A085 Folliculogenesis, oogenesis and superovulation

Differences in oocyte and cumulus cells gene expression in Nelore heifers with low and high antral follicle count

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Antral Follicle Count (AFC) has been associated with reproductive performance in cattle. In taurine females, higher AFC is positively associated with pregnancy rates, and is linked to greater in vivo and in vitro embryo production, both in number and efficiency. Contrarily, in indicine females, AFC is negatively correlated with pregnancy rates after IVP-ET or TFAI. How the transcriptional patters in ovarian follicular cells correlate with the AFC remains unknown. This study aimed to investigate the expression of 95 genes in oocytes and cumulus cells in females of low AFC (LAFC) and high AFC (HAFC) using Nelore heifers (Bos taurus indicus) as model. Nellore heifers (n=48) had the ovarian follicular wave synchronized. On Day 5, AFC was determined by ultrasound examination. The bottom 10 and top 10 females were assigned to experimental groups LAFC (number of follicles<30, AFC = $24 \pm$ 4.73) and HAFC (number of follicles >60, AFC = 72.3 ± 15.67), respectively. On Day 5 cumulus-oocyte complexes (COCs) were retrieved by ovum pick-up. Cumulus cells and oocytes were separated by pipetting. Twenty samples (pools) of 10 oocytes (one per animal) and 20 samples (pools) of cumulus cells from 10 COCs (one per animal) were used for RNA extraction, cDNA synthesis and gene expression assessment of 94 genes by RT-qPCR. Means of normalized gene expression, using PPIA as the reference gene, were compared by Student's t-test and were considered significant when P<0.05. In oocytes, a total of 11 genes were differentially expressed, including eight upregulated in LAFC group (GAPDH, HSF1 BMP15, HAS2, EGFR, NPR3, H1FOO and IGFBP2) and three downregulated in LAFC (TFAM, XBP1 and PRDX3) compared with HAFC. Collectively, these genes are related to COC differentiation, meiotic control, epigenetic modulation, follicular recruitment and cellular responses to stress. In cumulus cells a total of 27 genes were differentially expressed between the groups. Cumulus cells from LAFC animals had higher expression of genes associated with meiotic control (EGFR, RGS2, NPR3 and NPR2) and epigenetic modulation (DNMT3A, HDAC2 and PAF1), as well as genes associated with energetic metabolism, cellular responses to stress and others (CDK6, PA2G4, CASP9, STAT3, XBP1, HSP90AA1, HSPA5, HSPD1, SOD1, IGF1R, GSK3A, ATPL5, TFAM and PFKP). Contrarily, FSHR, GAPDH, SREBF1, NFKB2, ARO and PTGS2 were downregulated in LAFC compared with HAFC group. These results indicate that distinct cellular compartments within ovarian follicles have dissimilar transcriptional patterns comparing LAFC and HAFC females. Such variations are potentially linked to reproductive performance and are suggestive of differential modulation in terms of cumulus-oocyte complex differentiation, meiotic control and oocyte competence acquisition. Financial support: Sao Paulo Research Foundation (FAPESP; grants 2012/50533-2 and 2018/13155-6) and CAPES - Brazil - Finance Code 001.