breeds) in order to enable the development of strategies to reduce the labour input and carbon hoof print per kg of output. The breeding of more efficient sheep has been hampered internationally by the lack of sheep artificial insemination, as the only effective method for use with frozen-thawed semen is a laparoscopic procedure, whereby, semen is injected directly into the uterus but this requires veterinary expertise, is welfare unfriendly and sociable unacceptable. The only exception to this is in Norway, in which vaginal deposition of frozen-thawed semen yields good pregnancy rates. Research in Ireland has demonstrated this is due to the breed of the ewe used in Norway, whereby sperm can transverse the cervix in greater numbers than in other breeds, leading to higher pregnancy rates. SusSheP interrogated the differences across breeds in cervical physiology with a view of developing a more sociably acceptable AI method.

Methodology

We assembled a multi-disciplinary European wide consortium of experienced researchers, industry personnel and sheep farming representatives which shaped this cross-cutting innovative work towards interrogating problems that are holding back Irish and European sheep production. SusSheP used the most up-to-date methodologies in animal breeding, carbon/labour/systems modelling, life cycle assessment as well as carefully designed in vivo animal studies and in vitro molecular and functional studies.

We used existing Irish, UK and Norwegian databases to establish the main reasons for culling of ewes and to calculate their heritability. We used Go-Pro cameras worn by famers to study labour input information which was compiled alongside their technical and financial data which subsequently fed into a life cycle assessment analysis of a lowland SPSs using a range of stocking rates and ewes of varying prolificacy. In our survey of over 145 farmers, we identified the key factors contributing to farmer's attitude to change. We performed a multi-centre animal study for the collection of cervical mucus and tissue. This was followed by a detailed glycomic, proteomic, metabolomic as well as microbiome and sialic acid analysis of cervical mucus from up to six European ewe breeds and identified 124 O-glycans, 1425 proteins and

689 metabolites. In cervical tissue, we characterised over 10000 genes using RNAseq. We collaborated with key representatives from the animal breeding industry, knowledge transfer sectors as well as farmers with experience of performance recording and implementation of research findings. This facilitated the dissemination of results at seminar and workshops alongside a large number of scientific publications and presentations at scientific meetings.

Project Results

WP1: Tooth loss and mastitis were the main factors affecting ewe longevity, and both are heritable. There were no clear early life predictors for ewe longevity. Age at last lambing was identified as the best single trait for inclusion into sheep selection indexes. It has a heritability of 0.09-0.12. Sheep Ireland developed an App for on-farm recording of data and is now capturing ewe longevity phenotypes.

WP2: Using performance recording achieved higher genetic merit, increased economic margins, and reduced carbon emissions per kg of meat produced but incurred 10% extra labour. High prolific breeds did not significantly increase labour input, at key handling events, but resulted in higher economic margins, without a change in terms of kg CO2e/kg meat produced. Introducing PLF technology on extensive farms reduced labour by 20% and increased economic margins. It is possible to increase carbon and labour efficiency by changing ewe breed, breeding management or adopting technology on sheep farms.

WP3: There was a higher abundance of the sulfated glycan (Galβ1-3[SO3-GlcNAcβ1-6]GalNAc), fucosylated glycan (GlcNAcβ1-3(Fucα1-2Galβ1-3)GalNAc) and core 4 glycan (GlcNAcβ1-3[GlcNAcβ1-6]GalNAc) in the low fertility Suffolk breed compared with Norwegian White Sheep (NWS; high fertility). Biochemical analysis of cervical mucins showed higher

content of non-sialylated glycans in the high fertility ewe breeds (Fur and NWS) compared to the other ewe breeds.

Suffolk had higher levels of Neu5,9Ac2 compared to NWS. Gene expression analysis identified higher expression of MUC5AC, MUC5B, ST6GAL1, ST6GAL2 and lower expression of ST3GAL3, ST3GAL4 and SIGLEC10 in Suffolk compared to high fertility ewe breeds, indicating that specific alterations in sialylated mucin composition may be related to impaired cervical sperm transport. RNAseq of cervical tissue also revealed significantly enriched pathways involved in muscle contraction and development, that were downregulated in Suffolk compared to Fur and NWS during the follicular phase. This was supported by a lower abundance of desmosome proteins in Norwegian breeds suggesting different mechanical properties of the cervix, especially those involved in sperm transit such as ascending contractions. The immune response gene pathways were upregulated in Suffolk which also had the greatest microbial diversity. At the protein level, the Norwegian breeds exhibited higher amounts of antioxidant proteins (PRDX1, QSOX1, LPO).

WP4: Access to information and farmer age influenced farmer's attitude to change. A life cycle assessment analysis of a lowland based sheep system varying in stocking rate and ewe prolificacy demonstrated that increasing lambs weaned per ha through the increase in stocking rate had a negative effect on the environmental impact per ha and did not reduce the environmental impact intensity per kg live weight or kg carcass weight. In contrast, increasing lambs weaned per ha through improved ewe prolificacy reduced the environmental burden per kg live weight and kg carcass weight, with marginal increase in environmental impact per ha. For a fixed quantity of product, sheep systems with greater ewe prolificacy have lower environmental impact per ha and per unit product than systems with lower ewe prolificacy. WP5: There were 97 dissemination events over the course of the project.

