

## ➤ State of the art in understanding inter-organ crosstalk and physiology in farm animals

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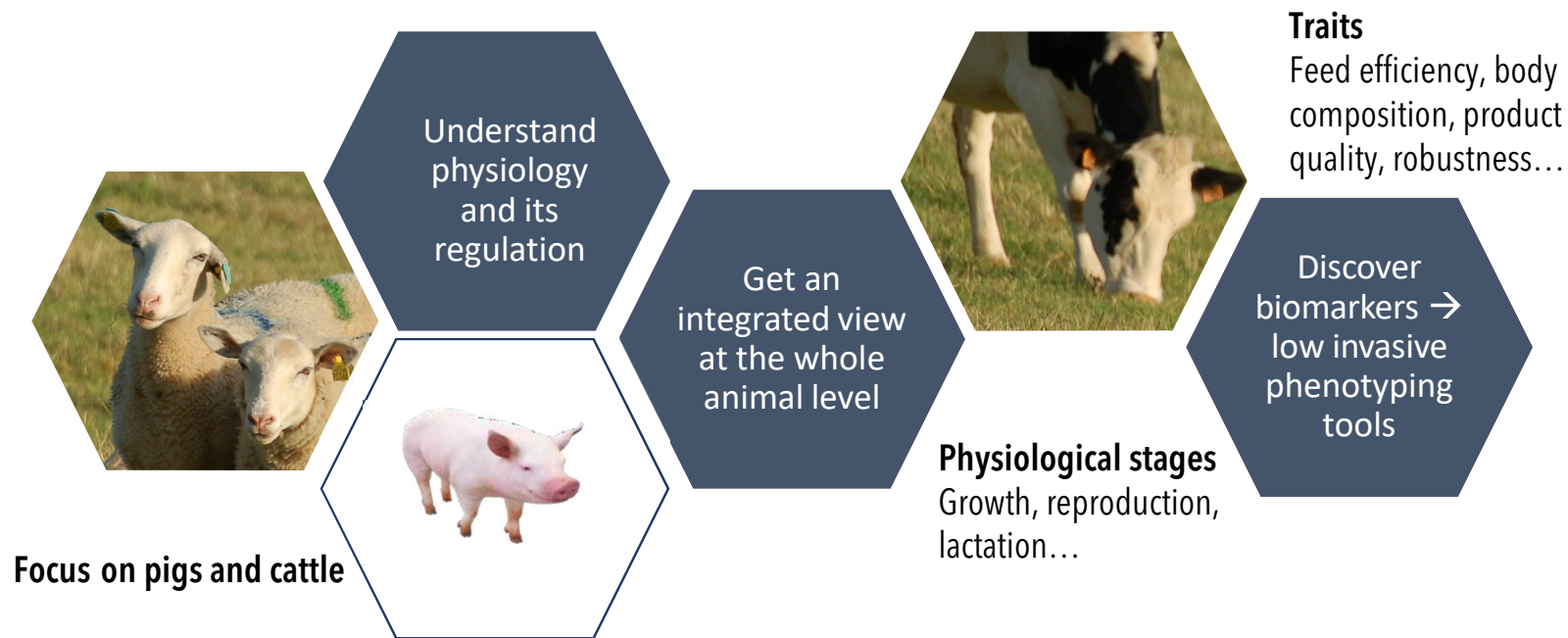
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**EAAP- 74th annual meeting, Lyon 2023 - Session 37**

## ➤ Revisiting Farm Animal Physiology

Managing phenotypic traits associated with health and performance of livestock animals across a wide range of production practices and climatic conditions



Not a novel issue, but new opportunities offered by recent advances in molecular biology, computation, and biosensors and *in vitro* technologies

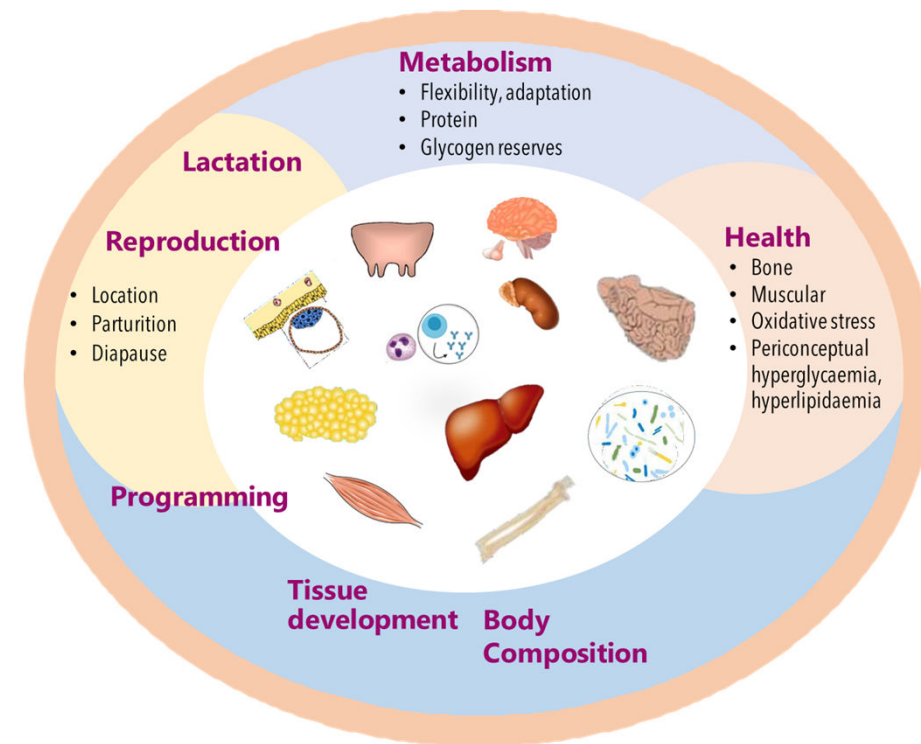


## ➤ Inter-Organ Crosstalk : An Intricate Network of Communication

A sophisticated network of communication makes it possible to maintain body homeostasis and homeorhesis when facing different conditions.

