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Determination of Optimal Housekeeping Genes for Transcriptional Study of the Endometrium

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Abstract:
Introduction: Selection of appropriate housekeeping genes are crucial for gene expression studies. These should be constitutive, with stable expression in the tissue tested. The human endometrium is a challenging tissue for transcriptomic studies due to dynamic changes in gene expression occurring throughout the menstrual cycle. We aimed to determine endometrial genes satisfying the criteria for optimal controls in gene expression studies.
Methods: A literature review was performed to select the 6 most commonly used housekeeping genes in endometrial qPCR studies. Differential expression analysis was undertaken in 2 RNA sequencing endometrial datasets derived from Gene Expression Omnibus (GEO) to test expression of these genes. Additionally, GEO was used to determine 3 more constitutive genes in these datasets. A targeted RNA-Seq protocol was employed to measure expression of the 9 selected genes in endometrial biopsies from 119 patients. Endometrial Receptivity Analysis timed the biopsies as: Pre-receptive (n=75), Receptive (n=38) and Post-receptive (n=6). An ANOVA test was applied to analyse gene expression among the different endometrial stages. P-values were corrected by False Discovery Rate (FDR).
Results: GAPDH, ACTB, PRDM4, UBE4A, ENOX2 and UBE2D2 from the literature and SRRM2, ACTG1 and RPL30 from GEO were selected as potential housekeeping genes. However, only UBE4A, SRRM2, ACTG1 and RPL30 showed constitutive gene expression throughout all experiments and endometrial stages. UBE2D2 was not constitutive in any (Table).

Table: FDR values calculated for the 9 selected housekeeping genes in the three experiments.

Housekeeping Genes	FDR Altmäe 2017 Dataset	FDR Sigurgeirsson 2017 Dataset	FDR Targeted RNA Sequencing
GAPDH	0.27	0.07	4.40E-08
ACTB	5.07E-03	0.77	1
PRDM4	2.89E-05	0.17	0.10
UBE4A	0.36	0.99	0.18
ENOX2	3.58E-04	0.08	1.35E-05
UBE2D2	0.01	3.85E-03	0.01
SRRM2	0.96	0.63	1
ACTG1	0.85	0.77	1
RPL30	0.84	0.72	0.40

Conclusion: Housekeeping genes commonly used in endometrial studies are not always constitutive and may cause errors in data interpretation. Genes should be carefully chosen according to the experimental design and the technology used. We propose SRRM2 and ACTG1 as housekeeping genes in RNA sequencing studies.
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