Section 2 - Research Outputs

Summary of Project Findings

SusSheP developed novel strategies to record and implement genetic selection for ewe longevity. While genetic selection is slow, it is permanent and cumulative and the work completed in WP1 will have long a lasting positive impact on European sheep production. Through the use of novel case studies across four European countries, we have clearly illustrated it is possible to increase carbon and labour efficiency by changing ewe breed, breeding management or adopting technology on sheep farms. Lower stocking rate systems had the lowest environmental impact per ha, irrespective of ewe prolificacy. Indeed, we have identified that for a fixed quantity of lamb meat, sheep systems with greater ewe prolificacy have lower environmental impact per ha and per unit product than systems with lower ewe prolificacy. In our survey of over 145 farmers, access to information and farmer age was identified as key, in farmers attitude to change. This is not only important for famers but also policy makers who can incentivise changes to

practice. We have made exciting discoveries which have brought cervical insemination with frozen-thawed semen closer to reality for farmers. We have identified specific sugars (glycans and sialic acids) as potential biomarkers for sperm transport, due to their ability to regulate mucus interaction with sperm and regulate the cervical immune response. The new knowledge generated, as well as the datasets and biobanked samples will be the foundation for further work which will focus on the immunological regulation of cervical sperm transport. SusSheP achieved all of its objectives and the results have been disseminated widely and has made a contribution to the competiveness of Irish and European Sheep Production. This was in no small part to the dedication of our cross disciplinary and multi-actor team of biologists, immunologists, geneticists, production experts and farmers that worked together.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students		0	1
Post Doctorates		0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
PhD Theses	1	Physical, Biochemical and Molecular Characterisation of the Cervix of European Ewe Breeds with Known Differences in Pregnancy Rates Following Cervical Artificial Insemination with Frozen-thawed Semen. Dr Laura Abril-Parreño. Submitted to the Department of Biological Sciences, Faculty of Science and Engineering, University of Limerick, Ireland, April 2021. Supervisors: Prof Sean Fair, Prof Kieran Meade and Dr Paul Cormican.
Publications in Peer Reviewed	9	1. Abril-Parreño L, Druart X, Fair S and Krogenæs A Metabolic signature of cervical mucus in ewe breeds with divergent

Scientific Journals

cervical sperm transport: the role of amino acids. Under review in Biology of Reproduction.

2. McLaren A, McHugh N, Lambe NR, Pabiou T, Wall E, Boman IA. Factors affecting ewe longevity on sheep farms in three European countries. Small Ruminant Research, 189, 2020, 106145, ISSN 09214488,https://doi.org/10.1016/j.smallrumres.2020.106145.
3. Abril-Parreño L, Krogenæs AK, Druart X, Cormican P, Fair S, Meade K. Cervical immune activation during the luteal phase may compromise subsequent trans-cervical ram sperm transport. Biology of Reproduction, 2022; ioac130.
4. Abril-Parreño L, Meade K, Krogenæs AK, Druart X, Cormican P, Fair S. Ewe breed differences in the cervical transcriptome at the follicular phase of a synchronised oestrous cycle. BMC genomics, 2022 May 11;23(1):363.
5. Abril-Parreño L, Morgan J, Krogenæs AK, Druart X, Cormican P, Gallagher ME, Reid C, Meade K, Saldova R, Fair S. Biochemical and molecular characterisation of sialylated cervical mucins in sheep.
6. Biology of Reproduction, 2022 Apr 26:ioac077.
7. Abril-Parreño L, Meade K, Krogenæs AK, Druart X, Fair S, Cormican P. Conserved and breed-specific differences in the cervical
8. transcriptome of sheep with divergent fertility at the follicular phase of a natural oestrous cycle. BMC genomics. 2021 Oct 20;22(1):752.
9. Abril-Parreño L, Wilkinson H, Krogenæs AK, Morgan J, Gallagher ME, Reid C, Druart X, Fair S, Saldova R. Identification and characterisation of O-linked glycans in cervical mucus as biomarkers of sperm transport: A novel sheep model. Glycobiology. 2021 Aug 10:cwab085.
10. Abril-Parreño L, Krogenæs AK, Byrne CJ, Donovan A, Stuen S, Caldas E, Diskin M, Druart X, Fair S. Ewe breed differences in cervical anatomy and cervicovaginal mucus properties: An international study. Theriogenology. 2021 Jan 15;160:18-25.
11. Morgan-Davies C, Kyle J, Boman IA, Wishart H, McLaren A, Fair S, Creighton P. A comparison of farm labour, profitability, and carbon footprint of different management strategies in Northern European grassland sheep systems. Agricultural Systems, 2021; 191, 103155. doi.org/10.1016/j.agsy.2021.103155
12. Fair S, Meade KG, Reynaud K, Druart X, de Graaf SP. The biological mechanisms regulating sperm selection by the ovine