- Tissue and organ systems functioning
  - Concerted action (between cells, between organs)
- Coordination of multiple functions
- Many types of signals used to ensure cell-to-cell communication locally or at long distance

→ *Focus on biochemical signals*

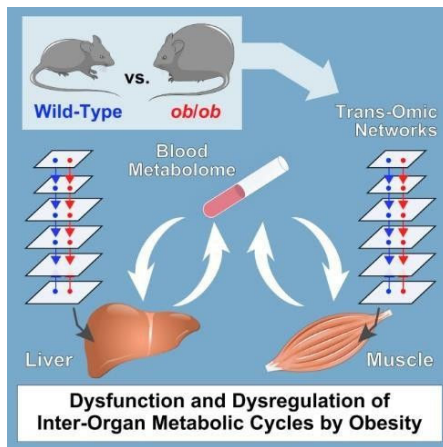


*The “symphony”  
of tissue and organ systems*

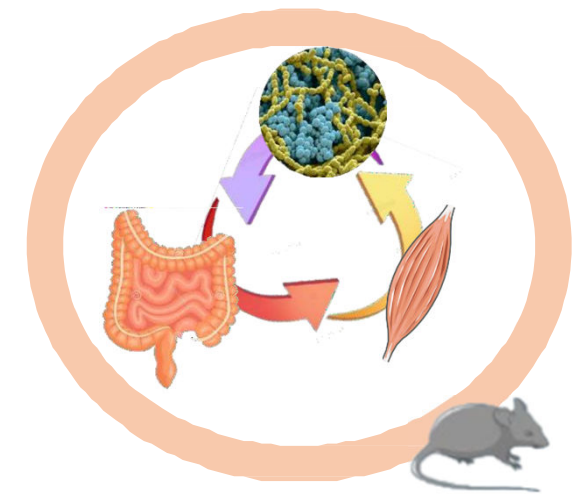
## ➤ Inter-Organ Cross-Talk: Holistic Dimension

A topic of increasing importance in physiology and pathophysiology

- Progress in omics and data science: a new way of doing physiology
- An further dimension : symbiotic interactions with resident microbiota (holobiont)



*Egami et al, 2021*

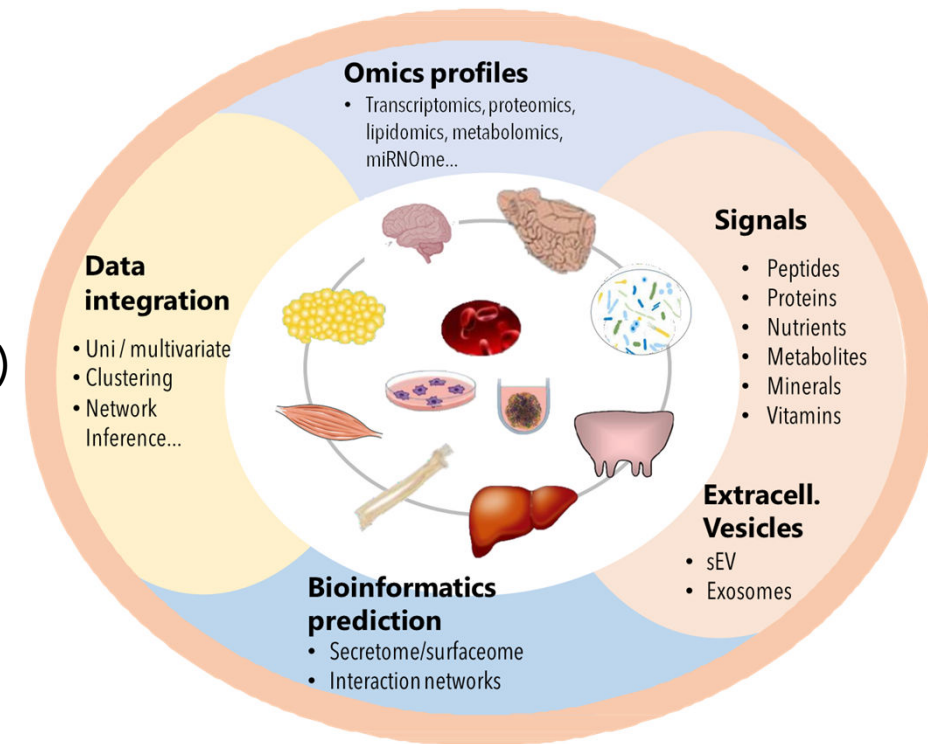


*Nay et al 2019*

## ➤ A Complex Scenario of Crosstalk Mediators

Understanding the systemic dimension of organ crosstalk requires taking into account the complexity of actors and their interactions

- To collect multiscale data: multi-compartment, multi-omics, longitudinal data...
- To integrate extensive and heterogeneous data (omic profiles, phenotypic data, environmental data...)
- To develop bioinformatics workflows for data mining and network analysis



# ➤ Adipose Tissue and Muscle Crosstalk

## Adipose tissue - muscle crosstalk modulates body composition and energy efficient use

### Nutrition, environment

- Common stem cell populations in both tissues
  - Composition of stem cell populations:
    - can be affected by several factors including nutritional factors and hygiene conditions
    - opposite effects between muscle and adipose tissue for some populations
- impact on the ratio of lean mass to fat mass

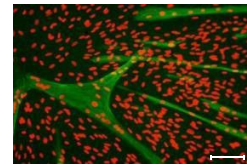


*Perruchot et al, 2013; 2021; Quéméner et al, 2022*

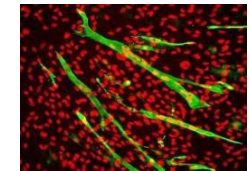
### Nutrition, genetics

#### 2D cell cultures : co-cultures or conditioned media

- Isolation of myoblasts and adipocytes
- Impact of adipocytes on myogenesis ; influence of adipocytes from different deposits or genetic types?

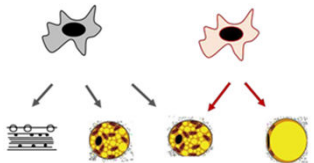


myoblasts  
+ differentiated adipocytes



myoblasts  
+ proliferative adipocytes

*Cassar-Malek et al, 2006*



Recent advances in the generation and characterization of induced pluripotent stem cells from pigs and ruminants → easier to work cell lines

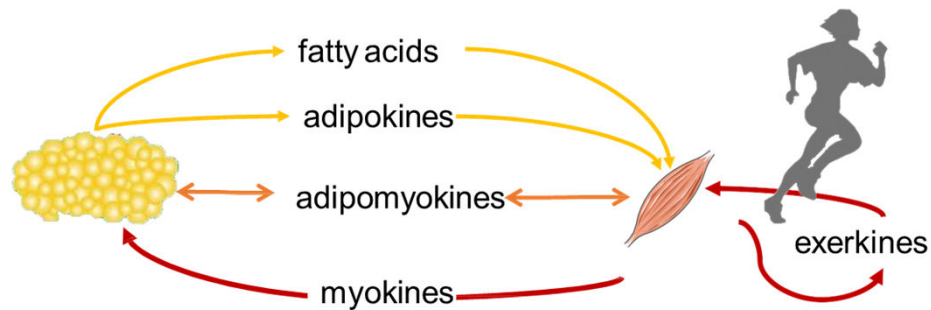
*Su et al, 2020, Zhang et al, 2022, Stout et al, 2023*

myogenesis    adipogenesis  
→ relative muscle and fat deposition



## ➤ Adipose Tissue and Muscle are Secretory Tissues

These tissues control their respective mass and homeostasis by exchanging multiple signals, including organokines\*.



