doi 10.18699/bgrs2024-7.3-15

Investigation of the epigenetic effects of IVF on circadian rhythms of descendents

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Key words: in-vitro fertilization; IVF; behavior; RNA sequencing; circadian rhythm; sleep

Motivation and Aim: During observations, performed in our laboratory, it has been noticed, that IVF descendents have a higher body mass than the control mice. After a sequence of behavioral experiments it was stated that the only parameter significantly differing among groups is the day-night distribution of mice activity: IVF group showed a higher activity rate during the light time, in which animals usually sleep. Thus, the aim of further nervous tissue analysis was to indicated, whether there are any epigenetic alterations in genes expression that can possibly provide this shift of activity and, if such, what molecular pathways are involved in it.

Materials and Methods: Samples for transcriptomic analysis were taken from the frontal cortex of both hemispheres of the mouse brain. Read quality assessment was performed using the FastQC tool [1], followed by trimming using Trimmomatic [2]. Genomic indexing was conducted using the Kallisto package [3] based on the Mus musculus Ensemble Mg39 reference genome. Subsequent analysis of the counted reads was performed using the Phantasus program [4] – PCA analysis was conducted, and differential expression analysis was performed using the built-in Limma program [5]. Enrichment of the results of differential expression analysis was carried out using the Enrichr database [6] and GSEA MSigDB [7].

Results: Subsequently, transcriptomic analysis was performed on 36 mouse brain samples – 8 samples from the control group and 28 from the test group. Fifteen thousand genes were identified with non-zero readings and were subsequently used in the analysis. Principal component analysis (PCA) revealed the presence of two distinct clusters separated by 1 component (1st component 19 %, 2nd component 10 %). Analysis of differential gene expression identified genes that significantly increased their expression in the test group of animals. Enrichment analysis of the 250 most highly expressed genes showed significant overlap with REACTOME database attributes, involved in circadian regulation and mitochondria functioning- REACTOME_CIRCADIAN_CLOCK, REACTOME_MITOCHONDRIAL_BIOGENESIS and REACTOME_TRANSCRIPTIONAL_ACTIVATION_OF_MITOCHONDRIA_BIOGENESIS. Especially, significantly increased expression was observed among genes-key players in the CLOCK complex, which is the main regulator of circadian rhythms.

Conclusions: Thus, we have demonstrated that in animals conceived through IVF, changes in circadian rhythm of metabolism are associated with alterations in the genetic regulation of this process.

Funding: This work was supported by grant RFBR No. 23-14-00179.

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