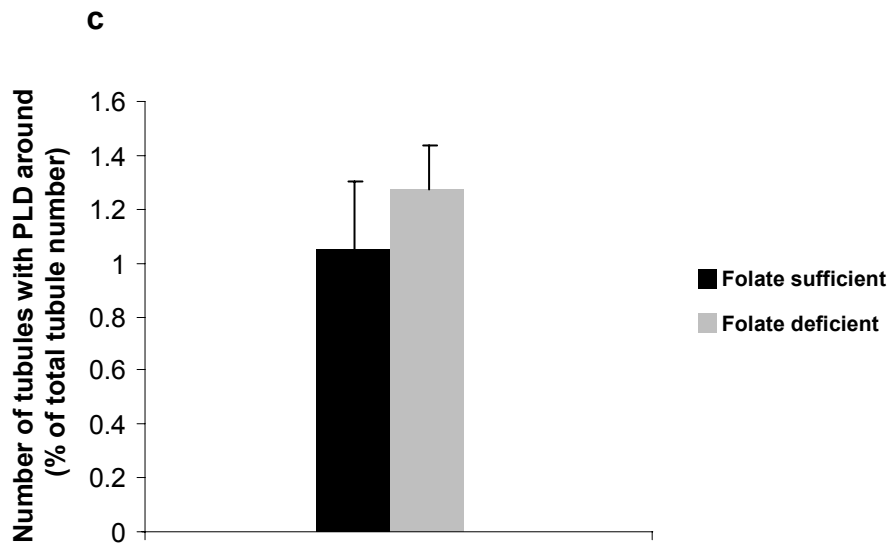
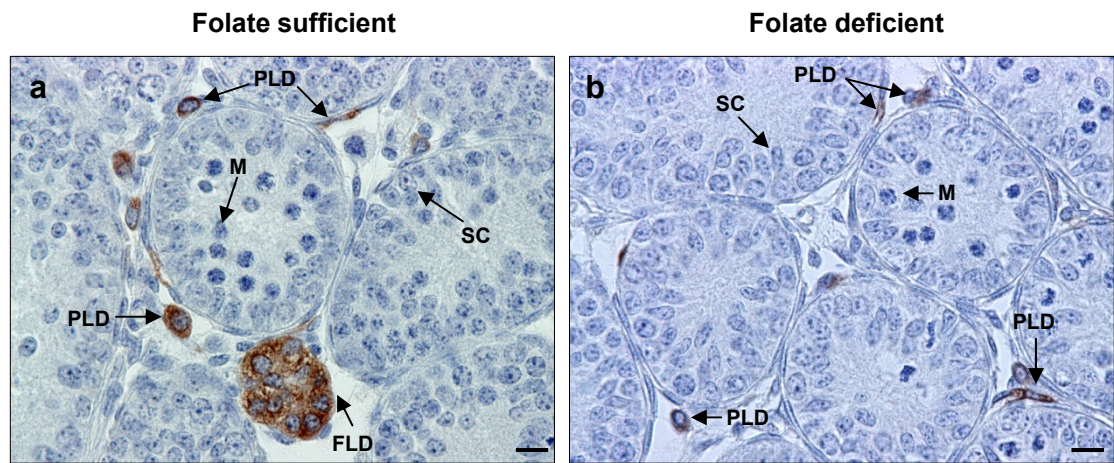