		cervix. Reproduction. 2019 pii: REP-18-0595.R1. doi: 10.1530/REP-18-0595. Review
Peer Reviewed Conference Papers	24	1. Nørstebø, S.F, Rodriguez-Campos, S., Umu, Ö.C.O., Abril-Parreño, L., Dalland, M., Gilfillan, G.D., Fair, S., Krogenæs, A. (2022, September 15- 17) The cervical microbiome of sheep breeds with different fertility rates [Conference presentation abstract]. 4th ICECVM 2022, Bari, Italy. 2. Abril-Parreño L, Druart X, Krogenæs AK and Fair S. Regulation of sperm transit across the ovine cervix. Oral presentation in the workshop entitled: Sperm in the female tract - what can we learn in vivo and in vitro? Chaired by Sean Fair. 38th Annual Association of Embryo Technology European Meeting, 10th and 14th- 16th of September 2022 3. Abril-Parreño L, Krogenæs AK, Druart X, Saldova R, Cormican P, Meade KG, Fair S. Ewe breed differences in cervical mucus biochemical and molecular composition: the role of sialylated cervical mucins. Annual meeting Association for Applied Animal Andrology. June 24th – 25th, 2022. Bologna, Italy. 4. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Differential glycosyltransferase gene expression in the ovine cervix may contribute to inter-breed differences in pregnancy rates following cervical insemination with frozen-thawed semen. European Society of Domestic Animal Reproduction. October 10th – 12th, 2021. Online. 5. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Cervical transcriptomic profiling of high and low fertility sheep breeds. 36th Annual Association of Embryo Technology European Meeting, 10th and 11th of September 2020. Online. 6. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Differential mucin gene expression in the ovine cervix contribute to breed differences in fertility. 71st Annual Meeting of European Federation of Animal Science, August 31st to September 4th, 2020. Online. 7. Abril-Parreño L, Meade KG, Cormican P and Fair S. Cervical transcriptomic profiling identifies breed- and phase-specific changes between high and low fertility sheep breeds. Annual Research and Innovation Day, December 17th, 2019. Department of Biological Sciences, University of Limerick, Limerick (Ireland). 8. Morgan-Davies C, Kyle J, Creighton P, Boman IA, Lambe N, McLaren A, Wishart H, Krogenaes A, Wall E, Padiou T, Diskin

MG, Meade KG, McHugh N, Abril-Parreño L, Caldas-Silveira E, Druart X and S Fair. SusSheP – Sustainable sheep production in Europe. European Federation for Animal Science (EAAP), 70th Annual Meeting, 2019. Ghent, Belgium.

9. Abril-Parreño L, Meade KG and Fair S. Cervicovaginal mucus properties and composition in sheep. Annual Research Day, December 11th, 2018. Department of Biological Sciences, University of Limerick, Limerick (Ireland).
10. Abril-Parreño L, Donovan A, Diskin MG, Fair S. Ewe breed differences in cervicovaginal mucus properties at a natural and synchronised oestrus. British Society for Animal Science, Annual Conference. April 9-11th, 2018. Croke Park, Dublin (Ireland).
11. Abril-Parreño L, Meade KG and Fair S. Ewe Breed Differences in Cervical Function. Annual Research Day, December 13th, 2017. Department of Biological Sciences, University of Limerick, Limerick (Ireland).
12. Fair S (2019) Sheep Breeders Round Table Conference. Presentation by S. Fair entitled 'Understanding the biology behind fertility'. East Midland Airport 26/11/2019
13. L. Abril-Parreño, K.G Meade, P. Cormican and S. Fair (2019) Cervical transcriptomic profiling identifies breed- and phase-specific changes between high and low fertility sheep breeds. Department of Biological Sciences Research and Innovation Day, December 17th, University of Limerick, Limerick, Ireland.
14. Abril-Parreño L, Cormican P, Krogenæs A, Meade KG and Fair S (2020) Characterisation of the cervical transcriptome in Irish and Norwegian sheep breeds known to have divergent pregnancy rates following cervical insemination with frozen-thawed semen. ICBF and Sheep Ireland Genetics Conference, January 17th, The Heritage Killenard, Laois, Ireland.
15. Abril-Parreño L, Wilkinson H, Krogenæs AK, Morgan J, Gallagher ME, Reid C, Druart X, Saldova R, Fair S. Glycomic profiling of cervical mucins in ewe breeds with differences in cervical sperm transport following cervical insemination. International Congress on Animal Reproduction. June 26th – 30th, 2022. Bologna, Italy.
16. Abril-Parreño L, Fair S. Biochemical and molecular characterisation of sialylated mucins and their impact on cervical sperm transport. Bernal Research Day, April 26th, 2022. Bernal Institute, University of Limerick, Limerick, Ireland.
17. Krogenæs AK, Abril-Parreño L, Druart X and Fair S. Ewe breed differences in the cervical mucus metabolome. European

