Parameters	Cohort 1 Mean ± SD* [range]	Cohort 2 Mean ± SD* [range]	Cohort 3 Mean ± SD* [range]	
Sample size (N)	186	109	154	
Smokers (N)	11	14	16	
Donor's age (years)	39.0±6.4 [25.7-65.8]	38.9± 5.1 [28.1-52.9]	37.8±5.4 [25.8-49.2]	
Body mass index (kg/m ²)	25.7±2.9 [19.4-36.8]	25.8±3.4 [17.3-40.3]	26.3±3.8 [17.5-38.7]	
Sperm concentration (million/ml)	28.6±33.4 [0.2-210]	38.1±40.6 [0.2-200]	43.2±51.6 [0.5-250]	

TABLE S1 Clinical parameters of the analyzed human sperm samples

* SD = standard deviation.

Assay	Primer	Sequence (5'-3') ^a	Number of CpGs	Annealing temperature
	Forward outer ^b	GTGTTTTGGGGTTGATTAGAGG		60°C
	Reverse outer ^b	ATCACCRATAAACCAAAACCCC		
Human	Forward	GTGTTTTTGGGTTGATTAGAGG		
rDNA upstream	Reverse	*CATCCCAAAACCCAACCTCTCC		
control element ^c	Sequencing 1	GGTTGATTAGAGGGTT	5	
	Sequencing 2	TTTTGGGGATAGGTG	13	
	Sequencing 3	TTYGGGGGAGGTATATTT	8	
	Forward outer ^b	GGTAGTTGTYGAGGGAGGGG		60°C
Human	Reverse outer ^b	AAAAAACRTCCCCAACCTCC		
rDNA	Forward	GTTTTYGTTGTGAGTTAGGTAGAGTTT		60°C
promoter ^a	Reverse	*AAAAAAACRTCCCCAACCTCC		
	Sequencing	GGTTTATGTGGGGGGAGAGGTTGT	9	
Human mouse	Forward	AGGTTTGTGATGTTTTTAGATGT		60°C
bovine, and	Reverse	*AAAACCTCACTAAACCATC		
marmoset	Sequencing 1	TTTGTGATGTTTTTAGATGTT	4	
18S rDNA	Sequencing 2	ATTAAGTTTTTGTTTTTGTATATA	4	
Human mouse	Forward	GGTTTTAAGTAGGAGGTGTTAGAAAAG		60°C
bovine, and	Reverse	*CAACCAAACACATACACCAAATATCT		
marmoset	Sequencing 1	GGATAATTGGTTTGTGG	7	
28S rDNA	Sequencing 2	GTTGGATTGTTTATTATTAATAGG	3	

TABLE S2 PCR and pyrosequencing primers for human, mouse, bovine, and marmoset repetitive elements

Human α-satellite DNA	Forward	*TGTAAGTGGATATTTGGATTATTGG		57°C
	Reverse	AACAATTTCAAAACTACTCCATCAA		
	Sequencing	СТСАААААТТТСТАААААТАСТТСТС	4	
	Forward	TTTTGAGTTAGGTGTGGGATA		60°C
Human LINE1	Reverse	*СТСАСТААААААТАССАААСАА		
	Sequencing	GTTAGGTGTGGGATATAGTT	4	
	Forward	GGGACACCGCTGATCGTATATTTTATTAAAAAATATAAA		52°C
Human	Reverse	ССАААСТААААТАСААТАА		
ALU ^e	Universal	*GGGACACCGCTGATCGTATA		
	Sequencing	AATAACTAAAATTACAAAC	3	
	Forward	GGAGAAGTGGTGGGTGGG		60°C
	Reverse	*CTCCTATATCACCAACCTAAAAAACCT		
Mouse	Sequencing 1	AGTGGTGGGTGGGTA	5	
promoter ^f	Sequencing 2	AGTGAGTGAATGTGG	4	
F	Sequencing 3	ATTGGTTTGTATGGTTGA	3	
	Sequencing 4	GATATTTAGTGGTGATAAGTTT	2	
	Forward	TTGGGGAGGTGGTTTAAAAATGA		60°C
Mouse	Reverse	*CCTCCAAAAACCCTCTCTAT		
rDNA core	Sequencing 1	GAGGTGGTTTAAAAATGAT	2	
promoter	Sequencing 2	GGATTTTAAAGGAATAATTGGT	3	
	Sequencing 3	GATAGGTTAATGAAAGAAAA	2	
Mouse	Forward	*AAATTATATTGGAGAATAGATTAGATGAGT		58°C
minor satellite	Reverse	TCCTTATTACTTTCCTCATTAATATACACT		
DNA	Sequencing	ACACTATTCTACAAATCCC	2	

