Epigenomic crosstalk in H3K27ac regulation and RNAseq: an integrative approach assessing metabolically modulated bovine embryos.

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In the intricate embryonic development dynamics, the chromatin landscape and the expression patterns are tightly coordinated responding to metabolic cues that shapes the fate of the implantation embryo. This study delves into the molecular choreography of bovine embryos, elucidating the interplay between the H3K27ac epigenetic marker and gene expression profiles under the influence of metabolic modulators - DCA (dichloroacetate, a pyruvate analog that increases the pyruvate to acetyl-CoA conversion) and IA (iodoacetate, which inhibits the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leading to glycolysis inhibition), from day 5 of culture. Both modulations were reported previously as affecting targeted metabolic pathways and acetylation levels for H3K27. The goal of this study was to integrate results from RNASeq and H3K27ac Cut&Tag (Cleavage Under Targets and Tagmentation) of inner cell masses from metabolically modulated bovine embryos and find crucial relationships between the datasets. For this, *in vitro* produced bovine embryos were cultured from day 5 in 3 experimental groups [synthetic oviductal fluid with amino acids (SOFaa) + 4% bovine serum albumin]: Control (CO - no additional supplementation), DCA (addition of 2mM of DCA) or IA (addition of 2µl of IA). Blastocysts were collected on Day 7 and their inner cell masses were submitted to RNASeq and Cut&Tag analysis. 3 ICM per group per replicate (3 replicates) were used for RNA extraction or for the Cut&Tag procedure. Samples were sequenced by the Illumina Genome Analyzer II (NextSeq PE40 high output). Results from both datasets were submitted to quality control with FastQC, genome alignment (Bos_taurus_UMD_3.1.1, NCBI) was done by Bowtie2, normalization and differential expression measurement of RNASeq data was performed by Deseq2. Peak-calling was done with MACS2 and the genomic annotation by ChipSeeker. Regarding the RNASeq dataset, comparing to CO, 855 transcripts were found differentially expressed in DCA, while 659 were found in IA. Assuming that H3K27ac is described as a permissive mark, the subsequent step was to consider unique peaks from each group confronted with DEGs, to retrieve the regulatory behavior among group comparisons. Therefore, for CO vs. DCA, 126 matches were found between CO peaks and CO upregulated DEGs, mainly associated with translation activity, mRNA processing and protein folding, while 184 matches were present between DCA peaks and

DCA upregulated DEGs, linking to glucose homeostasis, chromatin organization, cell differentiation and positive regulation of transcription from RNA polymerase, making up to 36% of complementary IDs across datasets. Concerning the contrast between CO vs. IA, 118 IDs were found upregulated in IA together with their mirroring peaks, aligned with molecular functions such as DNA topological change, mitochondrial ATP synthesis, positive regulation of DNA replication and translation. Along with 119 matches between upregulated DEGs in CO and their counterpart peaks pointing to *in utero* embryonic development, positive regulation of embryonic development and regulation of transcription from RNA polymerase II promoter, equally accounting for 36% of compatible IDs. This integrative approach revealed the important role H3K27ac in controlling gene expression and reinforces the metabolism as a key regulator of epigenomic remodeling during the early embryogenesis.

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