Metabolic adaptation + nutrient partitionning

*Gonzales-Gil et al, 2020*



Molecular BioSystems

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Cite this: *Mol. BioSyst.*, 2016, 12, 2722

Integrated data mining of transcriptomic and proteomic datasets to predict the secretome of adipose tissue and muscle in ruminants†

M. Bonnet,<sup>\*ab</sup> J. Tournayre<sup>ab</sup> and I. Cassar-Malek<sup>ab</sup>

- 1749 proteins secreted by both muscle and adipose tissues (adipomyokinome)
- 357 muscle-specific (myokinome) and 188 adipose-specific proteins (adipokinome)

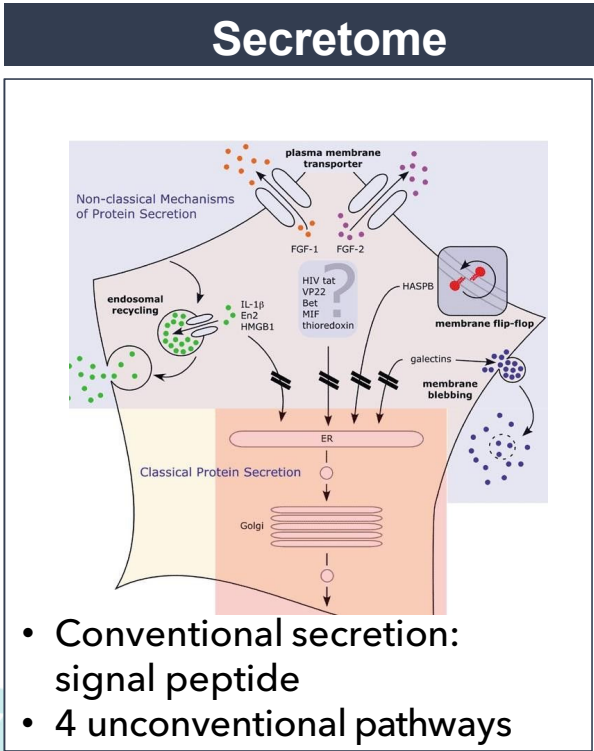
*Bonnet et al, 2016*

\* Peptides and proteins released from the cells (secreted proteome) = e.g. cytokines incl. interleukins, interferons, chemokines

# ➤ The Cellular Secretome / Surfaceome and Organ Crosstalk

## Identification of proteins involved in the adipose–muscle crosstalk using prediction of the secretome and surfaceome from protein sequences

Adipose tissue proteome  
(140 dpc)



13 predicted links

Interaction between plasminogen (adipokine) and α-enolase:  
→ regulation of myogenesis

Bonnet et al, 2020

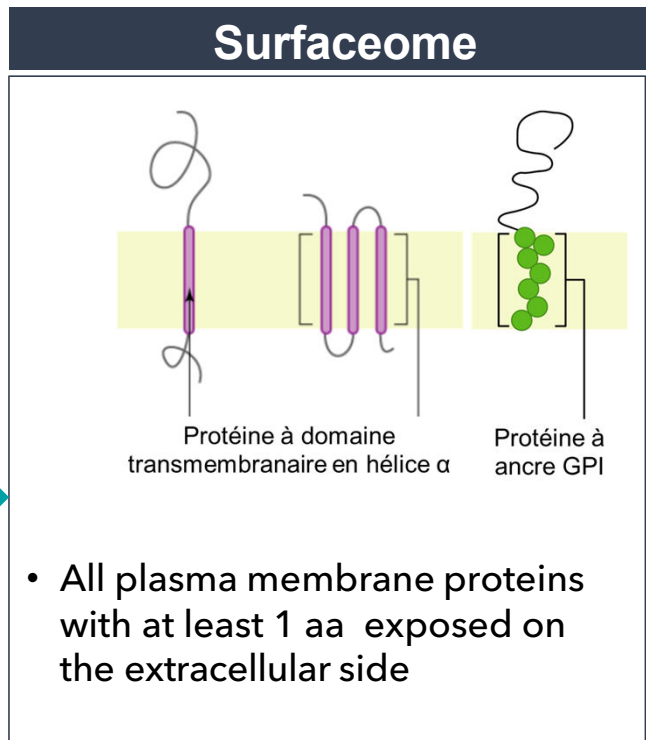


Protein-protein interaction



Kaspric et al, 2015  
Connault et al, 2021

Muscle proteome  
(140 dpc)





# ➤ Extracellular Vesicles (EVs) and Adipose Tissue - Muscle Crosstalk

## Role of the EV pathway in the adipose tissue-muscle crosstalk

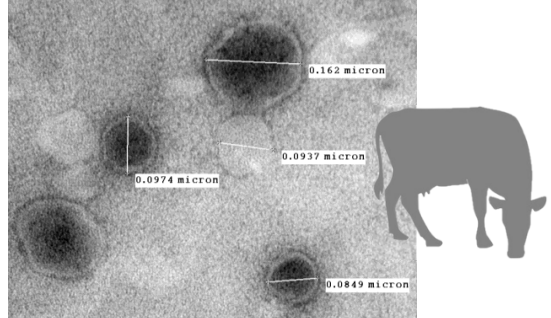
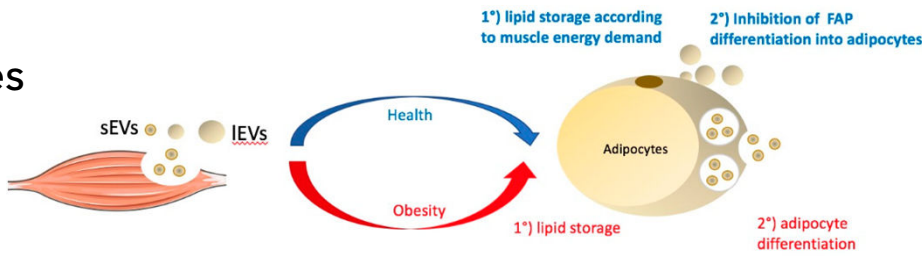
- Extracellular vesicles are important actors in the exchanges between tissues in healthy context and metabolic disease (as conveyers of cellular information)
- In vitro : muscle-released EVs control lipogenesis in adipose tissue → control of adipose tissue expansion by muscle

*Rome et al, 2019 ; Rome 2022*

- EVs isolated from plasma or milk may provide physiological signatures of production traits in farm animals.