Mouse major satellite DNA	Forward	*TGTGTGTGTGTATTTATTTTGGAAAGAA		58°C
	Reverse	AACATTTCTAAATATTCCACCTTTTTC		
	Sequencing	CAATATACATTTCTCATTTTTCAC	3	
	Forward	*GGTTGGGGAGGYGGTTTAAGTTATA		60°C
Mouse	Reverse	CTACCTATTCCAAAAACTATCAAATTCTT		
LINE1 T ^g	Sequencing 1	AATCCCAAACCAAAATA	2	
	Sequencing 2	CCTATTCAAACTAATTTCCTAAA	2	
	Forward	TGTTTTGTTTGGAAGGGGTTT		58°C
	Reverse	*AACTATATTTAAAACCAAAAATTTTTCC		
Bovine	Sequencing 1	GTTTGGAAGGGGTTTT	3	
DNA	Sequencing 2	GTGGGTGGTTTTATATT	3	
	Sequencing 3	GGTTTTTTTTGATAAGAATT	3	
	Sequencing 4	GAAGGGGTAGTTTTT	3	
	Forward	TTGGGTTTGGTGTATTGGAAGA		58°C
Bovine	Reverse	*ACTCCACCCCTATAAATACAAT		
testis satellite	Sequencing 1	GTAGGGTATTTTGATTTTAGA	2	
DNA	Sequencing 2	GGGTTGAGGTATGGAA	5	
	Sequencing 3	GGAATTTGGGGTTTTTT	2	
	Forward	GGTTTTTTAGGAGTTGGAT		57°C
	Reverse	*AAAATTTCCAACTATTTCTTAAATACCT		
Marmoset $\alpha_{-satellite}$ DNA	Sequencing 1	GGGTGAATTGGAATAA	2	
	Sequencing 2	TGAGAAATATTTGTTTTTAAAATTA	2	
	Sequencing 3	GGTTTTTAATGTGTGTGTATTTAATTT	1	

^a Primers indicated by a star are biotinylated at the 5' end.

^b Outer primers for nested PCR are only necessary, when working with very small amounts of DNA (equivalent to 10 sperm).

Primers adopted from the literature.

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- ^d Teschler, S., Gotthardt, J., Dammann, G., & Dammann, R.H. (2016). Aberrant DNA methylation of rDNA and PRIMA1 in borderline personality disorder. *International Journal of Molecular Sciences*, *17*, E67.
- ^e Yang, A. S., Estécio, M. R., Doshi, K., Kondo, Y., Tajara, E. H., & Issa, J. P. (2004). A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Research*, *32*, e38.
- ^f Shiao, Y. H., Leighty, R. M., Wang, C., Ge, X., Crawford, E. B., Spurrier, J. M., ... Anderson, L.M. (2012). Ontogeny-driven rDNA rearrangement, methylation, and transcription, and paternal influence. *PloS One*, *6*, e22266.
- ^g Walter, M., Teissandier, A., Pérez-Palacios, R., & Bourc'his, D. (2016). An epigenetic switch ensures transposon repression upon dynamic loss of DNA methylation in embryonic stem cells. *Elife*, *5*, e11418.