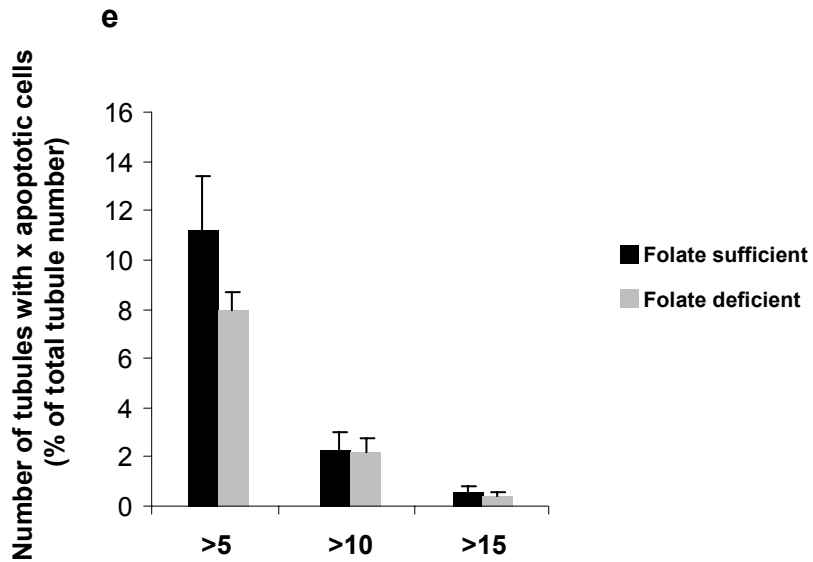
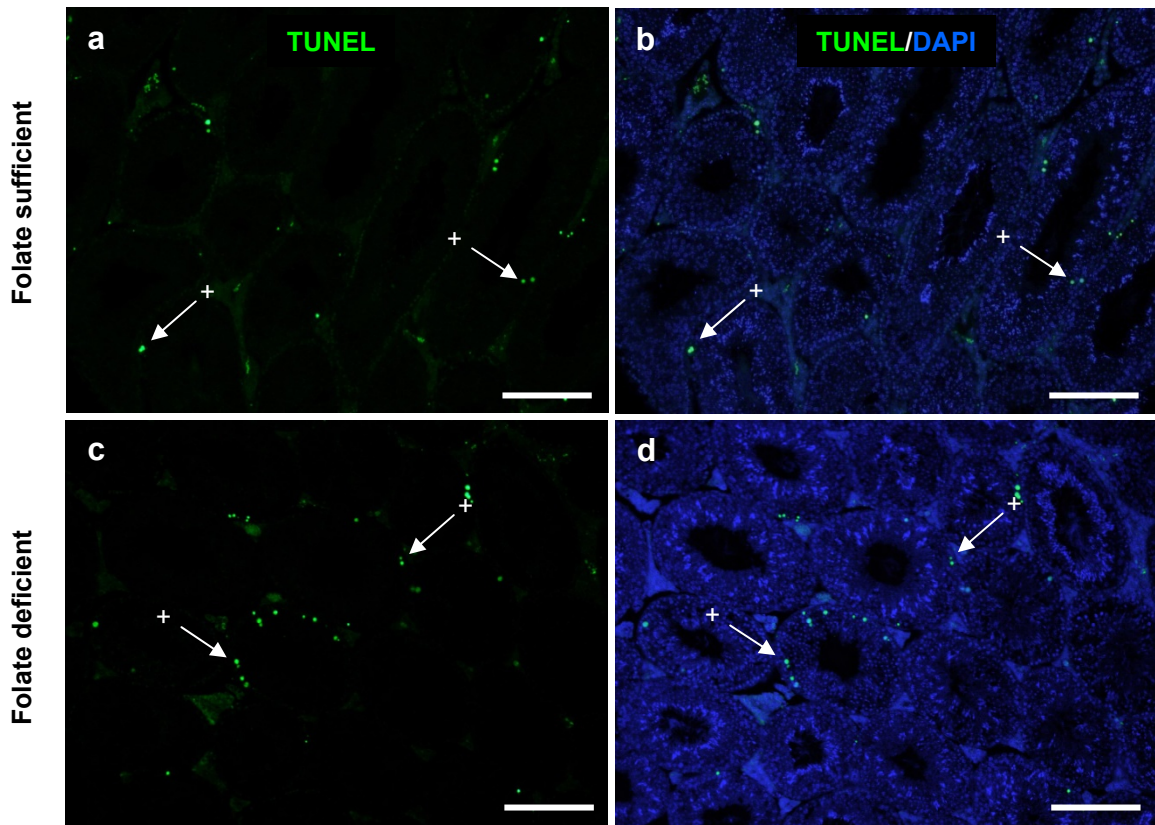
Supplementary Figure S1. Sertoli cell development is not affected by folate deficiency.

Post-natal day (PND) 12 testis cross sections from folate sufficient (FS) (a) and folate deficient (FD) mice (b) were co-labeled with anti-MIS (Müllerian-inhibiting substance, Sertoli cell [SC] marker, cytoplasmic, red) and anti-MVH (Mouse VASA homolog, spermatogonia [SG] marker, cytoplasmic, green) antibodies. Nuclei were stained with DAPI (blue). (c) The number of Sertoli cells present in the seminiferous tubules was the same in FS and FD testes. Means ± SEM of 3 determinations are shown. Bars represent 10µm.



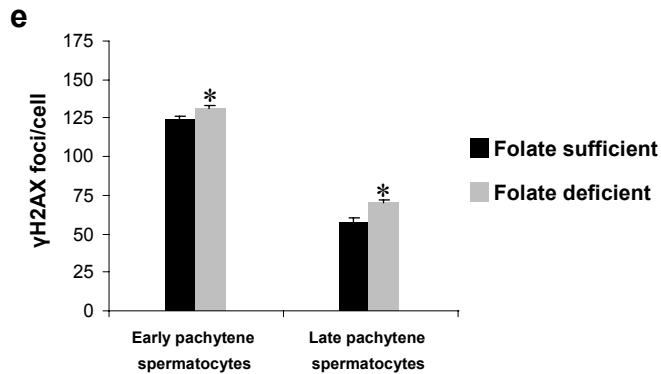
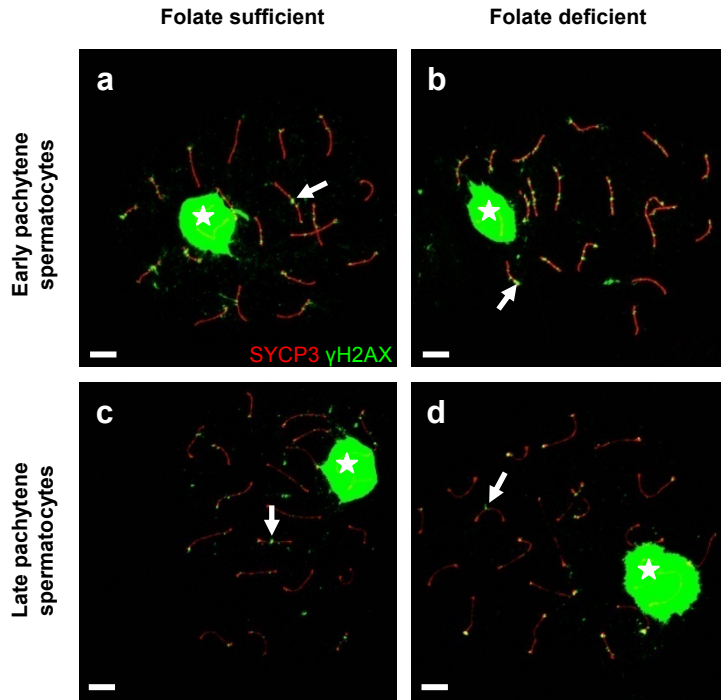
Supplementary Figure S2. Leydig cell development is not affected by folate deficiency.