Society of Domestic Animal Reproduction, October 10th – 12th, 2021. Thessaloniki, Greece.
18. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Characterisation of the cervical transcriptome in Irish and Norwegian sheep breeds known to have divergent pregnancy rates following cervical insemination with frozen-thawed semen. ICBF and Sheep Ireland Genetics Conference. January 17th, 2020. The Heritage Killenard, Laois (Ireland).
19. Abril-Parreño L, Meade KG, Cormican P and Fair S. Cervical transcriptomic profiling of high and low fertility sheep breeds. Bernal Research Day, December 5th, 2019. Bernal Institute, University of Limerick, Limerick (Ireland).
20. Abril-Parreño L, Donovan A, Diskin M.G, Meade KG and Fair S. Ewe breed differences in cervical anatomy and cervical mucus properties at a natural and synchronised oestrus. Teagasc Sheep Open Day. July 9th, 2018. Animal & Grassland Research and Innovation Centre, Athenry (Ireland).
21. Abril-Parreño L, Wall E, Pabiou T, Creighton P, Diskin MG, Meade KG, McHugh N and Fair S. (2017). European Research Area Network Research Project on Sustainable Sheep Production (SusSheP).
22. Abstract of a poster presentation presented at Teagasc Sheep Open Day. June 21st, 2017. Animal & Grassland Research and Innovation Centre, Athenry (Ireland).
23. C Morgan-Davies, P Creighton, IA Boman, T Blichfeldt, A Krogenaes, N Lambe, E Wall, T Padiou, MG Diskin, KG Meade, N Mchugh, X Druart, S Fair (2018) SusSheP – how to increase sustainability and profitability of European sheep production? Presented at the 69th annual meeting of the European Federation of Animal Science, 27th 31st August, Dubrovnik, Croatia.
24. McLaren A, McHugh N, Lambe NR, Pabiou T, Wall E, Boman IA (2020) Factors affecting ewe longevity on sheep farms in three European countries, Small Ruminant Research, Volume 189, 106145,

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Technology	Sheep Ireland developed a new App for the on-farm recording of data/phenotypes including those relating to ewe longevity	1
New Industry Collaborations Developed	We developed a number of new collaborations such as that with Dr Simen Foyn Norstebo, Norwegian University of Life Sciences (on the microbiome), Dr Colm Reid, University College Dublin (on mucins and Sialic acid), Dr Radka Saldova, National Institute for Biomedical Research and Training (Dublin, on Glycomics) and Professor Simon de Graaf at the University of Sydney (on Cervical Biology). During SusSheP we worked heavily with NSG in Norway which is the body responsible for genetic improvement in sheep and goats. We also collaborated with the Maternal Sheep Group in the UK.	6
New Processes	A new method for the release of O-glycans from cervical mucins was developed by NIBRT with input from Laura Abril Parreno. This can be applied to mucins extract from many bodily tissues.	1
New Products	Biobank of samples and datasets on cervical mucus.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	Through the use of novel case studies across four European countries, we have clearly illustrated it is possible to increase carbon and labour efficiency by changing ewe breed, breeding management or adopting technology on sheep farms. Lower stocking rate systems had the lowest environmental impact per ha, irrespective of ewe prolificacy. Indeed, we have identified that for a fixed quantity of lamb meat, sheep systems with greater ewe prolificacy have lower environmental impact per ha and per unit product than systems with lower ewe prolificacy.
Socio-Economic	In our survey of over 145 farmers, access to information and farmer age was identified as key, in farmers attitude to change. This is not only important for famers but also policy makers who can incentivise changes to practice. Our work on cervical AI in sheep has been the most comprehensive characterisation of the biology of the ovine cervix to-date and has provided us with a number of potential biomarkers which can improve the transport of frozen-thawed ram sperm across the cervix thus making cervical AI a reality for farmers. This will negate the need for laparoscopic insemination as it is becoming increasingly sociable unacceptable.
Industry	Sheep genetic improvement schemes across Europe are hampered by the low reliabilities associated with genetic breeding values which are a consequence of the lack of accurate commercial phenotypic data being recorded. In addition, sheep farmers have traditionally perceived performance recording as labour intensive and unfruitful. SusSheP has provided robust data on the benefits of performance recording and the use of PLF for the capturing of on-farm data. It has also initiated the on-farm recording of traits related to ewe longevity via the new Sheep Ireland App. Collectively, these will increase the economic viability of sheep farms.

