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

Véronique Cadoret, Peggy Jarrier-Gaillard, Feriel Yasmine Mahiddine, Fabrice Guerif, Rozenn Dalbies-Tran

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Gene expression throughout ewe folliculogenesis from the preantral to antral stages:  
gene clustering and connexin 43 protein profile by transpolarisation coupled to immunolocalization

Véronique Cadoret<sup>1,2</sup>, Peggy Jarrier-Gaillard<sup>1</sup>, Ferial Yasmine Mahiddine<sup>1</sup>,  
Fabrice Guerif<sup>1,2</sup>, Rozenn Dalbès-Tran<sup>1</sup>

<sup>1</sup> INRAE, CNRS, Université de Tours, PRC, Nouzilly, France

<sup>2</sup> CHRU de Tours, LBR, Tours, France

**Background:** 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:** 1389 follicles (80-800 µm) were collected from ovarian cortex strips of prepubertal ewes, and dispatched in 43 samples representing 5 follicular size classes (small, medium and large preantral, early and small antral). Expression of 40 genes was analyzed using microfluidic qPCR followed by hierarchical clustering. Protein expression was examined after tissue clearing by immunofluorescence.

**Results:** 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. *GJA1* increased slightly during follicle growth. Connexin43 was detected between granulosa/cumulus cells and at the periphery of the zona pellucida.

**Conclusions:** 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. Evaluating these factors in *in vivo* developed follicles sets the ground for validating biotechnologies of *in vitro* follicle culture, which aim at producing an oocyte of good quality for subsequent fertilization in the context of fertility preservation.

**Conflict of interest:** The authors have no conflicts of interest to disclose.