Post-natal day (PND) 12 testis cross sections from folate sufficient (FS) (a) and folate deficient (FD) mice (b) were stained using an anti-3 β HSD (3- β -hydroxysteroid dehydrogenase/ Δ -5-4 isomerase) antibody which is a marker for Leydig cells. (c) The number of progenitor Leydig cells (PLD, single cells stained in Brown) present in the interstitial tissue surrounding the seminiferous tubules was the same in the FS and FD testes. Fetal Leydig cells (FLD) were also not affected. M, indicates the meiotic germ cells and SC, the Sertoli cells. Means \pm SEM of 3 determinations are shown. Bars represent 10 μ m.



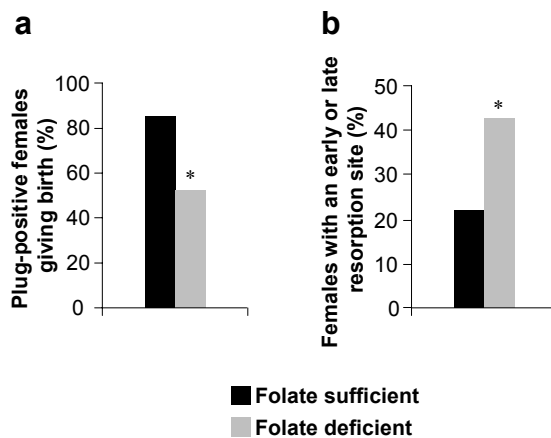
Supplementary Figure S3. Germ cell apoptosis rate is not altered by folate deficiency.

Testis cross sections from folate sufficient (FS) (a,b) and folate deficient (FD) (c,d) mice were submitted to a TUNEL assay to detect apoptotic cells (+, green); nuclei DNA was stained with DAPI (blue). (e) the number of apoptotic cells per tubule was assessed and classified in three categories (>5, >10 and >15). Means \pm SEM of 7 determinations are shown. Bars represent 100 μ m.



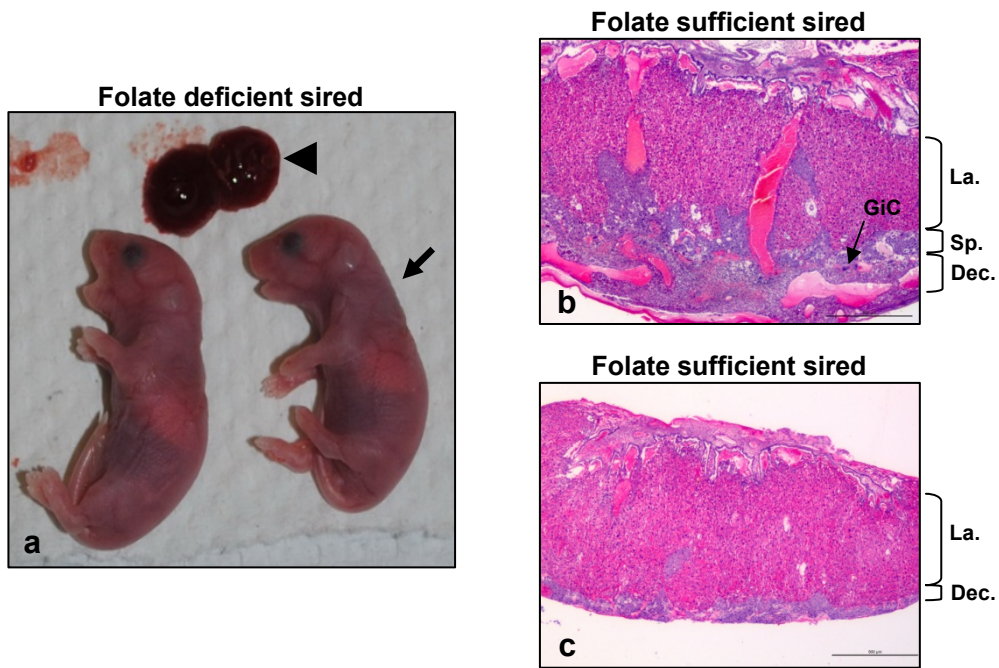
Supplementary Figure S4. Folate deficiency is associated with increased DNA double strand breaks (DSBs) in pachytene spermatocytes.