Dissemination Activities

Activity	Details
Workshops at which results were presented	• Kick off meeting workshop; University of Limerick, Ireland 24/4/2017 • End of Year 1 SusSheP workshop with the project team and collaborators; SRUC, Edinburugh 5/6/2018 • End of Year 2 SusSheP workshop with the project team and collaborators; INRA, Tours, France 4/6/2019 • End of Year 3 SusSheP workshop with the project team and collaborators; Virtual 9/6/2020 • End of project SusSheP workshop with the project team and collaborators; Virtual 2/12/2020 • E. Wall. Meeting with all sheep breed society representatives, Athenry Galway, Ireland. 13/2/2019 • E. Wall discussed SusShep project with Irish sheep industry stakeholders at industry meeting. 18/4/2018 • L Abril-Parreño, A Donovan, MG Diskin, KG Meade, S Fair (2018) Ewe breed differences in cervical anatomy and cervical mucus properties at a natural and synchronised oestrus. Teagasc Sheep Open Day, July 9th, Animal & Grassland Research and Innovation Centre, Athenry, Ireland. • L Abril-Parreño, E Wall, T Pabiou, P Creighton, MG Diskin, KG Meade, N McHugh, S Fair (2017) European Research Area Network Research Project on Sustainable Sheep Production (SusSheP). Teagasc Sheep Open Day, June 21st, Animal & Grassland Research and Innovation Centre, Athenry, Ireland.
Seminars at which results were presented	• Presentation by L. Abril Parreno entitled 'Characterisation of the cervical transcriptome in Irish and Norwegian sheep breeds known to have divergent pregnancy rates following cervical insemination with frozen-thawed semen'. ICBF and

	Sheep Ireland Genetics Conference, The Heritage Killenard, Laois, Ireland. 17/1/2020 • Presentation by S. Fair at the Sheep Breeders Round Table Conference 2019. Presentation entitled 'Understanding the biology behind fertility'. East Midlands Airport Hotel, UK. 26/11/2019 • McDermott K (2019) Presentation to Veterinary Students, University College Dublin, Ireland 10/4/2019 • Presentation by L Abril Parreno on Cervical transcriptomic profiling identifies breed- and phase-specific changes between high and low fertility sheep breeds. Department of Biological Sciences Research and Innovation Day, December 17th, University of Limerick, Limerick, Ireland. 17/12/2019 • Presentation by L Abril Parreno entitled 'Cervical transcriptomic profiling of high and low fertility sheep breeds'. Bernal Research Day, University of Limerick, Limerick, Ireland. 5/12/2019 • Presentation by L Abril Parreno entitled 'Cervical transcriptomic profiling of high and low fertility sheep breeds'. Bernal Research Day, December 5th, University of Limerick, Limerick, Ireland. 5/12/2019 • E. Wall. Meeting with all sheep breed society representatives, Athenry Galway, Ireland. 13/2/2019 • Kevin McDermott from Sheep Ireland gave a Presentation to Veterinary Students, University College Dublin, Ireland. 10/4/2019 • Presentation by M. Diskin on a Summary of sheep projects involving Teagasc. Athenry, Ireland 26/10/2018 • Thesis in Three presentation by L Abril-Parreño entitled 'Sperm interaction with the female reproductive tract: the role of sugars'. University of Limerick Research, University of Limerick, Limerick, Ireland. 20/3/2018. • Presentation by L Abril Parreno on Ewe Breed Differences in Cervical Function. Department of

	Biological Sciences Research and Innovation Day, Radisson Hotel, Limerick. 12/12/2018 • Presentation by E. Wall at the Sheep Ireland Genetics Conference 2018, Athlone, Ireland. 5/12/2018 • Diskin M, (2018) Summary of sheep projects involving Teagasc, Athenry, Ireland; 26/10/2018 • Wall E (2018) Sheep Ireland Genetics Conference 2018, Athlone, Ireland; 5/12/2028 • Wall E (2018) Meeting with all sheep breed society representatives, Athenry Galway, Ireland 13/2/2018 • Presentation by L Abril-Parreño entitled 'Ewe Breed Differences in Cervical Function'. Department of Biological Sciences Research Day, University of Limerick, Limerick, Ireland. 13/12/2017

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	• SusSheP website; https://www.sheep.ie/?page_id=2387; 200 following; 289 followers; 154 tweets; 629 likes • End of Project SUSAN seminar; promotional video made and uploaded onto SusSheP website https://www.sheep.ie/?p=6070 • Year 3 SusSheP newsletter; https://www.sheep.ie/wp-content/uploads/2021/01/SusShep-Year-3-newsletter_final.pdf • Year 2 SusSheP newsletter; https://www.sheep.ie/wp-content/uploads/2020/02/Newsletter-for-SusSheP-August-2019.pdf • Year 1 SusSheP newsletter; https://www.sheep.ie/wp-content/uploads/2018/09/SusSheP-leaflet_Sept-2018-final.pdf
Identify any knowledge transfer activities executed within the project.	Stakeholder meetings: • Diskin M, (2018) Summary of sheep projects involving Teagasc, Athenry, Ireland; 26/10/2022

