Session 15 *Gwenola.Tosser@toulouse.inra.fr*

Unravelling the mechanisms of mammalian ovarian follicular development and atresia: a cattle vs pig transcriptome and proteome study


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Introduction:

In mammals, ovarian folliculogenesis leading to the ovulation of completely mature oocytes is a long and complex process that is regulated at different levels. However, it is already known, that between cattle and pig, the pattern of expression of some well-known genes either are very similar or strongly differ during follicular growth or atresia. Our strategy to discover genes or gene network involved in follicular antral development or atresia was to compare antral small, large, healthy or atretic follicles from cattle and pig both at the transcriptomic and the proteomic levels.

Work progression:

Follicles from ten cows and ten sows were individually dissected. The granulosa cells and the follicular fluid were then pooled within the same animal to generate 54 RNA samples and 40 proteic samples, respectively.

Transcriptome results:

Granulosa cell samples	Healthy Small Follicles	Atretic Small Follicles	Healthy Large Follicles
sow	6 (7) (1-2mm)	7 (13)	6 (8) (7-8mm)
cattle	4 (7) (3-5mm)	6 (11)	5 (8) (15-20mm)

Table 1: Origin of RNA samples used for microarray hybridisation

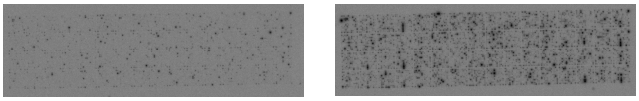

Figure 1 : Bovine (left) and porcine (right) complex probe Hybridisation on GPL3729 (porcine) microarray

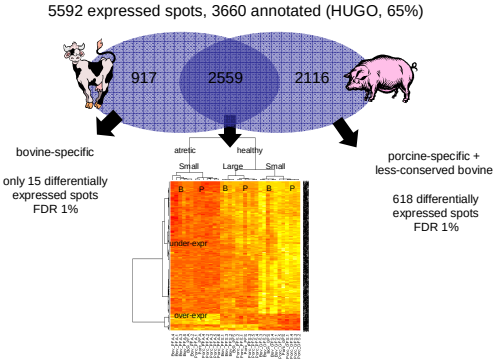

Figure 2 : Number of specifically and commonly expressed genes and statistical analysis (ANOVA, Bonferroni p-val<0.01) of the microarray data, showing 254 differentially expressed spots (242 genes, 49 unknown).
The heatmap shows that 1)follicular status an 2) species allows the classification of the follicles. Yellow color indicates under-expressed genes, whereas red colour shows over-expressed genes.

Proteome results:

Follicular fluids were subjected to 2D PAGE (100µg protein per gel; siver nitrate staining). Gels were compared within each species by using the Progenesis software, in order to visualize differential proteins between stages.

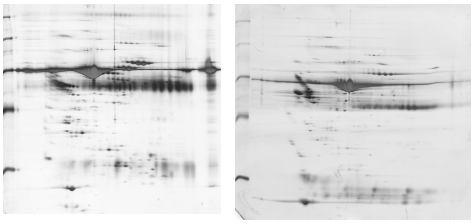

Figure 3 : Representative bovine (left) and porcine (right) follicular fluid 2D-PAGE.

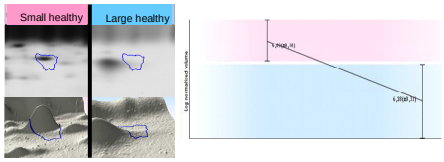

Figure 4 : exemple of semi quantitative analysis (bovine spot 12) with the Progenesis Samespot software.

	Healthy Small x Large	Atretic Small x Large	Large Healthy x Atretic
cow	13	12	3
sow	9	7	3

Table 2: Numbers of differentially expressed protein spots among the various physiological stages studied.

Conclusion:

For the transcriptome data, complementary statistical analysis are underway, to determine a minimum set of discriminant genes between species and follicular status. For the proteome data, identification of differentially expressed protein spots is underway, as well as comparison between species. We hope that integration of transcriptomic and proteomic studies will lead us to identify ovarian factors that are involved either in the healthy / atretic status of follicles or in the mono / polyovular feature of species.

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