Milk small EVs/adaptation to lactation

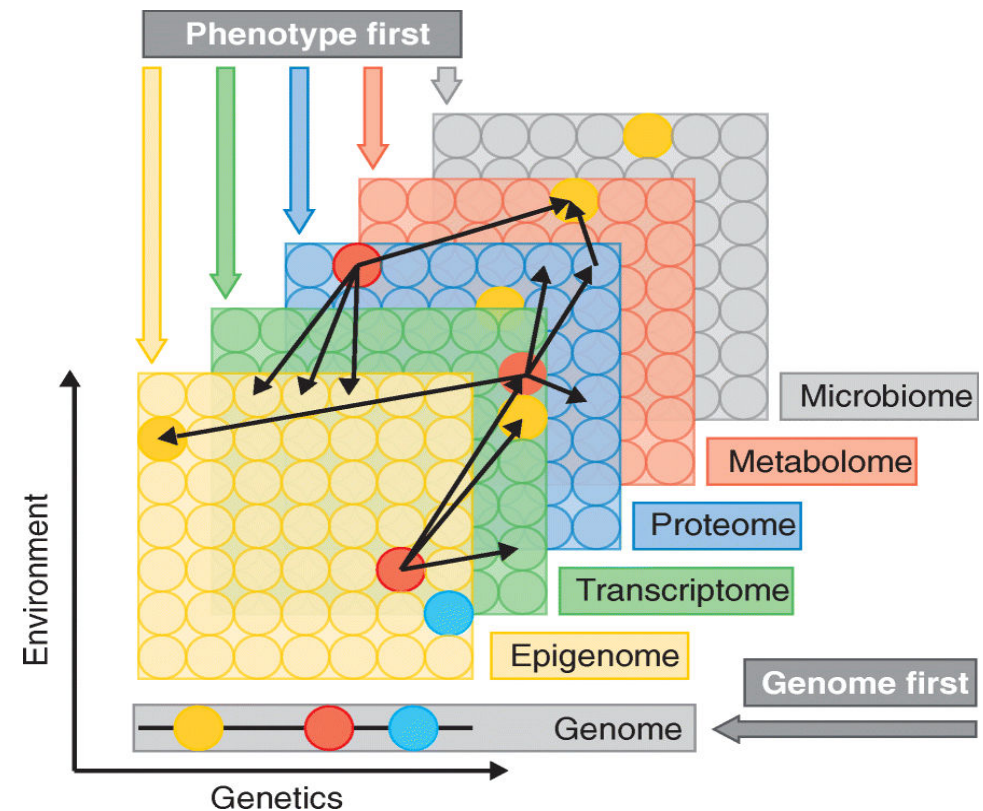
*Boby et al, 2023 (EAAP com)*



## ➤ Multiple Omics Profiling: Digging Deeper into Organ Crosstalk

Rapid advances in omics and computation are enabling multi-omics deep phenotyping

- Vertical access to different omics layers will help capture the complex molecular interplays of physiology and disease
- However most of these omics datasets are still assessed individually.



*Hasin et al, 2017*

# ➤ Unravelling Organ Crosstalk through Omics Profiling of tissues

Identification of co-expressed genes/proteins in tissues and the biological pathways associated with variations in feed efficiency

## Non productive functions

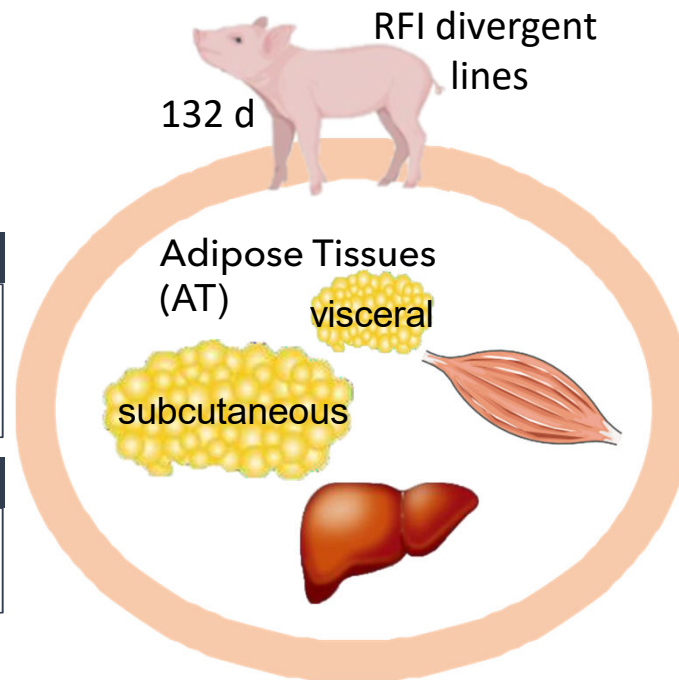
Most efficient animals

### Shared by the 4 tissues

- ↘ Immunity
- ↘ Defense response  
(↘ maintenance metabolism)

### Tissue specific modulation

- ↘ Oxidative stress in response to a health challenge in the AT



## ≠ energy use strategies

Most efficient animals

### Tissue specific modulation

- ↘ Oxidation of FAs and ↗ glycogen use in muscle
- ↗ Oxidation of FAs in the AT  
(= short-term energy strategy)

*Gondret et al., 2017; Sierzant et al., 2019; Messad et al., 2019*

Similar approach in growing cattle

*Cassar-Malek and Bonnet, 2018; + study in progress*



# ➤ Unravelling Organ Crosstalk through Omics Profiling of Biofluids

## Plasma and milk protein profiling in cows strongly divergent for milk fat secretion

⇒ *Divergence in DMI, energy balance, whole-body insulin sensitivity and nutrient partitioning*

- Plasma : 15 DAP involved in the interplay between nutrient availability and the endocrine system or the immune function  
*incl. adiponectin with insulin-sensitizing effect*
- Milk : 38 DAP involved in lipid metabolism/secretion and in inflammatory/immune processes