TABLE S3 Correlation between single CpG methylation (in human sperm rDNA, α-satellite DNA and LINE1 elements) and donor's age

	rDNA up control el	stream lement	rDNA pr	omoter	18S r	DNA	28S rI	DNA	α-satelli	te DNA	LIN	E1
	Pearson's r	<i>p</i> value	Pearson's r	<i>p</i> value	Pearsons'r	p value	Pearson's r	<i>p</i> value	Pearson's r	<i>p</i> value	Pearson's r	<i>p</i> value
CpG1	0.34	< 0.0001	0.57	< 0.0001	0.31	< 0.0001	0.49	< 0.0001	0.30	< 0.0001	0.22	0.003
CpG2	0.46	< 0.0001	0.59	< 0.0001	0.24	0.002	0.43	< 0.0001	0.26	0.001	0.31	< 0.0001
CpG3	0.60	< 0.0001	0.51	< 0.0001	0.34	< 0.0001	0.38	< 0.0001	0.29	< 0.0001	0.30	< 0.0001
CpG4	0.41	< 0.0001	0.54	< 0.0001	0.34	< 0.0001	0.45	< 0.0001	0.28	< 0.0001	0.27	< 0.0001
CpG5	0.50	< 0.0001	0.60	< 0.0001	0.46	< 0.0001	0.49	< 0.0001				
CpG6	0.65	< 0.0001	0.60	< 0.0001	0.34	< 0.0001	0.43	< 0.0001				
CpG7	0.63	< 0.0001	0.45	< 0.0001	0.43	< 0.0001	0.29	< 0.0001				
CpG8	0.50	< 0.0001	0.60	< 0.0001	0.40	< 0.0001	0.40	< 0.0001				
CpG9	0.54	< 0.0001	0.62	< 0.0001			0.43	< 0.0001				
CpG10	0.60	< 0.0001					0.48	< 0.0001				
CpG11	0.56	< 0.0001										
CpG12	0.59	< 0.0001										
CpG13	0.48	< 0.0001										
CpG14	0.65	< 0.0001										
CpG15	0.58	< 0.0001										
CpG16	0.56	< 0.0001										
CpG17	0.48	< 0.0001										
CpG18	0.43	< 0.0001										

Sperm cohort 1 (N = 186)

CpG19	0.46	< 0.0001
CpG20	0.27	< 0.0001
CpG21	0.30	< 0.0001
CpG22	0.28	< 0.0001
CpG23	0.32	< 0.0001
CpG24	0.45	< 0.0001
CpG25	0.55	< 0.0001
CpG26	0.54	< 0.0001

Sperm cohort 2 (N = 109)

	rDNA U	UCE	rDNA pr	omoter	18S r	DNA	28S rI	DNA	α-satellit	e DNA	LINI	E1
	Pearson's r	p value	Pearson's r	<i>p</i> value	Pearsons'r	p value	Pearson's r	<i>p</i> value	Pearson's r	p value	Pearson's r	p value
CpG1	0.44	< 0.0001	0.58	< 0.0001	0.32	0.002	0.46	< 0.0001	0.26	0.013	0.27	0.019
CpG2	0.52	< 0.0001	0.60	< 0.0001	0.30	0.004	0.42	< 0.0001	0.29	0.005	0.35	0.002
CpG3	0.55	< 0.0001	0.57	< 0.0001	0.37	< 0.0001	0.48	< 0.0001	0.31	0.002	0.24	0.038
CpG4	0.44	< 0.0001	0.58	< 0.0001	0.32	0.001	0.44	< 0.0001	0.26	0.011	0.27	0.029
CpG5	0.43	< 0.0001	0.53	< 0.0001	0.41	< 0.0001	0.43	< 0.0001				
CpG6	0.62	< 0.0001	0.46	< 0.0001	0.32	0.002	0.45	< 0.0001				
CpG7	0.59	< 0.0001	0.42	< 0.0001	0.37	< 0.0001	0.31	0.002				
CpG8	0.50	< 0.0001	0.64	< 0.0001	0.42	< 0.0001	0.35	0.001				
CpG9	0.53	< 0.0001	0.59	< 0.0001			0.39	< 0.0001				
CpG10	0.55	< 0.0001					0.45	< 0.0001				
CpG11	0.52	< 0.0001										
CpG12	0.53	< 0.0001										
CpG13	0.50	< 0.0001										

