

Effect of folate deficiency on promoter methylation and gene expression of *Esr1*, *Cav1*, and *Elavl1*, and its influence on spermatogenesis

Supplementary Material

Supplemental Table S1: Characteristics of 20 subjects for RT-PCR and BSP assay.

Characteristic	Low folate (n= 10) ^a	High folate (n=10) ^a	P-value ^b
Seminal plasma folate	15.83 (11.27—17.08)	43.07 (40.44—43.66)	0.00
Demographic			
Age (y)	31 (25—39)	31 (26—40)	0.83
BMI (kg/m ²)	21.6 (19.6—26.0)	22.1 (18.4—27.5)	0.77
Semen parameters			
Ejaculate volume (mL)	4.4 (2.5—6.0)	3.4 (2.0—9.0)	0.47
Sperm density (10 ⁶ /mL)	55.87 (19.07—91.44)	182.11 (97.03—227.08)	0.00
Sperm count (x 10 ⁶)	240.31 (61.01—394.40)	514.57 (279.63—1998.09)	0.02
Sperm progressive motility (%)	46.84 (32.39—65.37)	46.77 (35.98—64.13)	0.96
Sperm normal morphology (%)	43.84 (32.33—61.23)	46.34 (28.94—50.24)	0.81
Duration of abstinence (d)	5 (3—7)	3 (2—7)	0.15

^aAll variables were given as median (range).

^bindependent student's t-test was used to determine the difference.

Supplemental Table S2: List of PCR primers

Sample	Gene	Forward Primer	Reverse Primer	Length of Product (bp)
Human sperm	<i>Esr1</i>	GCCAACGCGCAGGTCTA	GCCGCAGCCTCAGA	62
	<i>Cav1</i>	GCGACCCTAACACCTCAAC	ATGCCGTCAAAACCTGTGTGTC	91
	<i>Elavl1</i>	TGAACTACGTGACCGGAAG	CCCAAACCGAGAGAACATGTC	194
	<i>β-actin</i>	GTCCACCGCAAATGCTTCTA	TGCTGTCACCTTCACCGTTC	190
Testis of mice	<i>Esr1</i>	GTCGCCTAGCCCGCTGAT	CATGTTTCCTTCTCGTTACTGC	214
	<i>Cav1</i>	TAAATCACAGCCCAGGAAAC	TGAGATGCTGGGGTCGC	232
	<i>Elavl1</i>	AGGTTCCCTCCGAGCCCATC	GCGTCTTGCCCAAGTTGT	246
	<i>β-actin</i>	CTGAGAGGAAATCGTGCGT	CCACAGGATTCCATACCCAAGA	208

Supplemental Table S3: List of BSP primers

Sample	Gene	Forward Primer	Reverse Primer	Length of Product (bp)
Human sperm	<i>Esr1</i>	AGATTAGTATTTAAAGTTGGAGGTT	ATATAAAAAATCATAATCATAATCC	283
	<i>Cav1</i>	TTTAAGAATTTTTTGTATTTTGG	TATCCTAAAACCTCAATCTCACCTAC	206
	<i>Elavl1</i>	TTTGTGGATTTTTYGGGGGA	ACGAACCRATATAAACRCCRA	164