(a-d) Co-immunofluorescent staining for SYCP3 (red) and γ H2AX (green) on isolated pachytene spermatocytes. SYCP3 is a marker of the meiotic chromosomal axis and γ H2AX is a marker of DNA DSBs. Arrows indicate DNA DSBs, and stars indicate the sex body. (e) The values shown are means \pm SEM. Statistical comparison between FS (n=3) and FD (n=3) mice was done using a subsample model test (*p<0.05). Bars represent 10 μ m.

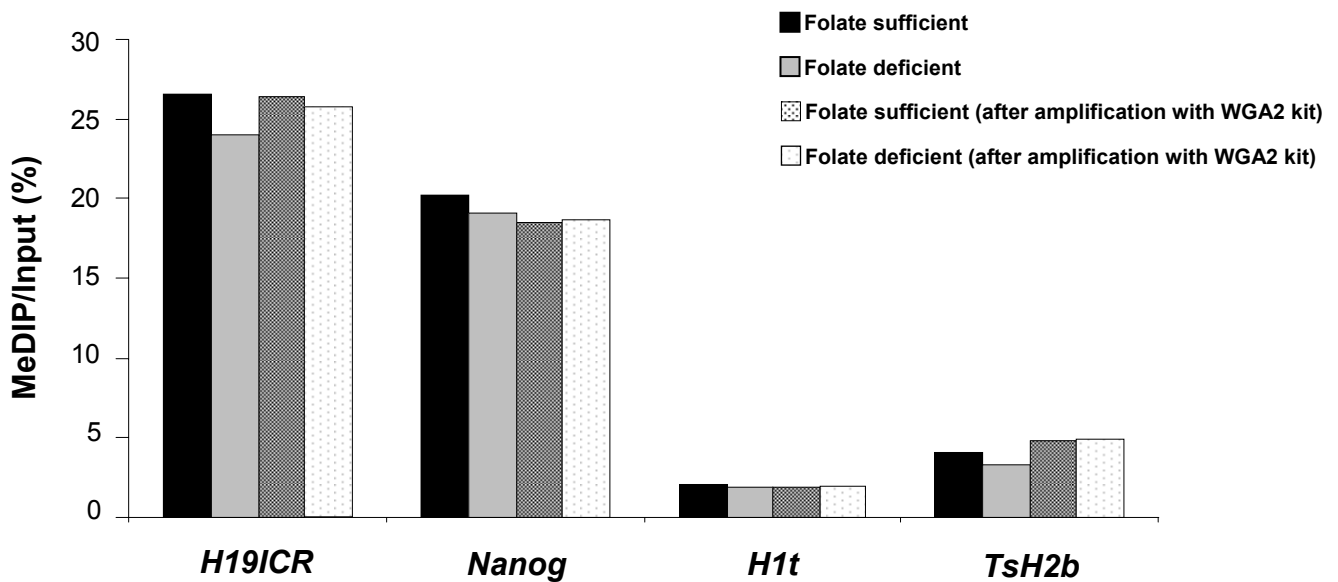


Supplementary Figure S5. Paternal folate deficiency compromises fertility and adversely affected pregnancy outcomes.

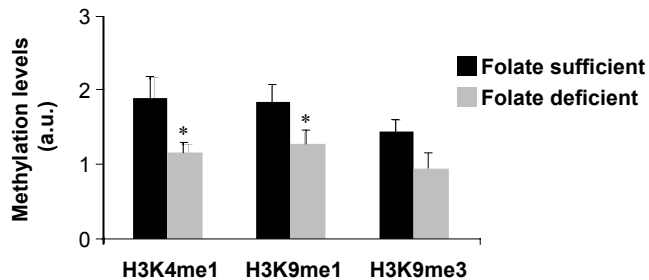
(a) Folate sufficient (FS) or folate deficient (FD) males were bred to CD-1 females and their litters examined at 18.5 days post conception. The pregnancy rate of females mated with a FD male decreased in comparison to an FS mating. (b), Females mated with a FD male had increased pregnancy resorption rates. Early resorption was identified by the presence of early-remnants of a placenta and no obvious signs of a fetus whereas late resorption was characterized by visible remnants of a dead fetus. All data were calculated and shown on a per male basis; * $p < 0.05$ by Fisher's exact test (a) and Chi-square test (b). Means of 20 (FS) and 21 (FD) determinations are shown.