CpG14	0.50	< 0.0001
CpG15	0.53	< 0.0001
CpG16	0.50	< 0.0001
CpG17	0.46	< 0.0001
CpG18	0.50	< 0.0001
CpG19	0.39	< 0.0001
CpG20	0.27	0.008
CpG21	0.23	0.028
CpG22	0.27	0.008
CpG23	0.28	0.006
CpG24	0.49	< 0.0001
CpG25	0.52	< 0.0001
CpG26	0.45	< 0.0001

Assay	Primer	Sequence (5'-3')*	Amplicon length	Variant	Number of CpGs	Annealing temperature			
rDNA	Forward	GGAGTTAGYGGGGTGGGGTTGT	272 hn	A/G	38	56°C			
region 1	Reverse	ACTAAAAAATTAAACCTCC	272 op	11/0	50	50 0			
(external transcribed spacer)	Amplified sequence	3GAGCCAGCGGGGTGGGGTTGTCGCGGCCGCCCGGGCGCCCGCAGCGGAGAGCGCACGGGGGCACGGTG 3CCCTCGCCGCCTTCCCCGCCGCCCCGGGGTGGGTCAGAGACCCGGACCCGGGCCGGCACCGGGAGTCGGG ACGCTCGGACGCGCGAGAGA <u>A/G</u> CAGCAGGCCCGCGGGGCCCGGGCGGCGCGCGCGCGGGGCGGGCGGGCGGGCGGGCGGGG							
rDNA	Forward	TATTYGGAGGTTTAATTTTTTAG	225 hn	۸/C	25	56°C			
region 2	Reverse	ТАТАТССТААААТТААССАААААААСССС	233 op	A/U	23	50 C			
control element and core promoter)	Amplified sequence	GGAGGCCCAACCTCTCCAGCGACAGGTCGCCAGAGGACAGCGTGTCAGCAATAACCCGGCGGCCCAAAAT GCCGACTCGGAGCGAAAGATATACCTCCCCCGGGGGCCGGGAGGTCGCGTCACCGACCACGCCGCCGGCCC AGGC <u>A/G</u> ACGCGCGACACGGACACCTGTCCCCAAAAACGCCACCATCGCAGCCACACACGGAGCGCCCGGG GCCCTCTGGTCAACCCCAGGACACAC							

TABLE S4Primers for deep bisulfite sequencing of human rDNA

* The A/G variant in the amplified sequence is underlined.

Region	CpG number	Correlation coefficient	p value				
Mus musculus $(N = 80)$							
rDNA spacer promoter	14	Pearson's $r = 0.19$	0.09				
rDNA core promoter	7	Pearson's $r = 0.23$	0.044				
18S rDNA	8	Pearson's $r = 0.25$	0.024				
28S rDNA	10	Pearson's $r = 0.36$	0.001				
Mouse minor satellite	2	Pearson's $r = 0.57$	< 0.0001				
Mouse major satellite	3	Pearson's $r = 0.24$	0.037				
Mouse LINE1-T	4	Pearson's $r = 0.27$	0.016				
Bos taurus (N = 36)*							
18S rDNA	8	Spearman's rho $= 0.45$	0.006				
28S rDNA	10	Spearman's rho $= 0.32$	0.058				
Bovine alpha-satellite	12	Spearman's rho $= 0.62$	< 0.0001				
Bovine testis satellite I	9	Spearman's rho $= 0.76$	< 0.0001				
Callithrix jacchus (N = 16)							
18S rDNA	8	Spearman's rho $= 0.02$	0.9				
28S rDNA	10	Spearman's rho $= 0.22$	0.4				
α-satellite DNA	5	Spearman's rho $= 0.63$	0.009				

TABLE S5 Correlations between donor's age and mean repeat methylation indifferent animal models

* Including samples from 9 bulls at three different ages and 3 bulls at two different ages.