	• Wall E (2018) Sheep Ireland Genetics Conference 2018, Athlone, Ireland; 5/12/2022 • Wall E (2018) Meeting with all sheep breed society representatives, Athenry Galway, Ireland 13/2/2019 • McDermott K (2019) Presentation to Veterinary Students, University College Dublin, Ireland 10/4/2019 • Presentation by L Abril Parreno entitled 'Cervical transcriptomic profiling of high and low fertility sheep breeds'. Bernal Research Day, 2019 December 5th, University of Limerick, Limerick, Ireland. Technical Publications: • Newsletter year 1- 2018: https://www.sheep.ie/wp-content/uploads/2018/09/SusSheP-leaflet_Sept-2018-final.pdf • Newsletter year 2- 2019: https://www.sheep.ie/wp-content/uploads • /2020/02/Newsletter-for-SusSheP-August-2019.pdf • Newsletter year 3- 2020: https://www.sheep.ie/wp-content/uploads /2021/01/SusShep-Year-3-newsletter_final.pdf • Project video SusShep: https://www.youtube.com/watch?v=P9KDeklnynI&list=PLFnlwoEx7IG9shR4sPEwlRlxZZudiGQ2u&index=4 • Fair S (2020) Elucidating the reasons why cervical insemination with frozen-thawed ram semen works in Norway but not elsewhere. ERA-NET SusAn Newsletter, SusAn 5th Newsletter Other KT activity • Dalton L (2022) Understanding the FemaleReproductive Tract's Role in Fertilization

	ACSCentral Science 8 (1), 3-6 DOI:10.1021/acscentsci.1c01595. • neoMimix: The New Sperm Selection Technology (2022) Interview of Prof Sean Fair by Jonathon McCrea on Irish national radio Futurelife programme; Newstalk on 7/3/22 http://ow.ly/39Vv50Ic3rI Sponsored by SFI • Interview of Prof Sean Fair on ReproRadio n June 2022; www.reproradio.com. This is an international podcast series.
List any impacts resulting from the knowledge transferred during the project.	• One highly trained PhD student with 5 first authored peer reviewed papers who has already secured postdoctoral work. • Ewe longevity traits now being routinely recorded at farm level. This facilitates breeding decisions by sheep farmers as well as feeding into genetic evaluations. • Data showing that lower stocking rate systems had the lowest environmental impact per ha, irrespective of ewe prolificacy will have implications for policy makers. For a fixed quantity of lamb meat, sheep systems with greater ewe prolificacy have lower environmental impact per ha and per unit product than systems with lower ewe prolificacy. • Technical back up given to Michael Crosse, Irelands largest sheep milk producer (https://www.sheepmilk.ie/) on how to collect and process ram semen and perform cervical AI in sheep. • A greater awareness of farmers, industry, third level students and the scientific community (UCD and UL) of the biology of the ovine cervix and the challenges of developing AI in sheep.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€393,305.00	A commercialisation fund from Enterprise Ireland. Title: Advancing Sperm Selection for use in Assisted Human Reproduction through Microfluidics. The work done in SusSheP on how sperm interact with the cervix and its secretions facilitated securing this grant.
Exchequer National Funding	€1,900,000.00	S. Fair is co-PI on an SFI investigators grant. Title: An integrated multidisciplinary approach to revolutionise dairy cattle breeding, through the application of state of the art technology to advance the identification, sexual maturation fertility and availability of semen from genetically elite sires. This was €1.9 m of which €624,322 was to S. Fair.
Exchequer National Funding	€208,000.00	S. Fair secured two IRC funded PhD scholarships. 1. Characterising the immunomodulatory properties of sperm from high and low fertility bulls 2. Advancing sperm selection for use in assisted human reproduction The data generated in SusSheP was used to leverage both of these awards.

Future Strategies

Laura Abril Parreno has recently been awarded a prestigious "Juan de la Cierva" postdoc fellowship in Spain and is preparing a Marie Curie postdoc application for September 2022.

N. McHugh and S. Fair applied to the Teagasc Walsh Fellowship programme for a PhD stipend to expand the ewe longevity work.

S. Fair has a new PhD student started (July 2022) who will be pioneering the development of an in vitro ovine cervix. We will collaborate with biomedical engineers, regenerative

medical scientists and clinicians. This is the first part of a larger plan to develop an in vitro biomimicry model of the ovine cervix which will facitate (i) the assessment of the interaction of sperm with the cervical immune response (ii) the study of biomarkers of interest to promote cervical sperm transport both from a sheep and human perspective and (iii) the testing of compounds for blocking sperm transport in humans for the development of on-demand, non-hormonal based contraceptives'. S. Fair plans on submitting a large proposal to the SFI Frontiers for the Future Programme in the next 6 months and will also target DAFM and Horizon 2020 should the opportunity arise.