*Delosière et al., 2023; Vheskhini et al., 2022*



## Blood profiling in growing pigs



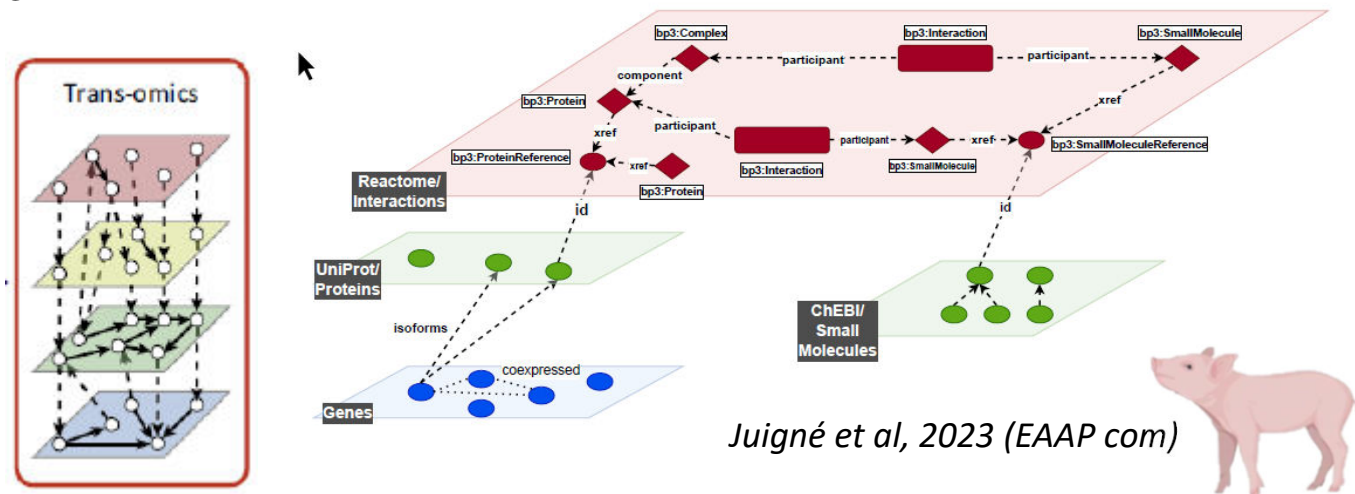
- At the mRNA level (transcriptomics profiling) and the metabolic level (NMR profiling)
- Biomarkers and predictors of gain:feed ratio, ADG or body fat content have been proposed

*Messad et al., 2021; Juigné et al., 2023*

## ➤ Advanced Statistical Methods and Machine Learning Algorithms

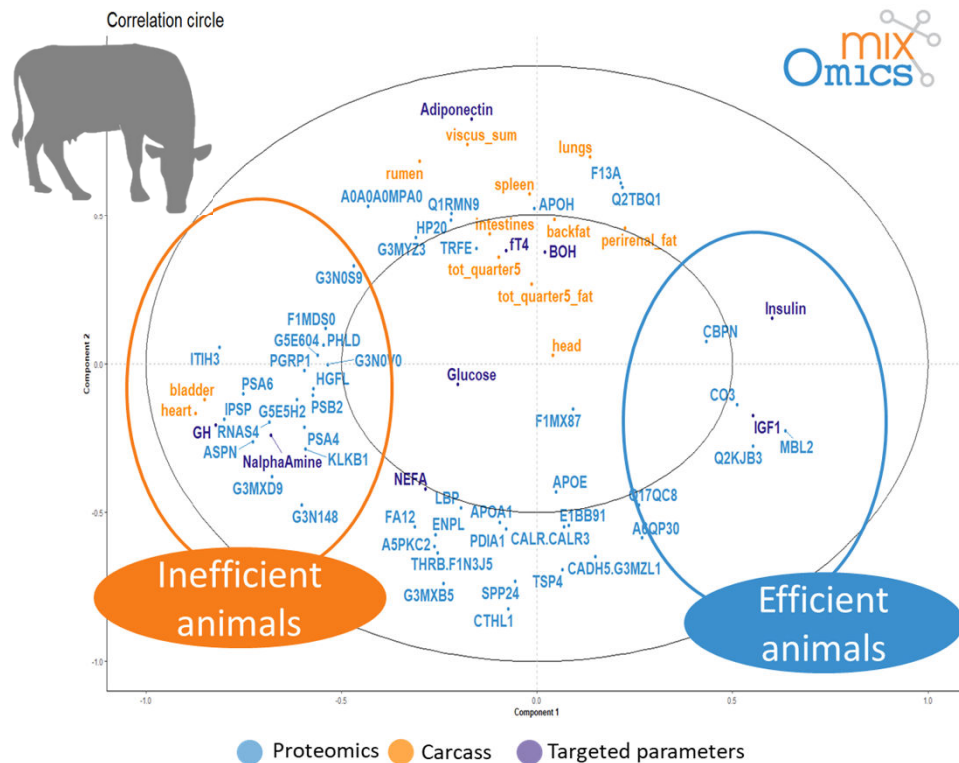
The development of mathematical methodologies used for the interpretation of organ crosstalk and physiology is needed.

- Analysis of large datasets powered by machine learning algorithms
- Integration of data from multiple sources powered by advanced statistical methods (e.g. multivariate, multi-block analyses)
- Informatics analysis of interaction between entities at different levels by using graphs of reactions



## ➤ An Example of Heterogenous Data Integration

### Contribution of plasma proteins to the phenotypic signature of feed efficiency (RFI) in Charolais young bulls



### Multiblock sPLSDA integration

Biosignature combining 25 proteins, 3 carcass traits, and 8 hormones or metabolites

Efficient bulls when compared to inefficient bulls:

