

Supplementary data

Manuscript: Dysregulated Autophagy Leads to Oxidative Stress and Aberrant Expression of ABC Transporters in Women with Early Miscarriage

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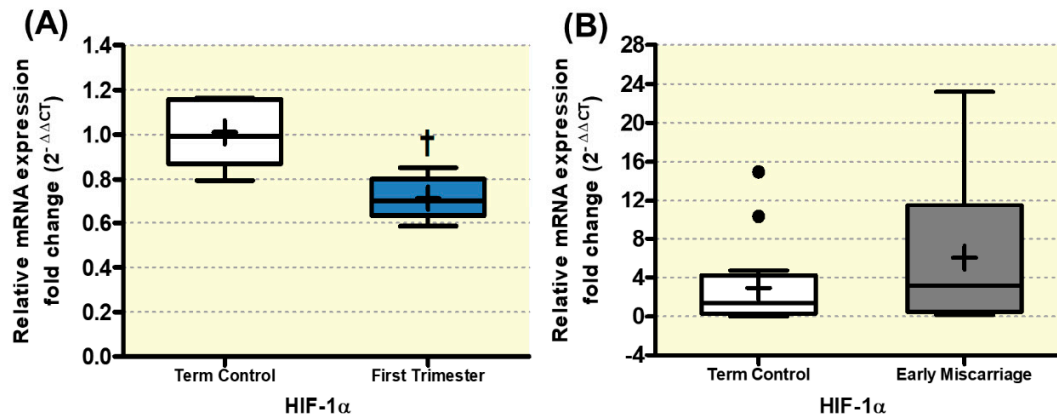


Figure S1. qRT-PCR analysis of hypoxia-inducible factor 1 (HIF-1 α) in placental tissues. (A) 1st and 3rd trimester controls (n=6 and n=5, respectively), **(B)** EMC vs term controls (n=20 and n=19, respectively). Data are presented as mean (x), median (-), and Tukey box and whiskers (1.5-times interquartile range). mRNA expression by RT-qPCR are presented as $2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct$ = Ct value of target gene— CT value of the mean of house keepers, i.e B-actin, GAPDH, YWHAZ, B2MG, L19, hTBP, hsUBQ) and normalized to the mean of controls. Significantly reduced HIF-1 α expression was observed in 1st trimester as compared to term controls. Significant differences were evaluated by using two-tailed Mann-Whitney test; 1st vs 3rd trimester (A) and EMC vs term controls (B): † ($P \leq .01$).

Table S1. Primer sequences used to quantify autophagy, ABC transporters and reference genes by RT-qPCR

Gene	Forward sequence, 5'-3'	Reverse sequence, 5'-3'	Gene Accession Number	fragment length [bp]
Autophagy				
<i>BECN1</i>	CCCGTGGGAATGGAATGAGA TTA	CCGTAAGGAACAAGTCGGTAT C	NM_003766.5	113
<i>Bcl-2</i>	GCGACTCCTGATTCATTG	CTTCCTCTGTGATGTTGTATT	NM_000657.3	157
<i>LC3-I</i>	TCCCGGACCATGTCAACAT	ACCATGCTGTGCTGGTTCAC	NM_181509.3	106
<i>LC3-II</i>	ACCATGCCGTCGGAGAAG	ATCGTTCTATTATCACCGGGAT TTT	NM_001085481.3	115
<i>P62</i>	AGGCGCACTACCGCGAT	CGTCACTGGAAAAGGCAACC	NM_003900.5	51
ABC Transporters				
<i>ABCA1</i>	CCACATTTTTGCCTGGGACG	AGCGATTCTCCCCAAACCTT	NM_005502.4	88
<i>ABCG1</i>	AACATGGAGGCCACTGAGA C	GGCCACCAACTCACCCTAT	NM_207629.2	228
<i>ABCG2</i>	GAGGCAAATCTTCGTTAT	CCATCACAACATCATCTT	NM_001348989.2	144
Hypoxia				
<i>HIF-1α</i>	TCCATGTGACCATGAGGAA A	CCAAGCAGGTCATAGGTGGT	NM_001243084.2	251
Reference Genes				
β -actin	AACTCCATCATGAAGTGTG ACG	GATCCACATCTGCTGGAAGG	NM_001101.5	234
<i>GAPDH</i>	GCTCCTCCTGTTGACAGTC A	ACCTTCCCCATGGTGTCTGA	NM_001357943.2	80
<i>YWHAZ</i>	CCGTTACTTGCTGAGGTTG	AGTTAAGGGCCAGACCCAGT	NM_001135699.2	143
<i>B2MG</i>	GATGAGTATGCCTGCCGTGT G	CAATCCAAATGCGGCATCT	NM_004048.4	114
<i>L19</i>	CCAACTCCCGTCAGCAGAT C	CAAGGTGTTTTTCCGGCATC	NM_000981.4	101
<i>TBP</i>	GTTCTGGGAAAATGGTGTGC	GCTGGAAAACCCAACCTTCTG	NM_001172085.2	100
<i>UBC</i>	TCGCAGCCGGGATTTG	GCATTGTCAAGTGACGATCAC A	NM_021009.7	64

Gene names: *BECN1*, Beclin-1; *Bcl-2*, B-cell lymphoma 2; *LC3-I*, Microtubule-associated protein light chain 3-I; *LC3-II*, LC3-phosphatidylethanolamine conjugate; *P62*, SQSTM1 gene (Sequestosome-1); *ABCA1*, ATP Binding Cassette Subfamily A Member 1; *ABCG1*, ATP-binding Cassette Subfamily G Member 1; *ABCG2*, ATP-binding cassette superfamily G member 2 (BCRP, breast cancer resistance protein); *HIF-1 α* , hypoxia-inducible factor 1; β -actin, Beta-actin; *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *YWHAZ*, 14-3-3 protein zeta/delta; *B2MG*, beta-2 microglobulin; *L19* (RPL19), ribosomal protein L19; *TBP*, TATA-box-binding protein; *UBC*, ubiquitin.