Project Publications

Peer reviewed papers

1. Abril-Parreño L, Druart X, Fair S and Krogenæs A Metabolic signature of cervical mucus in ewe breeds with divergentcervical sperm transport: the role of amino acids. Under review in Biology of Reproduction
2. McLaren A, McHugh N, Lambe NR, Pabiou T, Wall E, Boman IA. Factors affecting ewe longevity on sheep farms in threeEuropean countries. Small Ruminant Research, 189, 2020, 106145, ISSN 09214488,https://doi.org/10.1016/j.smallrumres.2020.106145.
3. Abril-Parreño L, Krogenæs AK, Druart X, Cormican P, Fair S, Meade K. Cervical immune activation during the lutealphase may compromise subsequent trans-cervical ram sperm transport. Biology of Reproduction, 2022; ioac130.
4. Abril-Parreño L, Meade K, Krogenæs AK, Druart X, Cormican P, Fair S. Ewe breed differences in the cervicaltranscriptome at the follicular phase of a synchronised oestrous cycle. BMC genomics, 2022 May 11;23(1):363.
5. Abril-Parreño L, Morgan J, Krogenæs AK, Druart X, Cormican P, Gallagher ME, Reid C, Meade K, Saldova R, Fair S.Biochemical and molecular characterisation of sialylated cervical mucins in sheep. Biology of Reproduction, 2022 Apr 26:ioac077.
6. Abril-Parreño L, Meade K, Krogenæs AK, Druart X, Fair S, Cormican P. Conserved and breed-specific differences in thecervical transcriptome of sheep with divergent fertility at the follicular phase of a natural oestrous cycle. BMC genomics. 2021 Oct 20;22(1):752.
7. Abril-Parreño L, Wilkinson H, Krogenæs AK, Morgan J, Gallagher ME, Reid C, Druart X, Fair S, Saldova R. Identificationand characterisation of O-linked glycans in cervical mucus as biomarkers of sperm transport: A novel sheep model. Glycobiology. 2021 Aug 10:cwab085.
8. Abril-Parreño L, Krogenæs AK, Byrne CJ, Donovan A, Stuen S, Caldas E, Diskin M, Druart X, Fair S. Ewe breed differencesin cervical anatomy and cervicovaginal mucus properties: An international study. Theriogenology. 2021 Jan 15;160:18-25.
9. Morgan-Davies C, Kyle J, Boman IA, Wishart H, McLaren A, Fair S, Creighton P. A comparison of farm labour,profitability, and carbon footprint of different

management strategies in Northern European grassland sheep systems. Agricultural Systems, 2021; 191, 103155. doi.org/10.1016/j.agsy.2021.103155

10. Fair S, Meade KG, Reynaud K, Druart X, de Graaf SP. The biological mechanisms regulating sperm selection by theovine cervix. Reproduction. 2019 pii: REP-18-0595.R1. doi: 10.1530/REP-18-0595. Review

Thesis

Physical, Biochemical and Molecular Characterisation of the Cervix of European Ewe Breeds with Known Differences in Pregnancy Rates Following Cervical Artificial Insemination with Frozen-thawed Semen. Dr Laura Abril-Parreño. Submitted to the Department of Biological Sciences, Faculty of Science and Engineering, University of Limerick, Ireland, April 2021. Supervisors: Prof Sean Fair, Prof Kieran Meade and Dr Paul Cormican.

Conference proceeding Oral Presentations

1. Nørstebø, S.F, Rodriguez-Campos, S., Umu, Ö.C.O., Abril-Parreño, L., Dalland, M., Gilfillan, G.D., Fair, S., Krogenæs, A. (2022, September 15-17) The cervical microbiome of sheep breeds with different fertility rates [Conference presentation abstract]. 4th ICECVM 2022, Bari, Italy.
2. Abril-Parreño L, Druart X, Krogenæs AK and Fair S. Regulation of sperm transit across the ovine cervix. Oral presentation in the workshop entitled: Sperm in the female tract - what can we learn in vivo and in vitro? Chaired by Sean Fair. 38th Annual Association of Embryo Technology European Meeting, 10th and 14th-16th of September 2022
3. Abril-Parreño L, Krogenæs AK, Druart X, Saldova R, Cormican P, Meade KG, Fair S. Ewe breed differences in cervicalmucus biochemical and molecular composition: the role of sialylated cervical mucins. Annual meeting Association for Applied Animal Andrology. June 24th – 25th, 2022. Bologna, Italy.
4. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Differential glycosyltransferase gene expressionin the ovine cervix may contribute to inter-breed differences in pregnancy rates following cervical insemination with frozen-thawed semen. European Society of Domestic Animal Reproduction. October 10th – 12th, 2021. Online.
5. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Cervical transcriptomic profiling of high and lowfertility sheep breeds. 36th Annual Association of Embryo Technology European Meeting, 10th and 11th of September 2020. Online.
6. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Differential mucin gene expression in the ovinecervix contribute to breed differences in fertility. 71st Annual Meeting of European Federation of Animal Science, August 31st to September 4th, 2020. Online.
7. Abril-Parreño L, Meade KG, Cormican P and Fair S. Cervical transcriptomic profiling identifies breed- and phase-specificchanges between high and low

fertility sheep breeds. Annual Research and Innovation Day, December 17th, 2019. Department of Biological Sciences, University of Limerick, Limerick (Ireland).