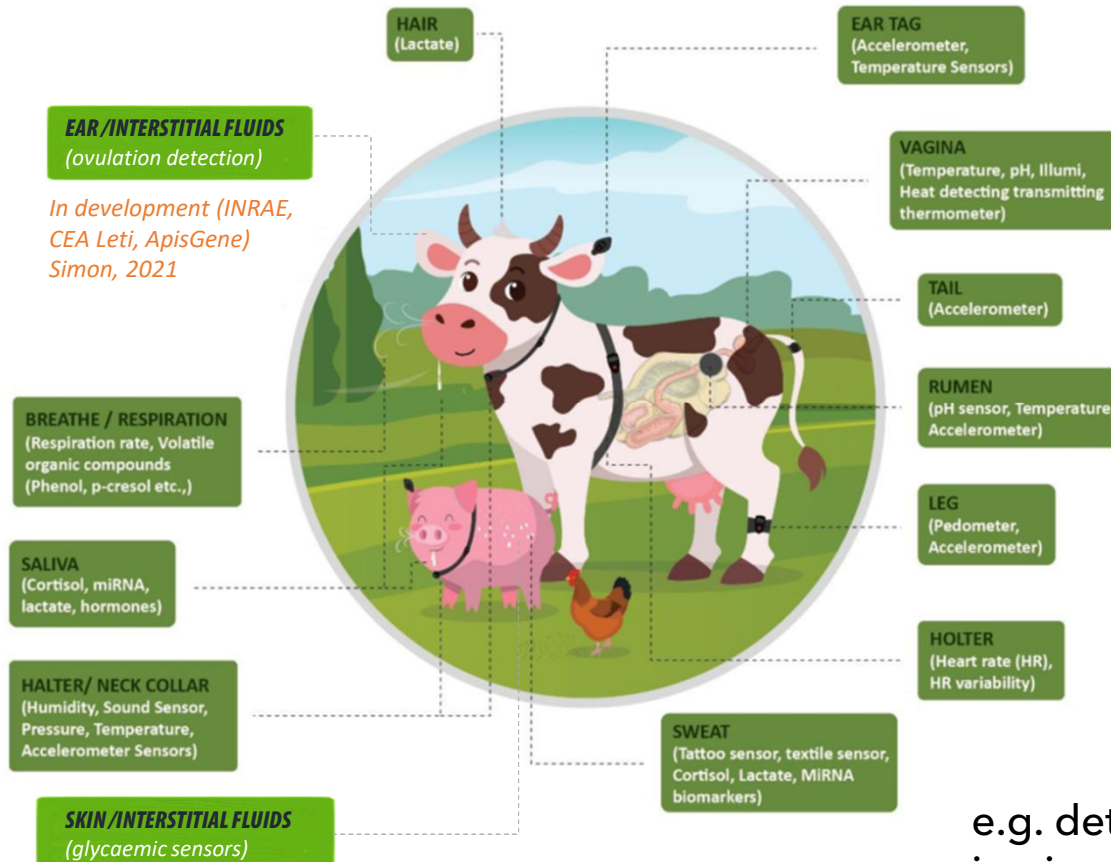
- Smaller bladder and heart
- Lower GH, adiponectin, FT4, alpha amino nitrogen and NEFA concentrations
- Higher insulin and IGF-I concentration
- 20 plasma proteins less abundant and 5 plasma proteins more abundant.

Proteomic data best discriminated RFI groups

*Cassar-Malek et al, 2023 (EAAP poster)*



## ➤ Using Biosensing Technologies for Real-time Monitoring



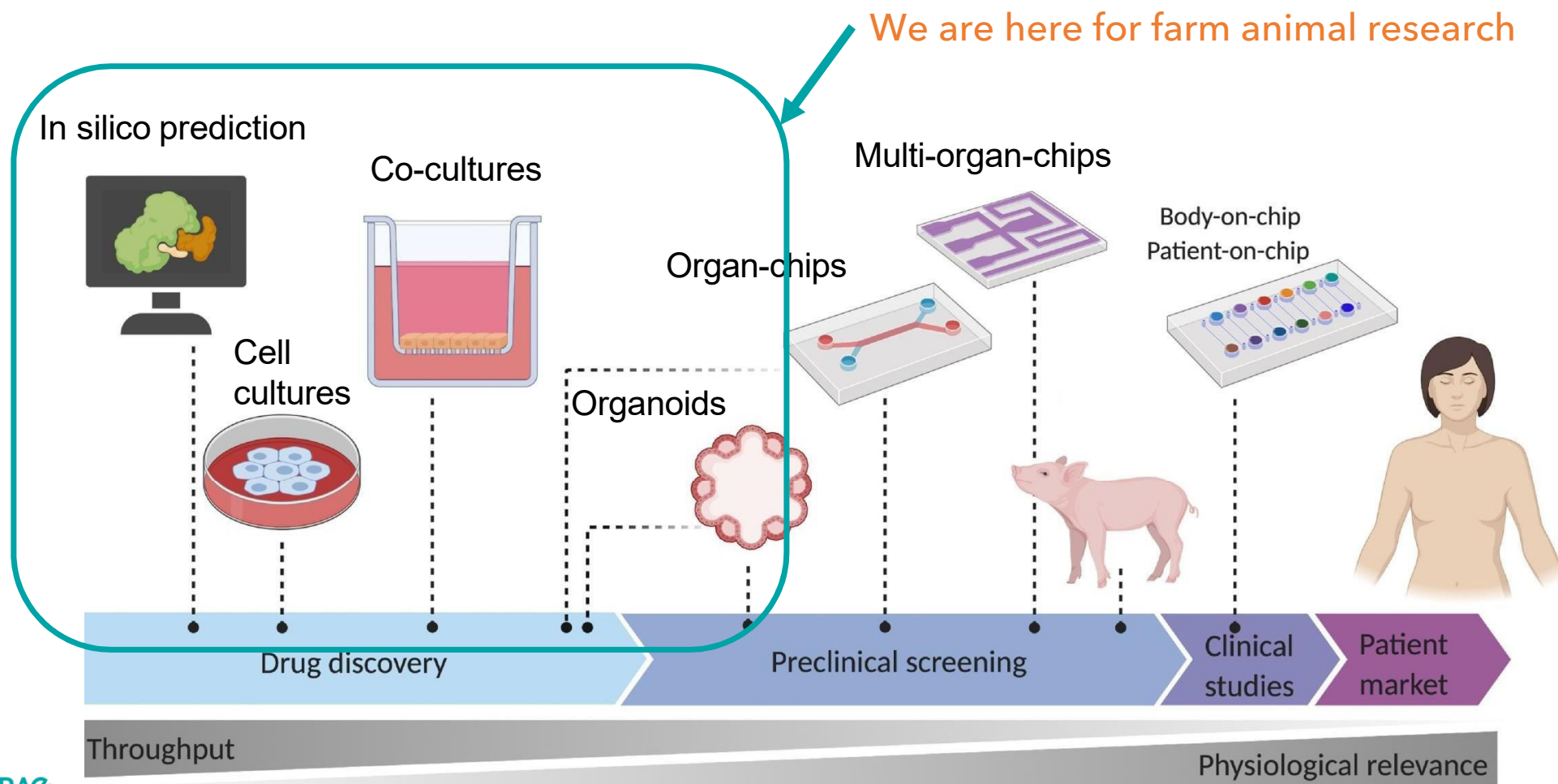
Adapted from  
Neethirajan 2020

Wearable /Implantable biosensors  
+ non wearable biosensors  
(e.g. inline milk sensing and blood analysis)

