Basal ewe folliculogenesis from preantral to antral stages: Gene clustering and Connexin 43 protein profile by transparisation coupled to immunolocalization

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Ovarian Club XVII Meeting
Building A Bridge Between Science And Clinical Practice
May 16-17, 2024 • Paris, France

Basal ewe folliculogenesis from the preantral to antral stages: Gene clustering and Connexin 43 protein profile by transparisation coupled to immunolocalization

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Introduction:

Basal follicular growth is a slow process controlled by a privileged molecular dialogue between the oocyte and somatic cells. A pivotal event is the formation of an antral cavity within the granulosa and the differentiation of the oocyte's closest cells into cumulus cells. Towards elucidating the mechanisms regulating these morphological and functional changes, we have analyzed expression of a panel of genes from the small preantral to the small antral follicular stages. Next, we have focused onto **Connexin43** encoded by the ***GJA1*** gene. Connexins are components of gap-junctions, channels that form between neighbour granulosa/cumulus cells, but also between **transzonal projections** (TZP) and the oocyte. The exchange of small molecules through gap-junctions throughout folliculogenesis is crucial for ultimately producing a **mature oocyte**.

Methods:

Ovaries were recovered year-round from peripubertal sheep at a local slaughterhouse.

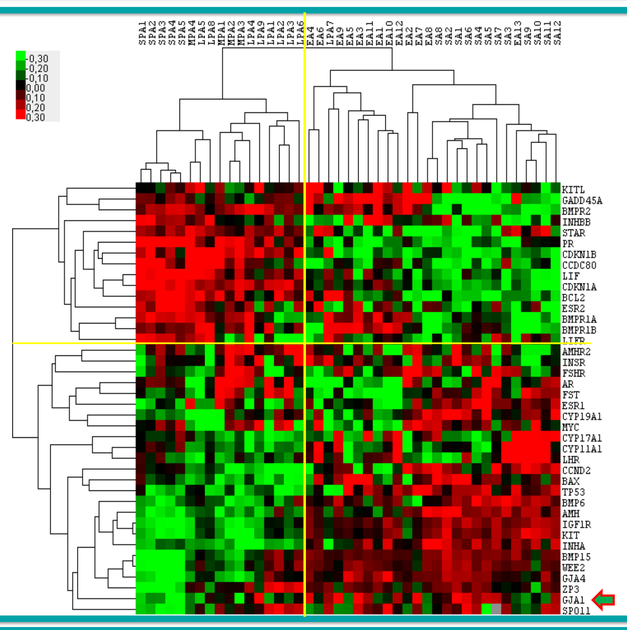
1389 follicles (80-850µm) were collected from **ovarian cortex strips** and dispatched into in **43 samples** representing **5 follicular size classes**:
small preantral (**SPA**), medium preantral (**MPA**), large preantral (**LPA**), early antral (**EA**) and small antral (**SA**), as previously described (Cadoret V et al., Reprod, 2017).

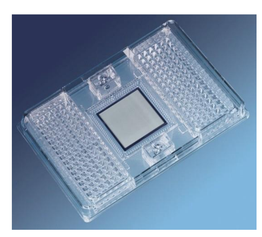
Expression of **39 genes** was analyzed using the microfluidic qPCR BioMark™ HD System from Fluidigm and subjected to hierarchical clustering.

Protein expression of **Connexin 43 (CX43) and TUBULIN alpha**, was examined by **immunofluorescence** on ovarian cortex strips and individual follicles after **CUBIC tissue clearing** and Methyl Green nuclear staining. **Transzonal projections of oocyte-cumulus complexes (OCCs)** were detected without previous tissue clearing.

Results 1: Gene expression

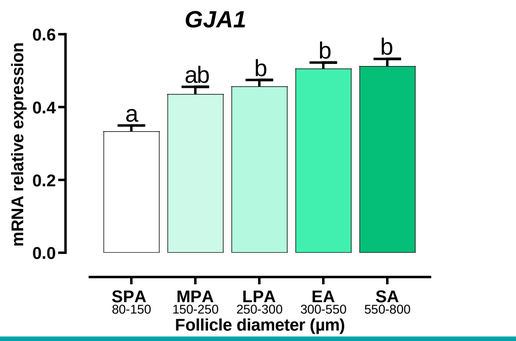
Hierarchical clustering segregated preantral and antral follicles and revealed changes in the expression of genes involved in cell proliferation and differentiation including hormone secretion, but also in the **communication between the oocyte, cumulus and granulosa cells like the *GJA1 gene* coding for CX43**



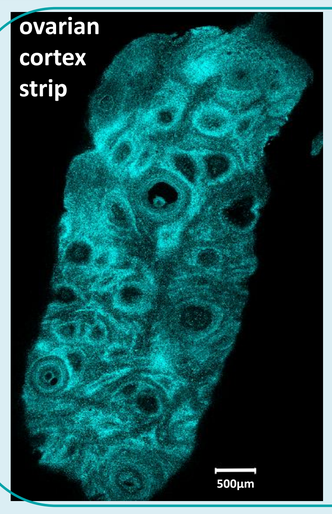


GJA1 expression increased slightly during follicle growth.

