

**Basal folliculogenesis from preantral to antral follicles:
Genes clustering and Proteins expression analysis by transparrisation
Focus on the expression of CX43
A model for the validation of *in vitro* follicle culture**

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Background: Basal follicular growth is a slow developmental process controlled by a privileged molecular dialogue between the oocyte and its surrounding follicular cells. A pivotal stage in this development is the concomitant formation of an antral cavity within the granulosa and their differentiation into cumulus cells, but the mechanisms regulating these morphological and functional changes remain poorly understood. For this purpose, we have analyzed a large panel of genes implicated in the main functions of the oocyte and follicular cells from the small preantral to the small antral follicular stages. Then, we have focused our protein study onto Connexin 43 encoded by the *GJA1* gene. These gap-junctions are channels allowing the transfer of small molecules between the oocyte and the corona radiata cells through the transzonal projections (TZP), and between the granulosa/cumulus cells themselves. Their regulation is crucial throughout folliculogenesis for the ultimate production of a mature oocyte.

Methods: In prepubertal ewes, 1389 follicles from 80µm to 800µm were collected from ovarian cortex strips and dispatched in 43 samples and 5 follicular size classes. The expression of 40 genes was analyzed using the qPCR BioMark™ HD System from Fluidigm and studied with a hierarchical clustering analysis. The protein expression was examined after transparrisation by immunofluorescence in cortex strips or individual isolated follicles.

Results: The hierarchical clustering analysis showed that the 3 classes of preantral follicles (PA) were clustered separately from the 2 classes of follicles with antrum. Follicle development and antrum formation were accompanied with changes in the expression of genes involved in follicular cell proliferation, apoptosis and granulosa cell differentiation but also in the communication between the oocyte, cumulus and granulosa cells. *In vivo*, the expression of the *GJA1* gene slightly increases during the growth of follicles (1.5 fold) and the CX43 is well detected specifically in the granulosa/cumulus compartment (excluding theca) and concentrated at the periphery of the oocyte along the zona pellucida closed to the TZP.

Conclusions: The destiny of the follicle is dependent on a delicate balance in the expression and actions of actors involved in proliferation, differentiation, apoptosis and between-cell communication inside the growing follicle. At the genomic level as well as at the protein level, this equilibrium must be evaluated *in vivo* for the validation of *in vitro* follicle development biotechnologies, in order to produce an oocyte of good quality for subsequent fertilization in the context of fertility preservation.

Conflict of interest: No