Sperm methylation of orthologous regions in 18S and 28S TABLE S6 rDNA in four analyzed species

	Methylation (%) Mean \pm SD ^a [range]	Methylation increase (%) in 10% of lifespan ^b
18S rDNA		
Human (N = 295)	14.2±3.4 [8.1-26.4]	1.83
Marmoset (N = 16)	22.1±6.9 [10.3-36.9]	0.25
Bovine $(N = 36)$	5.8±2.0 [2.8-12.1]	0.35
Mouse $(N = 80)$	18.1±3.3 [11.5-27.3]	0.81
28S rDNA		
Human (N = 295)	11.5±2.8 [5.7-21.4]	1.73
Marmoset (N = 16)	18.2±4.7 [10.6-27.8]	0.49
Bovine $(N = 36)$	8.1±2.1 [4.5-12.3]	0.41
Mouse $(N = 80)$	15.5±3.6 [8.2-25.1]	1.28

^a SD = standard deviation.
^b Lifespan is 80 years for humans, 12 years for marmoset, 20 year for bull, and 28 months for mouse.



FIGURE S1 rDNA methylation analysis of DNA aliquots equivalent to 10 human sperm. Scatter plots show the correlation between donor's age (x-axis in years) and mean methylation (y-axis in %) of the UCE (26 CpGs) and rDNA promoter (9 CpGs).. DNA aliquots of 8 donors covering an age range from 25 to 60 years with 5-year gaps were analyzed by bisulfite pyrosequencing in triplicates. The blue, green and red dots in the upper diagrams represent the three measurements for each sample; the black dots in the lower diagrams represent the mean of triplicate measurements. Pearson's correlations were used for statistical analysis of the 24 measurements as well as for the means of 8 triplicates. **** indicates p < 0.0001 and * p < 0.02.

CpG



9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28b 29 30 31 32 33 34 35 36 37 38 7 8 4 6

CpG methylation High Low

FIGURE S2A Single CpG methylation of the A variant of human rDNA region 1 (in the external transcribed spacer). Each row on the y-axis represents an individual sperm sample, 23 from young donors and 23 from old donors. The x-axis represents CpGs 1-38. The number within each box indicates the methylation percentage of a given CpG in a given sample, determined by deep bisulfite sequencing.

CpG

Low CpG methylation High

FIGURE S2B Single CpG methylation of the G variant of human rDNA region 1 (in the external transcribed spacer). Each row on the y-axis represents an individual sperm sample, 23 from young donors and 23 from old donors. The x-axis represents CpGs 1-38. The number within each box indicates the methylation percentage of a given CpG in a given sample, determined by deep bisulfite sequencing.