- Tracking activity, location or heat, and real-time monitoring of a wide range of physiological parameters (incl. hormone levels, metabolic markers, and immune function)

e.g. detection early signs of compromised health and welfare in pigs (Alameer et al, 2020) or in dairy cows under subacute ruminal acidosis (Wagner et al, 2020) using ML methods

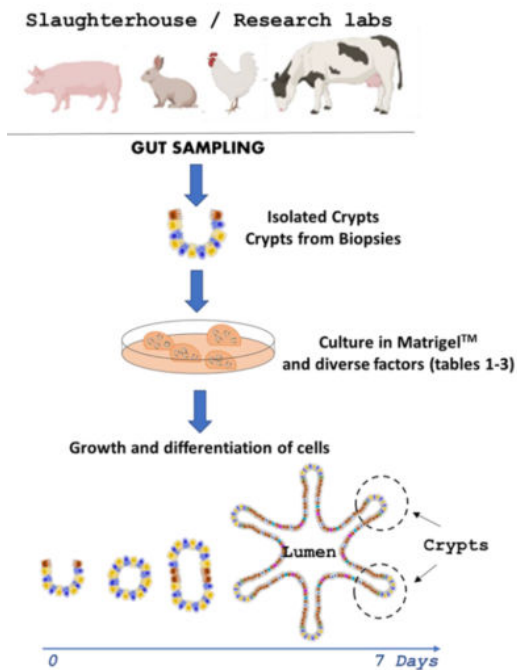
## ➤ Emerging Fields of Research: Highly Relevant *in vitro* Models



## ➤ Organoids: A New Frontier in Farm Animal Research

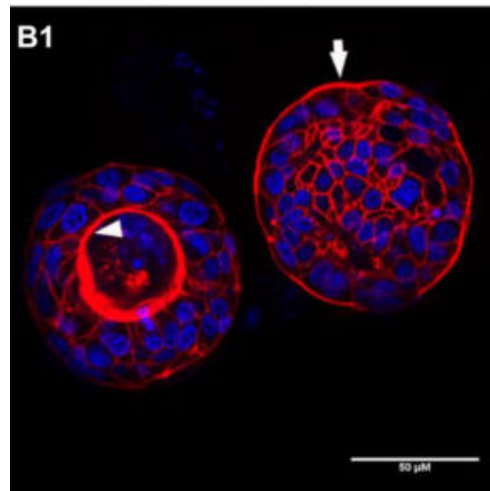
### Reducing animal experiments through organoids culture (3R alternative)

#### Engineered 3D versions of organs



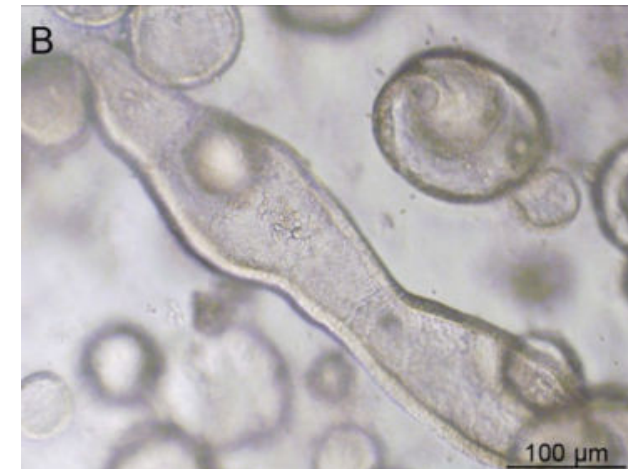
#### Piglet colon organoids

*Beaumont et al, 2021*



#### Bovine oviduct organoids

*Bourdon et al, 2021*



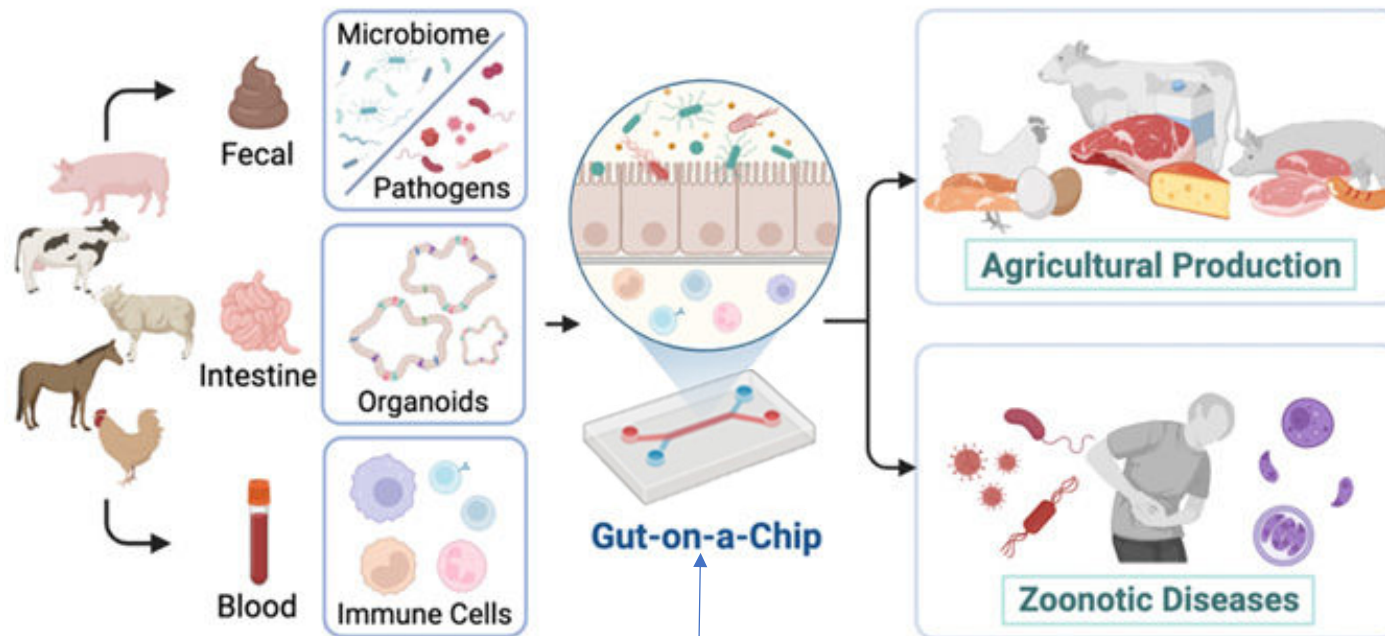
Organoids produced in farm animal species :