Supplementary Figure S6. Placental abnormalities in pregnancies sired by a folate deficient father. (a) The 18.5 day-post-conception (dpc) fetuses shared a fused placenta (arrowhead). The arrow indicates the runt fetus. Example of a 18.5dpc placenta cross sections from a fetus sired by a folate sufficient (FS) (b) and one by a folate deficient (FD) father (c). Placenta cross-sections stained with haematoxylin and eosin. The giant cell (GiC) layer indicated by an arrow in an FS-sired placenta was absent in the placenta of the fetus sired by an FD male. The spongiotrophoblast (Sp.), situated in-between the labyrinth (La.) and the maternal decidua (Dec.), was abnormally thin. Bars represent 500µm.



Supplementary Figure S7. Confirmation of sperm sample purity and enrichment of hypermethylated regions in the sperm MeDIP fractions. Real-time PCR was carried out on MeDIP and input samples of sperm DNA from folate sufficient (FS) or folate deficient (FD) males before and after amplification with a WGA2 kit. Regions amplified are known to be hypermethylated (*H19ICR*, *Nanog* promoter) or hypomethylated (*H1t*, *TsH2b* promoters) in sperm. Data are expressed as a ratio of DNA immunoprecipitated to input DNA. Means of 2 determinations are shown.



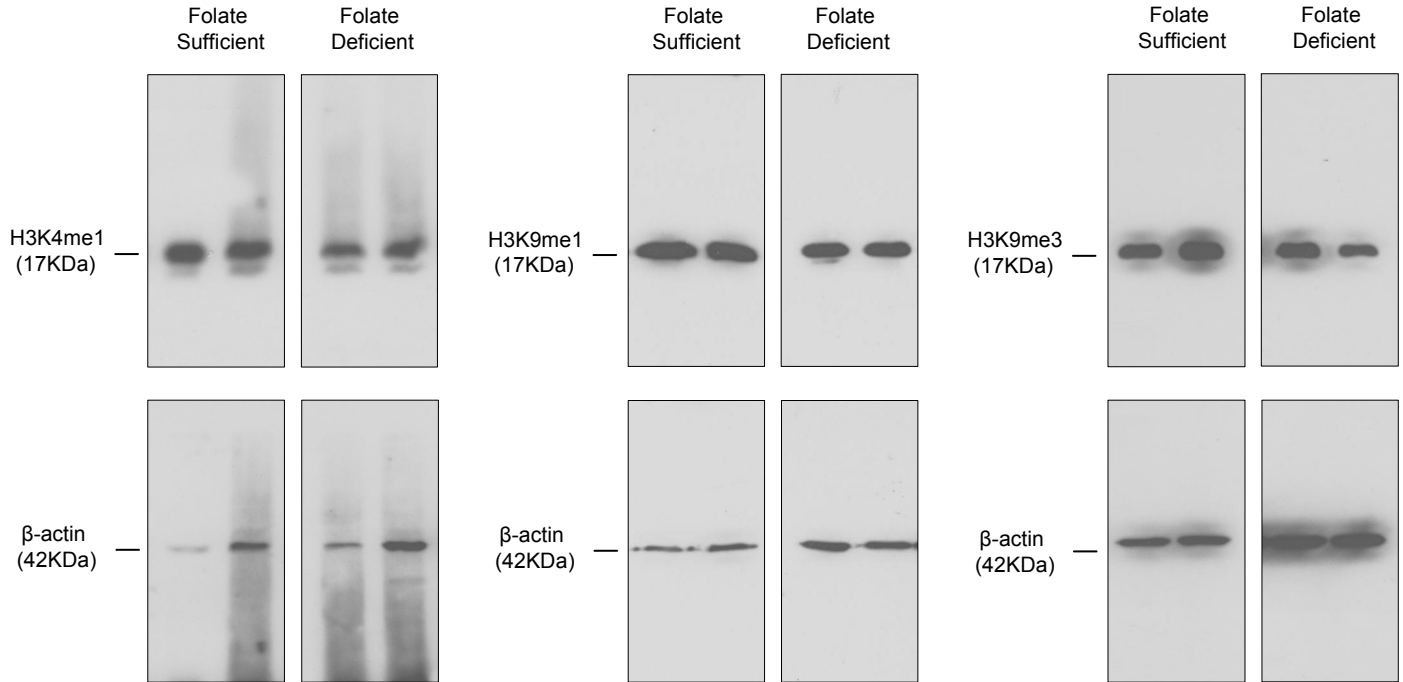
Supplementary Figure S8. Folate deficiency reduces sperm histone H3 lysine 4 (H3K4) and lysine 9 (K9) methylation.

Western blot analyses for levels of histone H3K4 mono-methylation (m1), H3K9m1 and H3K9 tri-methylation (m3) in sperm. Sperm from FD males had reduced H3K4me1, and K9me1 and H3K9me3 compared with FS sperm (* $p < 0.05$ by Student *t*-test) (FS, $n=7$; FD, $n=7$). H3K9me3 was also decreased in FD versus FS sperm ($p < 0.06$ by Student *t*-test) (FS, $n=6$; FD, $n=6$). Data are presented as the ratio of methylation over β -actin. The values are means \pm SEM.

H3K4me1

H3K9me1

H3K9me3



Supplementary Figure S9. Folate deficiency reduces sperm histone H3 lysine 4 (H3K4) and lysine 9 (K9) methylation.