U	þд																								
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
17	5	11	0	4	5	14	7	14	6	10	12	16	13	17	24	31	11	14	14	3	11	7	3	11	7
16	7	14	16	11	16	28	9	37	17	13	16	22	18	26	35	40	11	13	14	3	26	10	5	28	
16	5	8	4	2	3	7	4	10	5	6	8	14	9	12	15	26	8	12	14	3	8	6	3	9	
13	5	11	7	6	7	15	4	21	5	8	11	15	14	9	25	28	8	10	11	2	15	6	4	23	
11	4	9	6	3	6	14	5	20	10	6	9	15	12	14	23	27	7	11	10	2	13	7	5	18	
14	6	8	0	4	4	15	0	17	0	0	10	12	10	12	20	27	10	12	12	2	12	7	2	15	0
13	5	12	11	7	11	27	6	30	14	13	11	12	14	18	25	30	12	18	15	3	16	10	6	30	Ĕ
12	4	9	5	2	3	7	3	9	6	6	7	11	9	10	12	20	5	8	10	3	8	3	2	11	
19	8	12	9	6	8	14	7	19	11	11	12	17	13	17	26	31	10	11	15	4	14	7	4	16	o To L
19	6	14	14	11	14	27	9	27	16	12	17	23	21	24	31	40	14	16	17	3	21	6	5	25	
19	7	12	- 7	4	7	13	6	19	8	6	10	15	8	15	20	31	10	13	14	3	14	10	6	16	
15	2	9	6	3	2	9	2	12	7	8	10	10	15	14	21	28	10	12	14	3	7	0	4	14	
14	5	ő	6	3	5	ů	5	15	7	8	10	15	12	15	21	20	10	12	14	3	ú	6	4	13	
19	4	10	9	8	13	29	9	36	14	12	17	24	20	26	31	38	13	17	12	2	21	7	4	25	
17	7	11	8	3	7	12	5	17	9	8	12	16	12	16	21	27	9	13	15	3	13	8	4	15	Ś
13	5	8	5	2	5	9	4	13	7	6	9	15	11	13	18	26	8	11	12	2	9	6	3	13	_
13	4	8	6	4	6	11	5	15	7	7	7	16	13	14	19	28	8	12	13	3	13	- 7	4	15	
13	2	7	8	2	8	10	4	10	8	7	10	12	6	12	17	21	8	10	10	2	15	6	4	12	
15	6	ó	ŏ	7	8	15	5	18	10	10	13	16	14	17	22	30	8	10	12	2	13	7	5	15	J
23	8	12	13	5	9	23	ii	28	17	16	15	27	20	26	35	39	18	20	22	5	21	13	8	20	7
23	10	15	10	9	15	15	7	21	6	10	14	19	12	25	27	32	12	20	21	5	17	9	7	16	
36	13	18	8	5	8	15	10	16	10	15	17	31	18	26	29	52	27	29	31	6	16	14	8	16	
31	12	18	13	7	14	20	13	24	18	16	22	33	20	35	36	49	24	27	31	8	23	16	9	23	
50	25	40	41	21	36	51	28	54	20	25	32	34	33	41	61	73	50	52	28	11	51	20	17	51	
25	10	14	15	9	14	25	15	35	15	11	15	27	24	25	35	45	17	24	23	5	24	15	8	30	
35	13	18	8	6	8	16	10	19	11	16	20	35	21	29	31	52	27	30	32	6	17	13	8	18	
24	10	12	8	6	7	16	10	18	10	10	13	22	14	24	24	37	15	20	19	3	12	13	7	18	Ē
28	10	13	9	3	7	10	11	21	14	13	18	26	17	23	26	40	18	22	20	4	15	12	8	20	
26	11	15	11	5	11	20	10	25	13	14	19	29	23	27	30	49	22	22	23	5	20	14	9	29	Γd
20	11	16	7	4	7	12	8	18	12	14	10	28	16	24	27	43	10	25	25	5	13	10	6	14	0
39	22	30	28	18	27	40	25	51	25	21	24	37	27	31	43	59	31	37	39	10	33	23	17	41	B
36	14	23	17	11	14	30	16	33	17	21	24	39	28	37	46	63	31	34	36	8	28	18	10	31	ō
41	17	23	14	10	15	27	17	33	20	27	31	44	30	41	45	66	37	38	41	10	24	18	11	27	F
56	36	51	49	48	45	52	36	71	62	69	68	71	60	74	76	82	53	56	58	11	54	30	20	50	\mathbf{v}
33	13	20	21	18	19	33	19	39	10	24	23	39	26	36	42	57	24	28	27	9	32	17	13	34	
25	0	13	0	15	20	38	7	43	12	12	20	38	28	41	20	43	18	22	23	Á	33	17	8	38	
26	'n	15	ú	6	n	20	ģ	23	13	15	18	27	19	24	30	44	17	22	24	5	19	13	7	24	
28	12	15	9	5	8	15	9	23	14	15	18	31	20	2.6	31	45	19	25	27	6	18	14	8	21	J

Low CpG methylation High

CnC

FIGURE S2C Single CpG methylation of the A variant of human rDNA region 2 (in the upstream control element and core promoter). Each row on the y-axis represents an individual sperm sample, 23 from young donors and 23 from old donors. The x-axis represents CpGs 1-25. The number within each box indicates the methylation percentage of a given CpG in a given sample, determined by deep bisulfite sequencing.

