# Mass spectrometry imaging of lipids reveals the differences in fatty acid metabolism between ovarian and follicular compartments in porcine, bovine and ovine ovaries

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# Mass Spectrometry Imaging of lipids reveals the differences in fatty acid metabolism between ovarian and follicular compartments in porcine, bovine and ovine ovaries.


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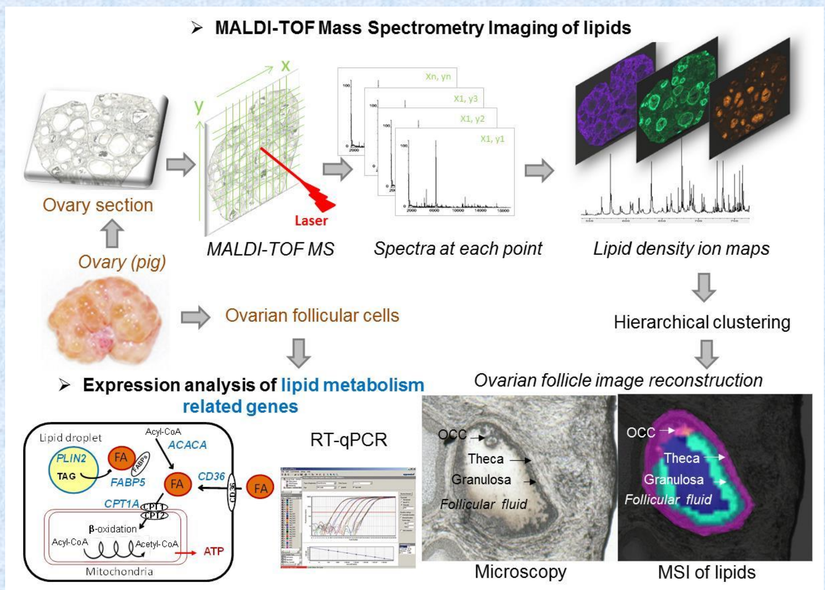
## INTRODUCTION

In mammals, an oocyte develops inside of **ovarian follicle** and this process is strongly supported by **surrounding follicular environment** consisting of somatic cells and follicular fluid. In antral follicle, the final stages of oogenesis require a lot of energy which is produced by follicular cells from different substrates including glucose, amino acids and fatty acids (**FA**). FA metabolism plays an important role in acquiring of oocyte developmental competence in human and in domestic animals.

**Objective** was to elucidate the specificity of FA metabolism at ovarian level by comparing lipid profiles and expression of FA metabolism-related genes in different ovarian compartments.