Western blot analyses for levels of histone H3K4 mono-methylation (m1), H3K9m1 and H3K9 tri-methylation (m3) in sperm. β -actin is used as a control. Representative bands from sperm extracts from 2 Folate Sufficient and 2 Folate Deficient males are shown.

Supplementary Table S1. No effects of folate deficiency on pubertal and adult testis, epididymides and body weight.

<i>Diet</i>	<i>Age</i>	<i>Mean body weight (g)</i>	<i>Mean testes weight (mg)</i>	<i>Relative testes weight (%)</i>	<i>Mean epididymides weight (mg)</i>	<i>Relative epididymides weight (%)</i>
FS	2 months	19.74±0.91	64.12±3.04	0.33±0.01	33.38±1.88	0.17±0.01
FD	2 months	21.06±0.50	63.67±1.70	0.30±0.01	32.85±2.80	0.15±0.01
FS	4 months	28.82±0.48	82.04±1.41	0.29±0.01	37.15±1.38	0.13±0.01
FD	4 months	29.11±0.53	82.51±1.41	0.29±0.01	39.11±1.45	0.14±0.01

2-mo: (Folate sufficient (FS), n=14; Folate deficient (FD), n=12)

4-mo: (FS, n=40; FD, n=37)

Means ±SEM are shown.

Supplementary Table S2. Increased abnormalities in fetuses sired by males exposed to a folate sufficient (FS) or deficient (FD) diet assessed at day 18.5 of gestation.

<i>Diet of the father</i>	<i>Embryo weight</i>	<i>Crown-rump length</i>	<i>Placenta weight</i>	<i>Placenta width</i>	<i>Embryo malformations</i>	<i>Placenta malformations</i>
FS	1.38±0.02	24.17±0.20	0.088 ±0.005	7.69±0.12	3/285	Small: 7/ 126
FD	1.40±0.02	24.18±0.15	0.087 ±0.004	7.54±0.09	14/328*	Small: 9/ 199 Fused: 2/ 199

Means ±SEM of 17 to 32 (FS) and 16 to 35 (FD) determinations are shown.

* $p < 0.05$ by Fisher's exact test.

Supplementary Table S3. Post-implantation loss increased in pregnancies sired by folate deficient males in comparison to folate sufficient sired pregnancies as assessed at day 18.5 of gestation.

<i>Diet of the male</i>	<i>Number of corpora lutea</i>	<i>Number of implantation sites</i>	<i>Pre-implantation loss (%)</i>	<i>Post-implantation loss (%)</i>
FS	13.16±0.56	10.92±0.48	15.90±3.41	2.71±0.82
FD	13.26±0.39	11.74±0.47	10.95±3.13	4.42±1.04 *

Means ±SEM of 32 (FS) and 35 (FD) determinations are shown.

* $p < 0.05$ by Kolmogorov-Smirnov test.

Supplementary Table S4. List of differentially methylated regions identified by MeDIP-array in the sperm of mice fed a folate deficient diet versus mice on a folate sufficient diet).

Location of the region differentially methylated	Closest gene	Distance from the TSS (in bp)	Function and associated abnormalities/disease	q value
chr10:73279052-73279112	<i>Pcdh15</i>	5549	protocadherin 15 Gene, mutation associated with deaf-blindness	0.04
chr10:80290089-80290138	<i>Oaz1</i>	712	ornithine decarboxylase antizyme 1 Gene, polymorphism associated with coronary heart disease	0.06
chr10:99958079-99958129	<i>Cep290</i>	7163	centrosomal protein 290 Gene, mutations affect multiple organ systems, most commonly the brain and spinal cord (central nervous system), retina, and kidneys.	0.07
chr11:44334824-44334879	<i>Rnf145</i>	1044	ring finger protein 145 Gene, Zinc ion binding	0.08
chr12:34108450-34108500	<i>Twistnb</i>	-6034	TWIST neighbor Gene, transcription factor associated with craniofacial defects and skeletal defects of limbs	0.08
chr13:67557465-67557522	<i>Zfp874</i>	-7075	zinc finger protein 874 Gene	0.09
chr15:78746099-78746148	<i>Pdxp</i>	1736	pyridoxal (pyridoxine, vitamin B6) phosphatase Gene, metabolism and signal transduction	0.08
chr15:91479170-91479223	<i>Lrrk2</i>	-24410	leucine-rich repeat kinase 2 Gene, signalling implicated in Parkinson disease	0.08
chr15:91280012-91280068	<i>Slc2a13</i>	-37392	solute carrier family 2 , member 13 Gene, transmembrane transport in brain involved in glucose methabolism	0.05