8. Morgan-Davies C, Kyle J, Creighton P, Boman IA, Lambe N, McLaren A, Wishart H, Krogenaes A, Wall E, Padiou T, DiskinMG, Meade KG, McHugh N, Abril-Parreño L, Caldas-Silveira E, Druart X and S Fair. SusSheP – Sustainable sheep production in Europe. European Federation for Animal Science (EAAP), 70th Annual Meeting, 2019. Ghent, Belgium.
9. Abril-Parreño L, Meade KG and Fair S. Cervicovaginal mucus properties and composition in sheep. Annual ResearchDay, December 11th, 2018. Department of Biological Sciences, University of Limerick, Limerick (Ireland).
10. Abril-Parreño L, Donovan A, Diskin MG, Fair S. Ewe breed differences in cervicovaginal mucus properties at a naturaland synchronised oestrus. British Society for Animal Science, Annual Conference. April 9-11th, 2018. Croke Park, Dublin (Ireland).
11. Abril-Parreño L, Meade KG and Fair S. Ewe Breed Differences in Cervical Function. Annual Research Day, December13th, 2017. Department of Biological Sciences, University of Limerick, Limerick (Ireland).
12. Fair S (2019) Sheep Breeders Round Table Conference. Presentation by S. Fair entitled 'Understanding the biologybehind fertility'. East Midland Airport 26/11/2019
13. Abril-Parreño L, Meade KG, Cormican P and Fair S (2019) Cervical transcriptomic profiling identifies breed- and phasespecific changes between high and low fertility sheep breeds. Department of Biological Sciences Research and Innovation Day, December 17th, University of Limerick, Limerick, Ireland.
14. Abril-Parreño L, Cormican P, Krogenæs A, Meade KG and Fair S (2020) Characterisation of the cervical transcriptome in Irish and Norwegian sheep breeds known to have divergent pregnancy rates following cervical insemination with frozen-thawed semen. ICBF and Sheep Ireland Genetics Conference, January 17th, The Heritage Killenard, Laois, Ireland.

Poster Presentations

1. Abril-Parreño L, Wilkinson H, Krogenæs AK, Morgan J, Gallagher ME, Reid C, Druart X, Saldova R, Fair S. Glycomicprofiling of cervical mucins in ewe breeds with differences in cervical sperm transport following cervical insemination. International Congress on Animal Reproduction. June 26th – 30th, 2022. Bologna, Italy.
2. Abril-Parreño L, Fair S. Biochemical and molecular characterisation of sialylated mucins and their impact on cervicalsperm transport. Bernal Research Day, April 26th, 2022. Bernal Institute, University of Limerick, Limerick, Ireland.

3. Krogenæs AK, Abril-Parreño L, Druart X and Fair S. Ewe breed differences in the cervical mucus metabolome. EuropeanSociety of Domestic Animal Reproduction, October 10th – 12th, 2021. Thessaloniki, Greece.
4. Abril-Parreño L, Cormican P, Krogenæs AK, Druart X, Meade KG, Fair S. Characterisation of the cervical transcriptome inIrish and Norwegian sheep breeds known to have divergent pregnancy rates following cervical insemination with frozenthawed semen. ICBF and Sheep Ireland Genetics Conference. January 17th, 2020. The Heritage Killenard, Laois (Ireland).
5. Abril-Parreño L, Meade KG, Cormican P and Fair S. Cervical transcriptomic profiling of high and low fertility sheepbreeds. Bernal Research Day, December 5th, 2019. Bernal Institute, University of Limerick, Limerick (Ireland).
6. Abril-Parreño L, Donovan A, Diskin M.G, Meade KG and Fair S. Ewe breed differences in cervical anatomy and cervicalmucus properties at a natural and synchronised oestrus. Teagasc Sheep Open Day. July 9th, 2018. Animal & Grassland Research and Innovation Centre, Athenry (Ireland).
7. Abril-Parreño L, Wall E, Pabiou T, Creighton P, Diskin MG, Meade KG, McHugh N and Fair S. (2017). European ResearchArea Network Research Project on Sustainable Sheep Production (SusSheP). Abstract of a poster presentation presented at Teagasc Sheep Open Day. June 21st, 2017. Animal & Grassland Research and Innovation Centre, Athenry (Ireland).
8. Morgan-Davies C, Creighton P, Boman IA, Blichfeldt T, Krogenaes A, Lambe N, Wall E, Padiou T, Diskin MG, Meade KG,Mchugh N, Druart X, Fair S (2018) SusSheP – how to increase sustainability and profitability of European sheep production? Presented at the 69th annual meeting of the European Federation of Animal Science, 27th - 31st August, Dubrovnik, Croatia.
9. McLaren A, McHugh N, Lambe NR, Pabiou T, Wall E, Boman IA (2020) Factors affecting ewe longevity on sheep farms inthree European countries, Small Ruminant Research, Volume 189, 106145, doi.org/10.1016/j.smallrumres.2020.106145