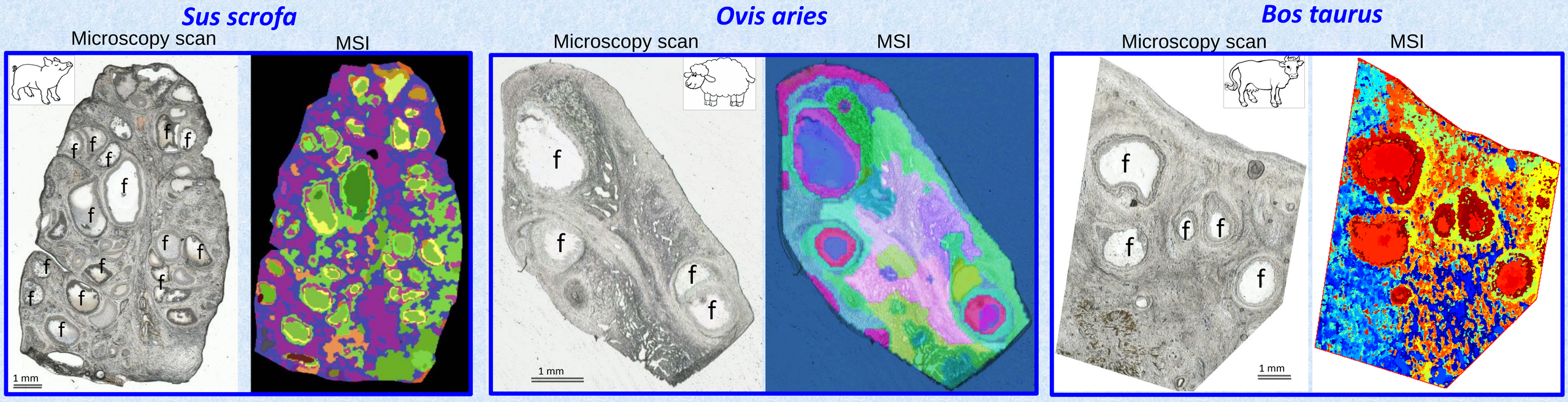
## EXPERIMENTAL APPROACHES



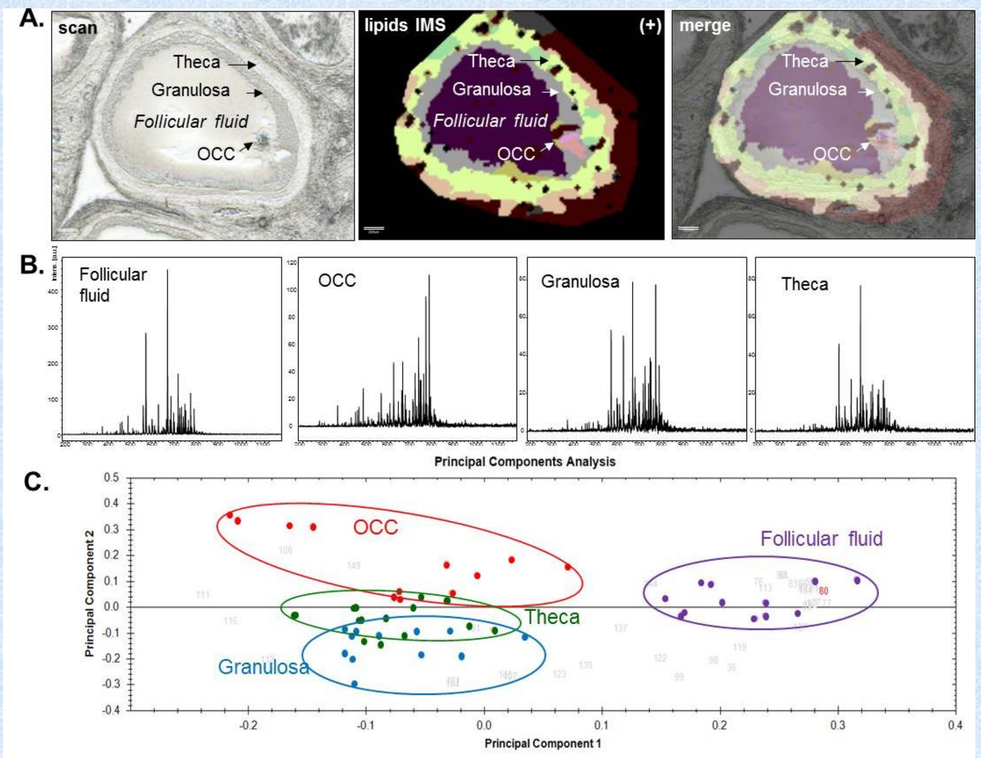

1) **Ovaries:** pig, sheep, cow; 10µm frozen sections
2) **Mass Spectrometry Imaging (MSI) of lipids:** UltrafleXtrem MALDI-TOF/TOF spectrometer (Bruker). Matrix : DHB or CHCA Resolution **:** 22 or 50 µm
3) **Clustering analysis :** FlexImaging or SCiLS Lab
4) **Real time PCR:** Reverse transcription of 100 ng total RNA & qPCR, 8 replicates.
5) **Statistics:** ANOVA, $p<0.05$.

## RESULTS

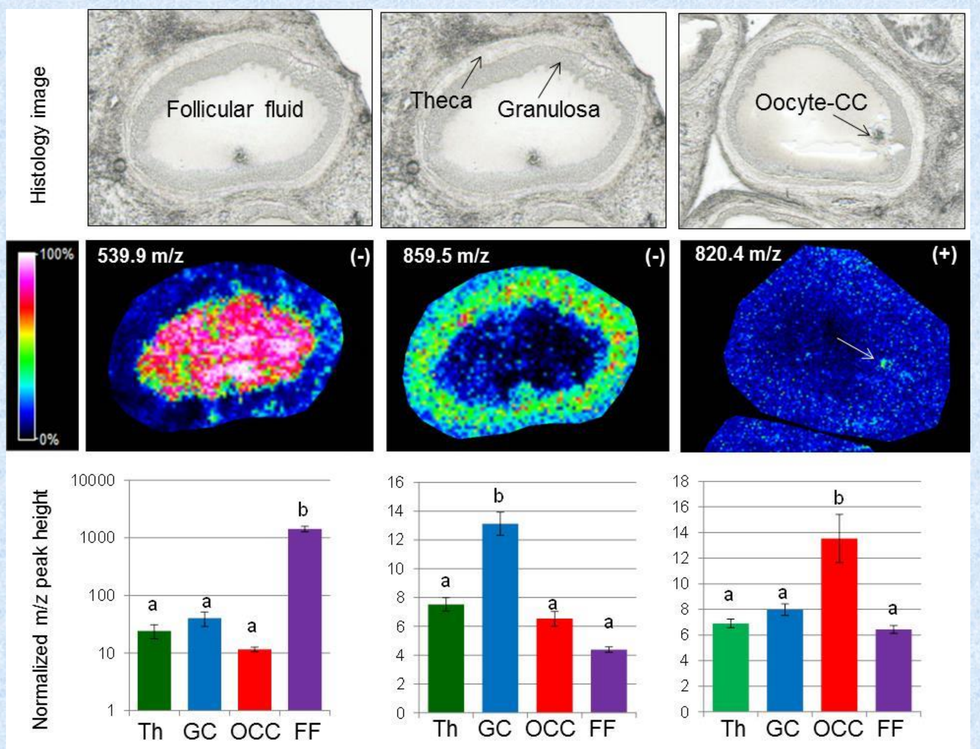
**1. Lipid MSI images** of ovarian sections were **reconstructed from lipid ion signals** (200-1200 m/z range). Ovarian section are shown as microscopy scan images, MSI are reconstructed images from lipid density ion maps. **Follicles (f)** are distinguished from interstitial tissues.