chr16:78384018-78384080	Btg3	-6612	B-cell translocation gene 3 Gene, target of P53, candidate tumor suppressor	0.1
chr17:22491647-22491696	6330416L07Rik	6663	RIKEN cDNA 6330416L07 Gene, predicted functions are nucleic acid and metal ion binding, gene regulation	0.07
chr17:23618886-23618938	AC154753.1	39766	function unknown	0.05
chr17:39981534-39981583	mmu-mir-715	477	mmu-mir-715, non-coding RNA post-transcriptional regulation	0.09
chr17:39982267-39982316	mmu-mir-715	1210	mmu-mir-715, non-coding RNA post-transcriptional regulation	0.06
chr17:39982172-39982221	mmu-mir-715	1115	mmu-mir-715, non-coding RNA post-transcriptional regulation	0.06
chr17:39981644-39981693	mmu-mir-715	587	mmu-mir-715, non-coding RNA post-transcriptional regulation	0.06
chr17:39981755-39981804	mmu-mir-715	698	mmu-mir-715, non-coding RNA post-transcriptional regulation	0.06
chr17:40384619-40384675	Crisp3	-5410	cysteine-rich secretory protein 3 Gene, epithelial cell expression, exocrine functions, upregulated in prostate and pancreatic cancer	0.07
chr17:92154652-92154706	SNORA17	57281	Small nucleolar RNA SNORA17, RNA biogenesis	0.08
chr17:92324177-92324232	SNORA17	-112244	Small nucleolar RNA SNORA17, RNA biogenesis	0.1
chr17:92242294-92242352	SNORA17	-30363	Small nucleolar RNA SNORA17, RNA biogenesis	0.05
chr17:92242219-92242278	SNORA17	-30288	Small nucleolar RNA SNORA17, RNA biogenesis	0.05
chr18:17154258-17154317	AC125166.1	-23273	function unknown	0.08

chr18:25320261-25320310	5730494M16Rik	6846	RIKEN cDNA 5730494M16 gene, high expression in testis, predicted function in cytoskeleton	0.05
chr19:13304457-13304506	Olf1463	-4261	olfactory receptor 1463 Gene, G protein-mediated transduction of odorant signals	0.07
chr1:161163423-161163480	Rfwd2	994	ring finger and WD repeat domain 2 Gene, p53 signalling pathway, over-expressed in breast and ovarian cancer	0.05
chr1:90130698-90130747	Heatr7b1	-3961	HEAT repeat containing 7B1 Gene, function unknown	0.07
chr2:126325205-126325258	Atp8b4	352	ATPase, class I, type 8B, member 4 Gene, predicted function is metabolism	0.1
chr2:164151125-164151176	AL590429.1	4253	function unknown	0.1
chr2:57910117-57910178	Ermn	-4872	ermin, ERM-like protein Gene, cytoskeleton organization and central nervous system development	0.05
chr2:85433263-85433319	Olf998	2592	olfactory receptor 998 Gene, transduction of odorant signals	0.05
chr2:89477720-89477773	Olf1249	-6693	olfactory receptor 1249 Gene, transduction of odorant signals	0.05
chr4:110072703-110072761	Agbl4	2336	ATP/GTP binding protein-like 4 Gene, metallocarboxypeptidase associated with Coeliac disease	0.05
chr4:111590106-111590157	Skint8	-1866	selection and upkeep of intraepithelial T cells 8 Gene, may act by engaging a cell surface molecule on immature T-cells in the embryonic	0.07

			thymus	
chr4:40188438-40188495	Ddx58	-1605	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 Gene, probable ATP-dependent RNA helicase, innate anti-viral immune defense	0.1
chr5:108254422-108254476	1700013N18Rik	-4823	RIKEN cDNA 1700013N18 gene, proposed function in protein modification	0.05
chr5:135035053-135035102	Gm52	886	predicted gene 52 Gene, function unknown	0.05
chr6:123808404-123808460	Vmn2r25	-5224	vomeronasal 2, receptor 25 Gene, signal transduction	0.08
chr6:28223524-28223582	Zfp800	-11621	zinc finger protein 800 Gene, predicted function is nucleic acid binding	0.08
chr6:29133530-29133587	Prrt4	-3974	proline-rich transmembrane protein 4 Gene, function unknown	0.05
chr6:57647426-57647482	Lancl2	-5141	bacterial lantibiotic synthetase component C-like 2 Gene, testis-specific G- protein coupled receptor	0.09
chr6:67488830-67488880	Tacstd2	-3155	tumor-associated calcium signal transducer 2 Gene, cell proliferation, associated with cancer	0.03
chr6:67488927-67488979	Tacstd2	-3253	tumor-associated calcium signal transducer 2 Gene, cell proliferation, associated with cancer	0.03
chr6:67489929-67489981	Tacstd2	-4255	tumor-associated calcium signal transducer 2 Gene, cell proliferation, associated with cancer	0.05
chr6:91107416-91107465	Hdac11	49	histone deacetylase 11 Gene, regulation of gene expression expressed in brain, testis and cancer	0.07

