Development and application of 3D livestock organoid models

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TAKE-HOME MESSAGE

Ruminant-derived stem cells from different sections of the GI tract differentiate into **tissue- and individual animal-specific organoids** that can be used to more **precisely model host-parasite interactions** in the lab within **target species**.

These organoids can be cultured to **overexpress rare cell types**, allowing the study of specialized cells *in vitro* at a **greater level of detail, resolution and precision than previously possible.**

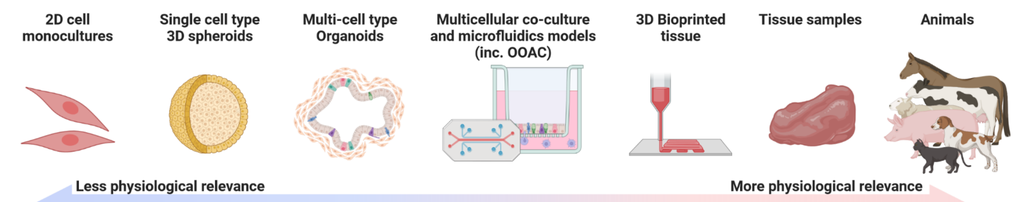
BACKGROUND

"**Organoids**" are stem cell-derived, three-dimensional, miniature versions of a specific tissue from a specific species. We have been developing organoid biotechnology based around **livestock species**, which we are applying to **model infectious diseases in the lab**.

This is providing unprecedented insight of host:pathogen interactions that was not previously obtainable in live animal models or pre-existing lab culture systems. It also **reduces reliance on live animals as experimental models** of infectious diseases. Improved understanding of how particular pathogens infect and persist within their host and more specifically, the molecules involved, will result in the identification of new drug and vaccine targets.

ORGANOIDS IN CONTEXT: BALANCING RELEVANCE, COMPLEXITY AND PRECISION

Host models of differing complexity in veterinary research

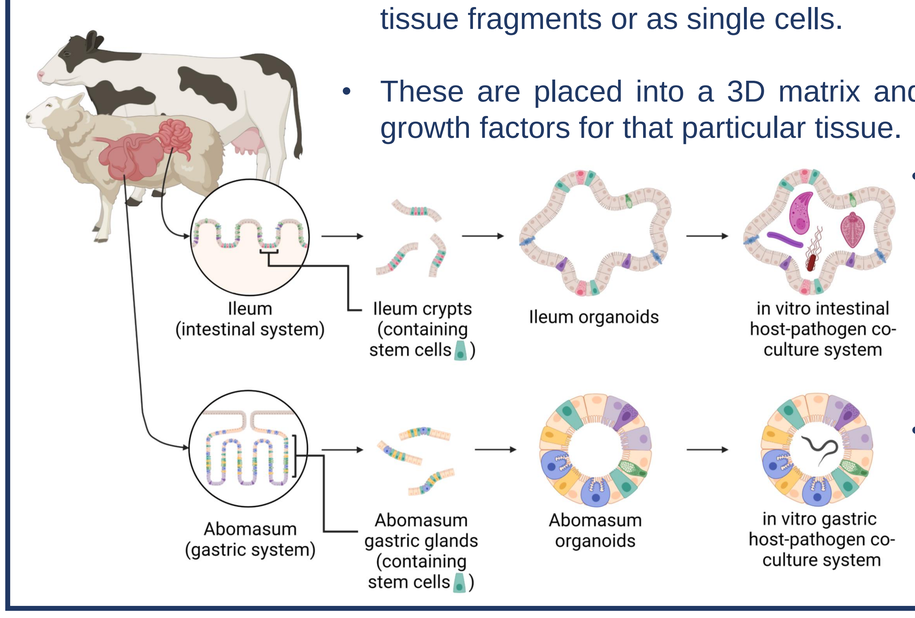


	2D cell monocultures	Single cell type 3D spheroids	Multi-cell type Organoids	Multicellular co-culture and microfluidics models (inc. OOAC)	3D Bioprinted tissue	Tissue samples	Animals
Ease of maintenance	++++	++++	++++	++	+	+	++
Relative cost	++	++	++	+++	++++	+++	+++
Physiological relevance	+	+	++	++	+++	++++	++++
3D Architecture	none	+	++	++	+++	++++	++++
Multicellularity	+	++	+++	+++	+++	++++	++++
Biological variation	+	+	++	++	+++	++++	++++