- Intestinal organoids in pigs, cattle, sheep, horse, and chicken
- Oviduct, mammary gland, lung (cattle)
- Gallbladder and testis (pig)

*Beaumont et al, 2021 ; Kar et al, 2021; Ma et al, 2022; Kawasaki et al, 2022*

## ➤ The Potential Applications of Organ-on-Chips in Farm Animals

Gut-on-a-chip systems could lead to deeper insights in physiological and pathological conditions through one health initiatives



*Tachibana and Ambrosini (2022)*

3R alternative

- Organoids or 3D cell culture
- Microfluidic chip

## ➤ The Step Forward : Multi-organ-a-chip systems (multi-OoC)

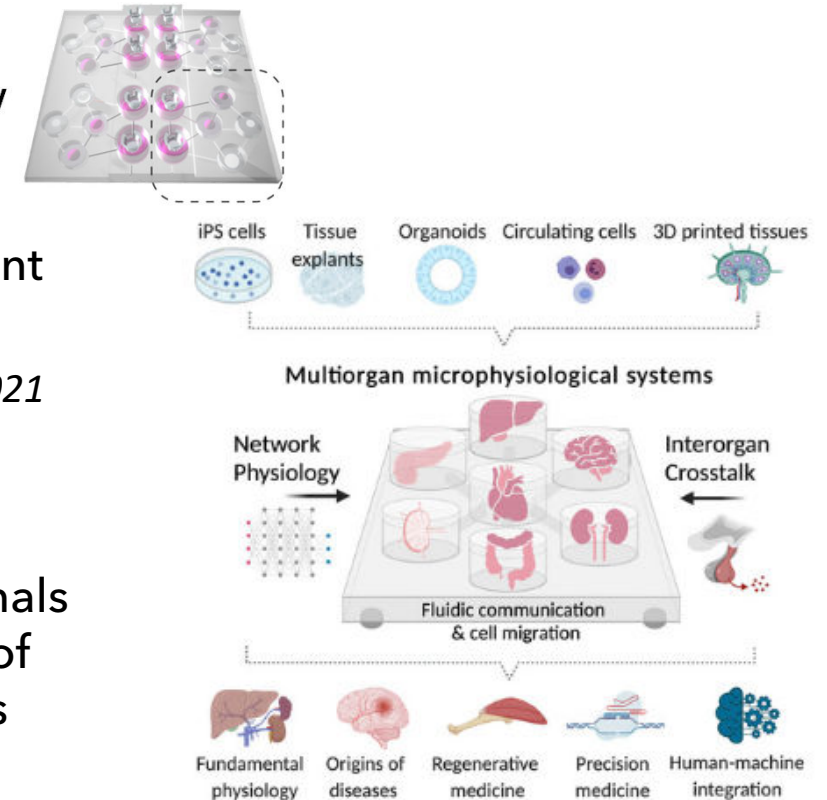
### Multi-organ physiological systems as tools to interrogate inter-organ crosstalk

Separate organ chambers connected by microfluidic flow channels precisely emulating blood circulation

Further development is needed before the full deployment of OoC and multi-OoC as routine bench-tools.

*Picollet-D'hahan et al, 2021 ; Vunjak-Novakovic et al, 2021*

**In farm animal research:** physiological response of animals exposed to challenges, effects of nutrients, progression of diseases... w<sub>o</sub> subjecting animals to harmful experiments (3R alternatives)

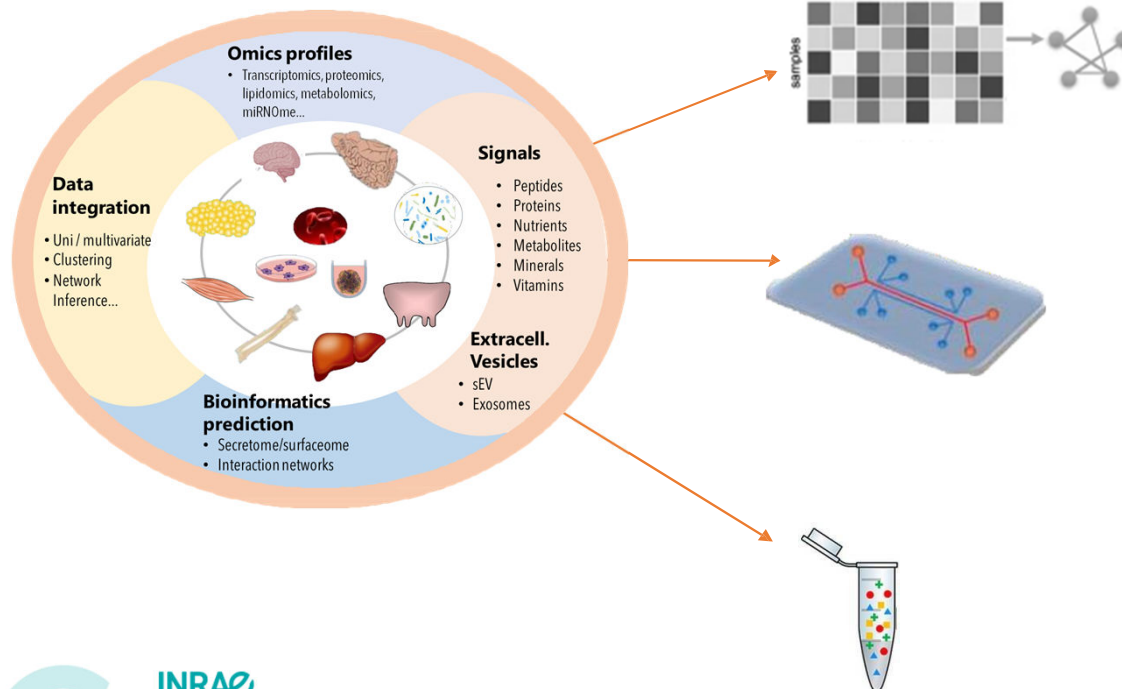


*Trapezar, 2021*



## ➤ Conclusion

There are several opportunities to revolutionize the study the inter-organ crosstalk in farm animal physiology by taking advantage of new technologies.



**There is still work to do in order to:**

- Collect, analyse and integrate large datasets generated by omics, biosensors, and even imaging technologies (powered by machine learning algorithms )
- Decipher integrated physiology while reducing animal experimentation (organs-on-chip, multi-oc)
- Discover low invasive biomarkers (incl. the EV source) and develop efficient and minimally invasive phenotyping tools



## ➤ Thank You for Your Attention

### EAAP- 74th annual meeting, Lyon 2023 - Session 37

