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# Targeted genome-wide DNA methylation profiling of ovarian granulosa cells from women with PCOS

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## Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrinopathy of obscure pathophysiologic origins, globally affecting 6-15% women of childbearing age. Emerging evidence on repercussions of environmental insults and changing lifestyles on fecundity and reproductive health have necessitated the study of tissue-specific epigenetic alterations in PCOS development. In semblance to follicular and oocyte defects observed in PCOS ovaries, targeted bisulfite sequencing was performed to generate the methylome signatures of ovarian granulosa cells (GCs) obtained from age-BMI matched women with PCOS (n=3) and healthy, regularly menstruating controls (n=3) using next generation sequencing approach. Paired end sequencing of samples was carried out on Illumina HiSeq 2500 ® platform and data were analyzed using the Bismark tool. Methylation levels of a few selected genes relevant to ovarian function were further validated in GCs obtained from 10 controls and 10 women with PCOS by pyrosequencing. Relative transcript levels of these genes were assessed by q-RT PCR using Taqman assays. In the methylome analysis, a total of 6486 CpG sites representing 3840 genes associated with pathways such as Wnt signaling, G-protein receptor signaling, angiogenesis, chemokine and cytokine mediated inflammation and integrin signaling showed differential methylation in PCOS. Of these, a total of 2977 CpG sites representing 2063 genes were identified as hypomethylated while 3509 CpG sites in 1777 genes were found to be hypermethylated. Additionally, differential methylation was also noted in several non-coding RNAs regulating vital ovarian functions and which are reported to be dysregulated in PCOS. This data provides compelling evidence in support of epigenetic alterations as etiopathogenic factors associated with ovarian dysfunction in PCOS.

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