Smith *et al*, 2022, *Vet Record*

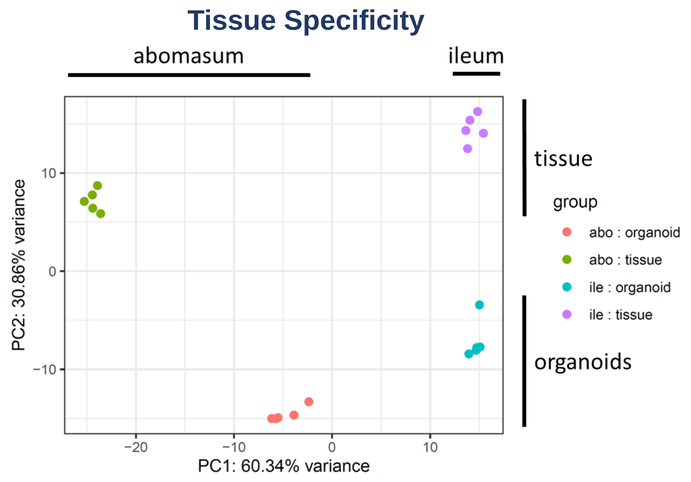
ORGANOID CULTIVATION

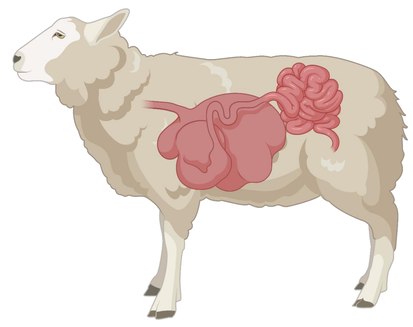
- Primary stem cells are extracted from the target tissue, either in tissue fragments or as single cells.
- These are placed into a 3D matrix and provided with relevant growth factors for that particular tissue.
- Cells develop and self-organize into a 3D organoid, as a miniature representative of the target tissue.
- As an example, the schematic depicts gastrointestinal organoid development.



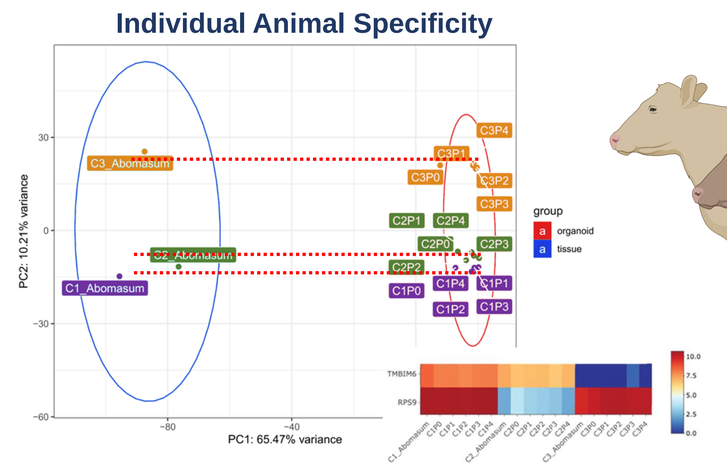
ORGANOID TRANSCRIPTOMES ARE REFLECTIVE OF SOURCE TISSUE AND DONOR ANIMAL

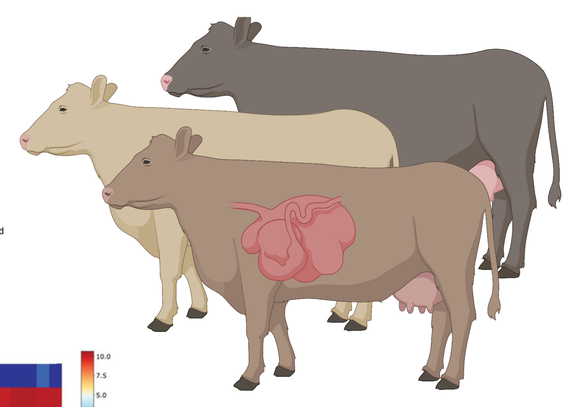
Organoids from different individual tissues retain **tissue-specific gene expression profiles** (e.g. abomasum vs ileum), despite being cultured in identical conditions. Furthermore, organoids also retain **specific gene expression profiles associated with a particular individual animal**, as some genes show animal-to-animal variation in their expression level and this variation is retained among organoids derived from these animals