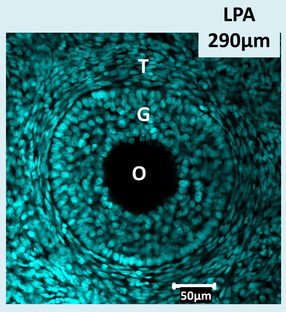
Gene expression is normalized with 3 reference genes : SDHA, RPL19 and beta-ACTIN.
One way ANOVA test and Tukey's multiple comparison post test.

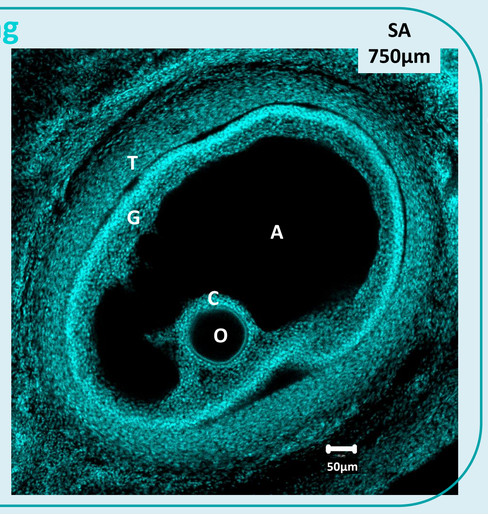


Results 2: Ovarian cortex and follicle clearing : CX43 expression



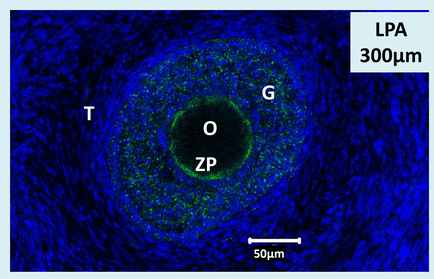
Cortex nuclear staining



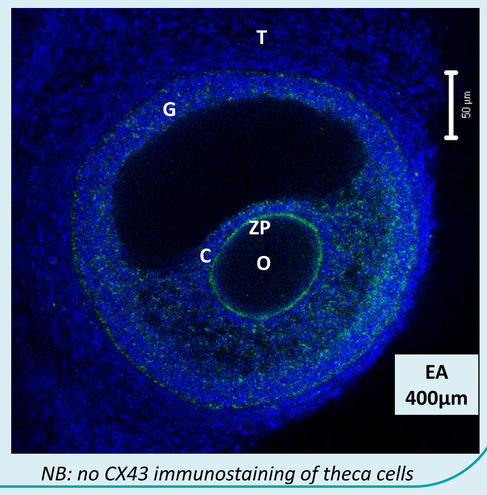


CUBIC tissue clearing allowed to study the follicles population and proteins localisation *in situ* up to a depth of 1 mm in the **ovarian cortex strips**

CX43 localisation in the cortex

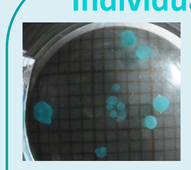


Cx43 was detected between granulosa/cumulus cells and at the periphery of the zona pellucida (ZP)