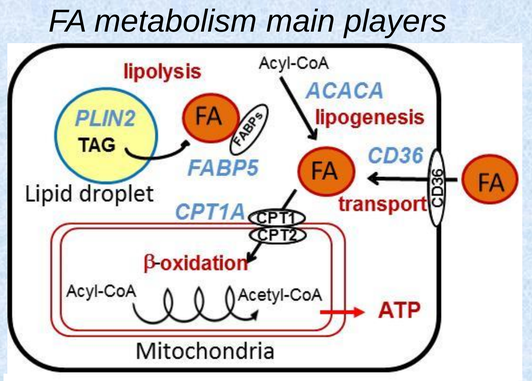

**2. Hierarchical clustering and Principle Component Analysis** of lipid spectra from individual follicles allowed discrimination of different follicular cells (granulosa, theca, oocyte-cumulus) and follicular fluid.

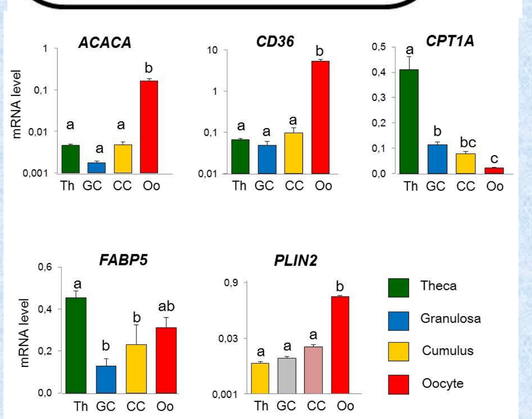



**3. Quantification** of **lipid species abundance** in different types of follicular cells and in follicular fluid showed variations in lipid content between follicular compartments. **Comparison of mRNA level** confirmed significant differences in lipid metabolism between ovarian follicular cells.




*FA metabolism main players*




**Lipid distribution in whole porcine follicle by targeted MALDI MSI analysis performed in positive ion mode.**

**(A)** Histological image of follicle (scan), its molecular reconstruction image (lipids MSI) and their superposition (merge) . Scale bars - 200µm. **(B)** Representative MALDI-TOF MS single spectra acquired directly from the region of interest (FF, OCC, GC and Th) of porcine ovary section in the m/z 200-1200 range. **(C)** PCA of lipids differentially abundant between follicular compartments. Each dot corresponds to single spectra extracted randomly from the structure of interest (purple – Follicular Fluid, red – Oocyte-Cumulus Complex, blue – granulosa cells, green - Theca cells). The grey numbers represent differential m/z species ($p<0.05$).

**Single ion density maps and quantification of three lipid species in porcine ovarian follicles.**

Lipid ion species measured at m/z 539.9, m/z 859.5 (in negative ion mode) and at m/z 820.4 (in positive ion mode) showed enriched abundance in different follicular compartments (FF-follicular fluid, GC –granulosa cells and OCC-Oocyte-Cumulus Complex, respectively). Histograms present the mean values ± SEM of the ion intensities measured at 12 positions throughout Th, GC, OCC and FF compartments.

**Gene expression analysis of FA metabolism-related genes in porcine ovarian cells.**

Transcripts coding for ACACA (Acetyl-CoA-carboxylase A), CD36 (FA transporter), CPT1A (carnitine palmitoyltransferase 1), FABP5 (FA binding protein 5) and PLIN2 (perilipin 2) were quantified in porcine follicular cells (Th, GC, CC and oocyte) by real time PCR. Histograms present relative mRNA level ± SEM.

## CONCLUSION

Different distribution of lipid species through the ovary, shown by MS Imaging, together with differential expression of FA metabolism related genes between the follicular cell types demonstrated that **lipid metabolism differs between ovarian and follicular compartments**.


Physiologie de Reproduction et des Comportements
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Plate-forme d'Analyse Intégrative des Biomolécules et de Phénomique des Animaux d'Intérêt Bio-agronomique
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