	nC	
U	þФ	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
17	6	9	5	3	5	9 14	5	12	7	7	10	15	12	14	18	29	9	14	14	3	10	7	4	12	ר
16	5	8	4	2	3	6	4	9	6	6	8	13	9	12	15	26	7	12	13	3	7	6	3	8	
12	4	9	7	4	6	14	4	16	6	5	8	12	13	12	19	23	6	9	9	2	12	5	4	17	
11	4	7	4	2	4	8	3	11	6	6	8	13	13	12	17	22	6	10	10	2	10	5	4	12	
15	6	0	6	4	6	14	5	14	7	-	10	15	13	15	20	28	8	12	13	3	12	7	4	15	
13	5	10	7	4	7	13	4	15	6	6	8	12	12	13	18	24	7	11	10	2	13	6	4	17	Ē
12	4	6	4	2	4	6	3	9	5	5	7	12	10	10	14	20	5	9	9	2	8	5	3	9	
16	6	10	5	3	5	9	4	11	7	6	9	13	11	13	17	25	7	12	12	3	10	6	4	11	en l
14	5	9	7	4	6	12	5	16	8	7	10	15	13	15	21	26	7	12	12	2	12	6	4	15	
14	6	8	5	3	5	8	4	12	7	7	10	14	15	13	19	26	8	13	13	3	10	6	4	10	Q
15	6	10	5	3	4	10	4	11	5	6	7	11	9	12	16	25	7	11	11	2	10	5	3	11	2
11	4	7	4	2	4	6	3	9	6	5	7	12	12	10	15	22	6	10	10	2	8	5	3	10	
14	5	8	5	3	5	9	4	12	7	7	9	14	12	13	17	25	7	12	12	2	9	6	4	12	2
13	6	8	6	3	2	9	- 4	12	7	7	8	13	12	12	17	22	7	11	11	2	10	7	4	12	
14	5	8	5	2	5	8	4	12	6	6	8	14	13	13	18	26	7	12	12	2	9	6	4	12	U 1
14	5	8	6	3	6	11	5	14	8	7	10	15	14	14	19	26	8	12	13	2	11	6	4	13	
14	6	8	5	3	4	7	4	10	6	6	8	13	10	11	15	23	6	11	12	3	10	6	4	11	
9	3	6	4	2	3	7	2	9	5	4	5	9	7	8	13	14	3	7	8	1	9	4	2	10	
13	10	9		4		11	4	14	7	7	9	14	12	14	18	23	0	10	21	2	11		4	13	5
27	12	17	10	8	ŷ	15	9	20	13	14	18	28	19	25	29	39	15	24	24	5	17	13	8	18	
30	13	16	7	4	7	11	8	16	10	12	15	25	15	23	25	38	13	22	23	5	13	12	6	13	
31	13	17	9	5	9	14	10	19	13	13	17	28	19	26	29	41	15	25	27	7	17	14	7	16	
33	14	18	10	6	10	16	12	18	13	14	19	28	21	26	29	49	20	28	29	7	16	13	8	17	
33	13	17	13	0	11	20	12	25	14	17	18	27	21	32	30	49	20	22	23	5	19	10	8	21	
32	13	17	9	5	8	15	10	20	12	15	19	31	18	27	31	45	17	28	29	6	15	13	8	17	
25	9	13	5	3	5	9	6	15	9	10	13	22	12	19	22	29	9	19	20	4	12	11	5	12	\subseteq
26	11	14	8	4	7	11	7	17	10	11	14	24	17	21	25	33	11	21	21	5	13	12	6	15	5
28	11	15	10	5	10	17	9	21	12	13	17	27	22	25	31	43	15	24	24	6	18	13	8	23	
20	13	16	7	4	6	18	10	17	14	14	17	27	15	20	26	41	15	25	24	6	20	13	6	13	Q
32	15	19	12	7	12	18	12	22	14	15	19	28	19	27	31	43	16	26	28	8	19	16	ğ	21	0
33	13	19	12	7	11	21	12	25	14	16	20	31	22	29	34	46	17	27	28	7	21	15	9	23	
38	17	22	14	9	13	22	15	26	17	21	25	37	26	35	39	60	25	36	36	10	21	17	10	22	0
29	13	16	9	5	7	13	9	19	12	14	17	28	18	25	28	40	15	25	26	6	16	14	7	17	
34	16	21	15	10	13	22	13	27	15	17	21	32	22	31	36	48	19	30	30	8	22	17	10	25	Ś
26	10	15	9	6	10	18	9	22	12	12	15	26	22	25	31	43	16	25	24	5	18	12	8	21	
27	12	16	14	8	13	23	11	26	14	15	18	27	20	26	33	42	14	23	24	6	21	14	8	26	
29	12	16	9	5	9	15	9	22	13	14	18	29	21	26	31	41	14	25	26	6	18	14	8	21	
32	15	19	9	5	8	15	10	20	12	15	19	30	18	27	31	43	17	27	29	7	18	14	7	17	J