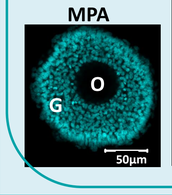


NB: no CX43 immunostaining of theca cells

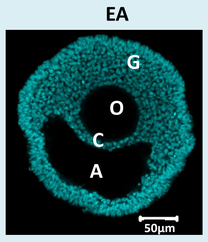
Individual follicle nuclear staining



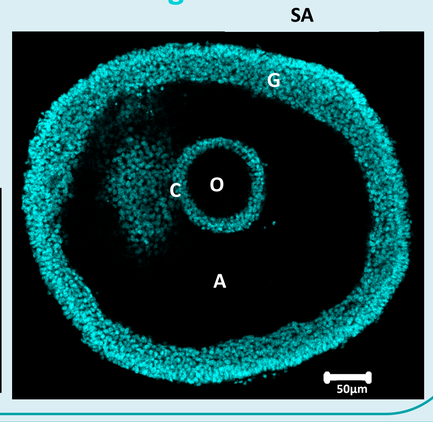
Medium Pre antral MPA



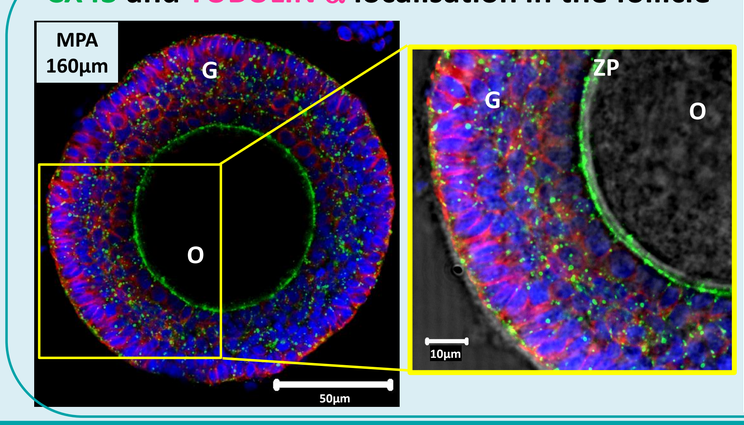
Early antral EA

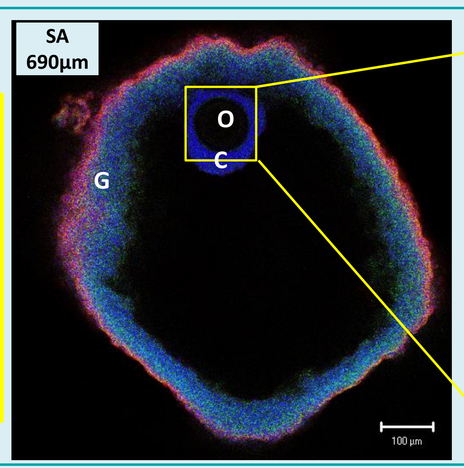


Small antral SA

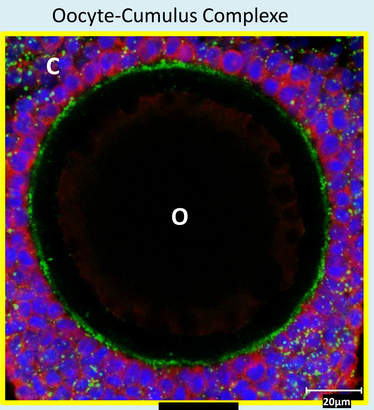


CX43 and TUBULIN α localisation in the follicle





Oocyte-Cumulus Complexe

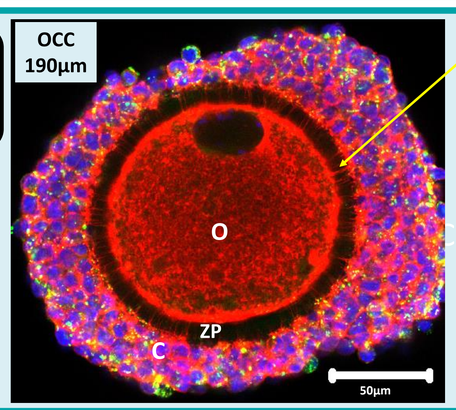


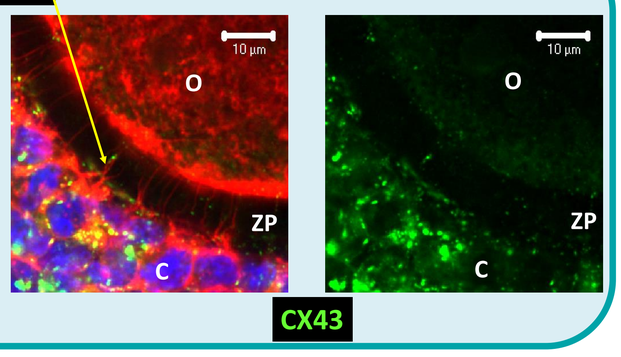
CX43

Results 3 : Oocyte-Cumulus Complexe (OCC) : CX43 localisation and TZP detection

Cx43 was detected in OCCs closed to the **Transzonal Projections (TZP).**

Transzonal projections of oocyte-cumulus complexe were highlighted after Actin F staining with Phalloidin Alexa 555 without tissue clearing





T: Theca cells
G: Granulosa cells
C: Cumulus cells
A: Antrum
O: Oocyte
ZP: Zona Pellucida
TZP: Transzonal Projection

Conclusion:

The destiny of a follicle relies on a delicate balance in the expression and activity of factors involved in proliferation, differentiation, apoptosis and cell-cell communication. Here, the **Cubic clearing method**, in combination with Methyl Green labelling and/or immunodetection, is established as a tool to observe and characterize follicles within the ovarian cortex of ewes and likely all large mammals including humans. It will also be applied to assess ***in vitro*** **follicle development following ovarian cortex culture or isolated follicle culture**, for research purposes or in the context of fertility preservation.

Acknowledgements
The authors thank Albert Arnould for ovary collection at the slaughterhouse, GeT PlaGe for the Fluidigm experiment and PIC for the confocal microscopy experiments.

Financement:
CRYOVAIRE