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Toward building the cow folliculome☆

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ABSTRACT

One of the goals of the EmbryoGENE network was to gather information on the conditions leading to competent oocytes. Using a combination of transcriptomic analyses we are building the foundation of the folliculome, which will take the form of a virtual follicle with gene expression profiling data spanning small to ovulatory or atretic follicles. The different models currently being established not only provide information on the follicular conditions leading to good outcome but also intermediary steps, including evolution towards atresia. The physiology of very few species has been covered to the extent of our database, which is the only one for mono-ovulatory species. The first interesting observation extracted from our data is related to the plateau phase of follicular development, which is not a linear intermediate between growth and ovulation but rather an important modification step of tissue ontogenesis during which growth switches to differentiation or atresia. The markers of cell division, matrix rearrangement, mesenchymal differentiation, oxidation, steroidogenesis and ovulatory changes identified confirm known changes but also several others are now hinting to a more complex picture of this dynamic tissue. In addition to biomarkers, we have insight into the multiple pathways involved during the last few days before ovulation. Our new ability to validate these networks in vitro using primary granulosa cells culture also contributes to the construction of a follicular blueprint. The amazing list of gene responding to FSH alone is a good start but a complete meta-analysis will provide the foundation of the bovine folliculome.

1. Introduction

The understanding of the follicular physiology is coming to a new paradigm. Like many of my colleagues, I was trained in ovarian physiology with the knowledge obtained by measuring the levels different products or hormones in the blood thanks to the development of biochemistry and Elisa techniques. Theses hormones were limited in numbers and with probably less than 40 of them, one could explain most of the stages and functions of the ovarian follicle. I must add that still today, these hormones, FSH, LH, estradiol, progesterone, GNRH, PGF2a either as measured or as used for treatments represents the key factors controlling follicular faith. Starting in the 80s, the progressive discovery of genes expressed in the ovary or the follicle, has progressively broadened our understanding of follicular complexity. Joann Richards, amongst others, was a pioneer in the discovery of several of these new genes found to be active in the ovary. This second wave added several new players in the regulation of follicular growth and differentiation in a one by one approach that is still today very much valuable to understand the role of individual gene in the process. We now have several hundred genes for which

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an ovarian function is known (see the ovarian kaleidoscope http://ovary.stanford.edu/) and many of them have been tested in knock-out models in mouse to generate a specific phenotype. The overall analysis of these new players reveals a level of complexity that was not expected and has created a new field: system biology. We now realize that genes are working in group and influence each other in a dynamic fashion. With the development of Genomics early in this century, the capacity to analyze all genes present in a tissue created a tsunami of information that is not organized yet, creating a completely new challenge for physiologists. This review will focus on new strategies to extract analyze and connect data together towards a better physiological understanding of the ovarian follicle.

2. Sample collection and preparation for microarray analysis

Since some of the datasets used in this review are not published, a summary of methods is provided for the LH treatment effect although the same tissues were used by Gilbert et al. (2011). Briefly, 2 h pre-LH, 6 h and 22 h post LH granulosa cells were kindly provided by Dr. S.J. Dieleman (Utrecht University, The Netherlands). The ovarian stimulation treatment and ovariectomy protocol are explained in Knijn et al. (2002, 2012) and sample processing for RNA extraction and amplification are described in detail by Gilbert et al. (2011). aRNA for each granulosa cell samples were hybridized in four replicates on Agilent-manufactured EmbryoGENE bovine slides and microarray analysis was performed like mentioned in Robert et al. (2011).

3. Granulosa vs theca vs cumulus vs oocyte

The follicle is a combination of several tissues and although the focus is generally on granulosa cells, the theca cell compartment plays a significant role in the growth and steroidogenic process (Fortune, 1986). We do not have theca cell data but Lane Christenson has published a recent paper where the microarray data revealed that of the 24,016 probe sets which represent, 11,548 independent annotated bovine genes and 7091 unannotated expressed sequence tags, the majority of the probe sets, 10,770 were present in all 3 cell types (mural, antral and theca) before the LH surge, respectively and the theca cells only showed 1247 unique probes (Christenson et al., 2013). The cumulus on the other side is considered as a sub-population of granulosa where some differentiation events are prevented by the proximity of the oocyte (Eppig et al., 1998; Diaz et al., 2007). Additionally, the oocyte has been shown to be in constant interaction with all parts of the follicle as its ablation results in atresia (Vanderhyden et al., 1993). This complex structure, the follicles, evolves during a 6 months period in bovine (Lussier et al., 1987) with each compartment being in constant changes. This is especially obvious in the last 10 days where final growth, selection, dominance and ovulation occurs in a rapid sequence if atresia is not triggered as in 99% of the follicles end up in this final path.

The phenotype of a cell or an organ, like a follicle, is the results of around 15,000 individual genes being expressed in a given cell at a given time. Using the EmbryoGENE platform, several tissues were analyzed in hundreds of arrays containing all bovine genes and thousands of isoforms. The comparison of these data sets reveals that roughly 13,000 transcripts are common between granulosa cells, oocytes and cumulus cells (Fig. 1). For the purpose of comparison we have considered a transcript present in a tissue if the probe is giving a value greater than 2 standard deviation above the back ground in at least 75% of all the array theta we have for each tissue. A total of 21,697 probes were then selected and included in the comparison. The transcripts that are tissues-specific represent a minority in each sub group. As expected more transcripts are common to cumulus and granulosa (1922) than granulosa and oocytes (116). The cumulus share more transcripts with the oocyte than the granulosa (688 vs 116). The oocyte has more unique transcripts than the granulosa (2541 vs 97) but surprisingly the cumulus also has a more distinct transcriptome than the granulosa with 2203 unique transcripts. Since the cumulus represent a derivation from the granulosa one would have expected less uniqueness.

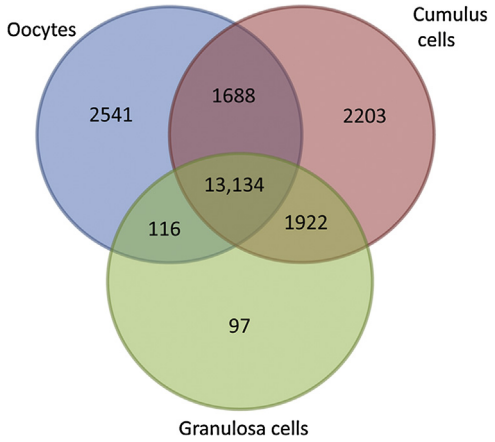

Fig. 1. Venn diagram showing the number of commonly express genes or variants between 3 different tissues based on the EmbryoGENE slide hybridization and a cut-off corresponding to twice the background level for target-probe intensity.

Recently our laboratory has published a dynamic study where the effect of coasting (waiting to harvest oocytes from stimulated follicles during 5 days and left to mature for 1–2–3 or 4 days with only endogenous LH in presence of a corpus luteum) has been analyzed with consecutive transcriptome survey from the same animals exposed to different coasting times. The number of observed common transcripts is close to 15,000 with only 500 genes changing from the growth phase (days 1–2) to the plateau-early atretic phase (days 3–4). Also the number of gene common between two successive stages decrease as time goes (384–87–55–26) as well as the gene unique to each stage (204–196–18 and 11) (Fig. 2) (Nivet et al., 2013). Simply stated, the atresia process requires less new genes than the growth process. These macro analyses are important to understand the dynamic of differentiation and how complex the process is. One must remember that these analyses are made on pools of cells and often on pools of animals