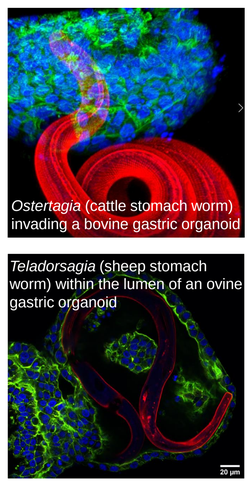
Smith *et al*, 2021 *Front Cell & Inf Micro*

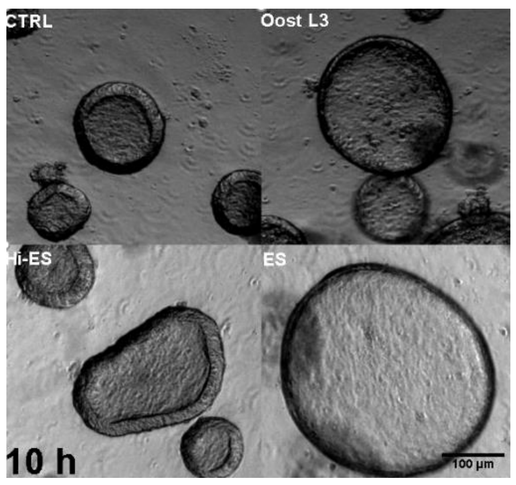




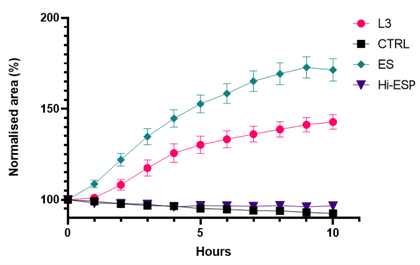
Faber *et al*, 2022 *Front Cell & Inf Micro*

MODELLING DISCRETE HOST:PATHOGEN INTERACTIONS IN ORGANOIDS

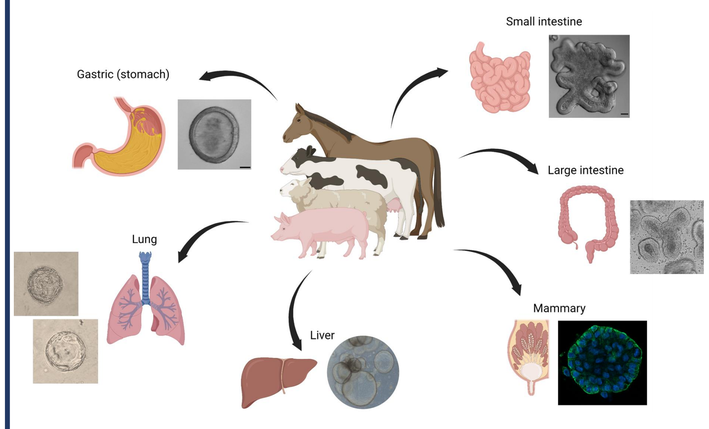




"Ballooning" in GI nematode and nematode ES-treated organoids: an example of a discrete host:pathogen interaction that can now be modelled in the lab – in this example, we hypothesize ballooning is a mechanism used by certain gastric parasites to invade host tissue



DEVELOPING ANIMAL ORGANOIDS FOR ALL DISEASE-RELEVANT TISSUES



As part of a RESAS-funded research programme, we are continuing to develop organoids from different livestock species and tissues that are commonly afflicted by infectious diseases.

We will apply these models to investigate host:pathogen interactions and novel technologies for diseases intervention (e.g. vaccines and vaccine delivery systems

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