Low CpG methylation High

FIGURE S2D Single CpG methylation of the G variant of human rDNA region 2 (in the upstream control element and core promoter). Each row on the y-axis represents an individual sperm sample, 23 from young donors and 23 from old donors. The x-axis represents CpGs 1-25. The number within each box indicates the methylation percentage of a given CpG in a given sample, determined by deep bisulfite sequencing.



FIGURE S3 Box plots presenting the epimutation rates (ERs) of alleles carrying the A versus the G variant in rDNA regions 1 (representing the external transcribed spacer) and 2 (upstream control element and core promoter). The bottom and the top of the box represent the 25^{th} and 75^{th} percentile, respectively. The median is represented by a horizontal line. Bars extend from the boxes to at most 1.5 times the height of the box. Outliers are indicated by an open circle and extreme outliers by a star symbol. The *p* values indicate significant differences of the ERs between 23 young versus 23 old donors and between rDNA region 1 versus 2, respectively. Mann-Whitney U tests were used for group comparisons.



FIGURE S4 Methylation of the rDNA upstream control element (UCE) and promoter in human fetal cord blood. Each dot represents one of 121 cord blood samples. The y-axis indicates mean methylation (in %) of 26 CpG and 9 CpG sites in the UCE and promoter, respectively. The x-axis indicates the age of the father (in years) at conception. There is no significant correlation (Pearson's r = 0.01; p = 0.92 and r = 0.06; p = 0.53, respectively) between cord blood rDNA methylation and paternal age.



Quantile	Absolute error
0%	0.001
5%	0.310
10%	0.607
15%	0.758
20%	0.981
25%	1.252
30%	1.430
35%	1.726
40%	1.923
45%	2.401
50%	2.911
55%	3.413
60%	3.693
65%	4.202
70%	4.610
75%	4.852
80%	5.469
85%	6.308
90%	6.570
95%	7.242
100%	12.546

FIGURE S5 Distribution of absolute error in the test data set. In total 154 test samples were analyzed. The cumulative distribution (i.e. the fraction of values $\langle = x \rangle$ is plotted on the y-axis versus the absolute error in years on the x-axis. Reference lines (dotted) are given for the 50% and 75% quantiles.



FIGURE S6 Repeat methylation in marmoset sperm increases with donor's age. Scatter plots show positive correlations between donor's age (x-axis in years) and mean methylation (y-axis in %) of 18S rDNA (8 CpGs), 28S rDNA (10 CpGs), and α -satellite DNA (5 CpGs) in marmoset sperm (N = 16). Spearman's correlations were used for statistical analysis of bisulfite pyrosequencing data. ** indicates p < 0.01.



FIGURE S7 Evolutionary conservation of the paternal age effect on sperm methylation of orthologous rDNA regions. Scatter plots show positive correlations between donor's age (x-axis in percentage of lifespan) and mean methylation (y-axis in %) of 18S rDNA (8 CpGs) and 28S rDNA (10 CpGs), respectively, in 80 mouse (purple dots), 36 bull (yellow), 16 marmoset (red), and 295 human (green) sperm samples. Mean methylation of all 427 analyzed samples (indicated by the black regression lines) significantly increased with donor's age (Pearson's r = 0.20; *p* < 0.0001 for 18S and r = 0.13; *p* < 0.01 for 28S rDNA).