chr7:113980076-113980128	<i>Olf703</i>	-8025	olfactory receptor 703 Gene, transduction of odorant signals	0.09
chr7:115126908-115126966	<i>Olf477</i>	-6944	olfactory receptor 477 Gene, transduction of odorant signals	0.05
chr7:115127023-115127072	<i>Olf477</i>	-6834	olfactory receptor 477 Gene, transduction of odorant signals	0.05
chr7:29322310-29322366	<i>AC136456.1</i>	2547	function unknown	0.06
chr7:29593309-29593358	<i>Hnrnp1</i>	-2623	heterogeneous nuclear ribonucleoprotein L Gene, regulation of mRNA splicing	0.1
chr8:102661089-102661144	<i>AC122514.2</i>	77100	function unknown	0.09
chr8:116162357-116162406	<i>Mon1b</i>	2682	MON1 homolog b (yeast) Gene	0.1
chr9:39022393-39022446	<i>Olf944</i>	-2525	olfactory receptor 944 Gene, transduction of odorant signals	0.09
chr9:39048473-39048533	<i>Olf144</i>	-2487	olfactory receptor 1537, pseudogene 1, transduction of odorant signals	0.05
chr9:39771133-39771186	<i>Olf976</i>	-6605	olfactory receptor 976 Gene, transduction of odorant signals	0.09
chr9:46154025-46154074	<i>4931429L15Rik</i>	-25980	RIKEN cDNA 4931429L15 gene, function unknown	0.08
chrX:101872497-101872550	<i>Zdhhc15</i>	-6120	zinc finger, DHHC domain containing 15 Gene, associated with mental retardation	0.07
chr6:17274409-17274468	<i>*Cav1</i>	16715	Caveolin 1, cell signal progression	0.18
chr14:45844046-45844097	<i>*Txndc16</i>	-4068	Thioredoxin Domain-Containing 16, cell redox homeostasis	0.2

The q-value is an adjusted p-value indicating false discovery rate (FDR) calculated by Wilcoxon rank sum-test. For all genes with a q-value < 0.1, 10% are expected to be false positives.

**Cav 1* and **Txndc16* were identified when relaxing the FDR from 0.1 to 0.25.

Supplementary Table S5. List of primers used for the MeDIP-qPCR, gene expression and pyrosequencing arrays.

MeDIP-qPCR primers		
Gene promoter region	Primers	sequence
<i>H19 ICR</i>	Forward	GCCTCAGTGGTCGATATGGTTT
	Reverse	AAAGGGACCCCTCCAGAA
<i>Nanog</i>	Forward	GGACTGATCGGCAAACCTTTGA
	Reverse	TGGTCCCAAACCTCCTGATCTTC
<i>H1t</i>	Forward	ACGTAGGTGCCATGGGTAAGA
	Reverse	CCCGCCTGAATCTCAAGAGA
<i>TsH2B</i>	Forward	CCCCGCTTCTCAACCTCAA
	Reverse	CAACGTCTCAAAACAGTTCCAAC

Gene expression primers		
Gene	Primers	sequence
<i>Cav1</i>	Forward	CGCGACCCCAAGCATCT
	Reverse	CTGCAATCACATCTTCAAAGTCAA
<i>Cav2</i>	Forward	GAGTCACGACCGGGATCCT
	Reverse	GATCCTCGAAGCCTAGCTTGAG
<i>Txndc16</i>	Forward	CACGCTAATGTGGCCTTCAGA
	Reverse	CAACATCATTCAACACCAGAACTC
<i>Ddx58</i>	Forward	GAGACAGAGAAGCTAGCCAAGGA
	Reverse	GTGCCGAATTCTCTGTTTTGAAT
<i>Parp1</i>	Forward	GCCGAAAGGAATGGGTAAC
	Reverse	CCTGTTTTTTGACCTTTAACTTCTTGA

Pyrosequencing primers		
Genomic location	Primers	Sequence
<i>Cav1-CpG1</i>	Forward	TAGGGGTTTTTAGGTAAAGTGT
	Reverse biotinylated	CTTTCAATTACTACTATACCCTTCCTAATA
	Sequencing	ATTTTTGTAGTGAGATAAAAT
<i>Cav1-CpG2</i>	Forward biotinylated	ATTAGGAAGGGTATAGTAGTAATTGAAAGA
	Reverse	ACCCTTAATCCTATAAAAACTTAATTCCC

	Sequencing	CCCCAATATAAAAAATACCA
<i>Cav1-CpG-rich</i>	Forward	GTTGTTAGAATTTTGGGGATGTG
	Reverse biotinylated	ATAACTAAAAAAAAACCTCTTAECTATTC
	Sequencing	GGGGATGTGTTTAGAT
<i>Txndc16-CpG1</i>	Forward	TGGAAATGGAGTTATATTTATTTAAAGTG
	Reverse biotinylated	CATATTAACATTTTTCTTCCAECTTCTA
	Sequencing	TTTTATTTGAAGAAGTAATAATAG
<i>Txndc16-CpG2</i>	Forward biotinylated	GGGTGTTGGGAATTGAATTT
	Reverse	ACAAATACCTAACTATTAACCATCTCTC
	Sequencing	CTATTAAACCATCTCTCCA
<i>Txndc16-Cpg-island</i>	Forward	GTGGAGGTTTGGGATATTGAATAAAG
	Reverse biotinylated	TATCTTTCCCTCTCCCTCAACT
	Sequencing	AAAGGGTGTTTTATTGT