

Supplementary Material

1 Supplementary Figures and Tables

Supplementary Table S1. List of primer sequences

Gene symbol	Gene name	RefSeq ID	Primer sequence	
<i>Cat</i>	catalase	NM_009804.2	F	5' -TCACTCAGGTGCGGACATTC - 3'
			R	5' -TAGTCAGGTTGGACGTCAGT - 3'
<i>Cdh2</i>	cadherin 2	NM_007664.5	F	5' -CTTGCTTCAGGCGTCTGTGGA - 3'
			R	5' - TTCGTGCACATCCTTCGGTAA - 3'
<i>Cldn11</i>	Claudin 11	NM_016674.4	F	5' -GGCGACATTAGTGGCCACAGCA - 3'
			R	5' -GCAGCGGCCAGCCAGTAA - 3'
<i>Ctnnb1</i>	catenin beta 1	NM_007614.3	F	5' - CGCCGCTTATAATCGCTCC - 3'
			R	5' - TTCACAGGACACGAGCTGAC - 3'
<i>Cyct</i>	cytochrome c, testis	NM_009989.3	F	5' -CGGCTGCTGTGATTGTGAATAC - 3'
			R	5' -TGTCTTGTGTTTCCCGCCTT - 3'
<i>F11r</i>	F11 receptor	NM_172647.2	F	5' - AGGTCATTATACAGCCAGCCC - 3'
			R	5' -GAAGGCATCCTGTGCAGCTA - 3'
<i>Gpx4</i>	glutathione peroxidase 4	NM_001037741.3	F	5' - TGTGTAATGGGACGATGCC - 3'
			R	5' - TCTCTATCACCTGGGCTCCT - 3'
<i>Ocln</i>	occludin	NM_008756.2	F	5' - CTTATCTTGGGAGCCTGGACAT - 3'
			R	5' - ATGCATCTCTCCGCCATACA - 3'
<i>Pm1</i>	protamine 1	NM_013637.4	F	5' -ACAGGTTGGCTGGCTCGACC - 3'
			R	5' -CGGCAGCATCGGTATCTGGCC - 3'
<i>Pm2</i>	protamine 2	NM_008933.1	F	5' -CCAGGGGCCTGGACAAGACC - 3'
			R	5' -TCTGTGGTGGTGGTGGCCC - 3'
<i>Sod1</i>	superoxide dismutase 1	NM_011434.1	F	5' - GGAACCATCCACTTCGAGCA - 3'
			R	5' -CTGCACTGGTACAGCCTTGT - 3'
<i>Sod2</i>	superoxide dismutase 2	NM_013671.3	F	5' - GAACAATCTCAACGCCACCG - 3'
			R	5' - CCAGCAACTCTCCTTGGGTT - 3'
<i>Sod3</i>	superoxide dismutase 3	NM_011435.3	F	5' -CTGACAGGTGCAGAGAACCTC - 3'
			R	5' - GCGTGTGCTCTATCTTCTCA - 3'
<i>Sycp1</i>	synaptonemal complex protein 1	NM_011516.2	F	5' -GCCCCATGCTCGAACAG GTTGC - 3'
			R	5' -ACAGTCTGCTCATTGGCTCTGAA - 3'
<i>Sycp3</i>	synaptonemal complex protein 3	NM_011517.2	F	5' -GGACAGCGACAGCTCACCGG - 3'
			R	5' -GGTGGCTTCCAGATTCCAGA - 3'
<i>Tjp1</i>	tight junction protein 1	NM_009386.2	F	5' -TCTTGCAAAGTATCCCTTCTGT - 3'
			R	5' -CAGAAATCGTGCTGATGTGCC - 3'
<i>Tnp1</i>	transition protein 1	NM_009407.2	F	5' -CCGAGCTCCTCACAAG GCGGT - 3'
			R	5' -CAGGGCAGAGCTCATTGCCGC - 3'
<i>Tnp2</i>	transition protein 2	NM_013694.4	F	5' -CCTGCAAGACCCAGCCACCG - 3'
			R	5' -GTTTCCGCCTCCTGACGCC - 3'

Table S2. Anogenital distance and organ weights (parental generation, P)

Group	n	AGD (cm)	Testes (g)	Epididymides (g)	Prostate (g)	Seminal vesicles (g)	Spleen (g)
Control	10	1.29±0.01	0.209±0.004	0.066±0.001	0.096±0.003	0.273±0.011	0.084±0.003
T2D	8	1.25±0.01	0.207±0.004	0.063±0.001	0.081±0.004**	0.244±0.012	0.076±0.003

Data are presented as the mean ± SEM. Differences between groups were tested by ANCOVA with body weight as covariate (Ancova – STATISTICA 7.0). **P<0.01. AGD, anogenital distance.

Table S3. Sperm parameters (parental generation, P)

Group	n	Concentration (1 * 10 ⁶ /ml)	Viability (%)	CMA3 (%)	Annexin V (%)
Control	10	22.20±2.31	80.94±1.23	97.50±0.34	53.15±1.93
T2D	8	16.16±1.90	83.58±1.77	98.19±0.37	57.06±3.42

Data are presented as the mean ± SEM. Differences between groups were tested by *t*-test (GraphPad Prism 7.0). CMA3, chromomycin A3

Table S4. Representation of the stages of the sperm production in the seminiferous epithelium

Group	I – III (%)	IV – VI (%)	VII – VIII (%)	IX – XII (%)
Control	10.41±2.27	23.50±1.95	30.23±2.16	35.86±5.35
T2D	15.73±4.45	25.79±3.06	27.93±5.33	30.54±2.73

Data are presented as the mean ± SEM (n = 10 controls, 206 tubules; 8 T2D males, 254 tubules).

Table S5. Anogenital distance and organ weights (the offspring generations, F₁ and F₂)

Group	n	AGD (cm)	Testes (g)	Epididymides (g)	Prostate (g)	Seminal vesicles (g)	Spleen (g)
Control F ₁	10	1.21±0.03	0.186±0.003	0.057±0.002	0.075±0.005	0.167±0.008	0.101±0.004
T2D F ₁	9	1.22±0.03	0.176±0.002**	0.057±0.002	0.087±0.005	0.160±0.007	0.091±0.004
Control F ₂	10	1.24±0.02	0.171±0.009	0.054±0.002	0.080±0.005	0.162±0.010	0.093±0.005
T2D F ₂	10	1.22±0.02	0.164±0.008	0.055±0.002	0.083±0.004	0.170±0.010	0.081±0.005

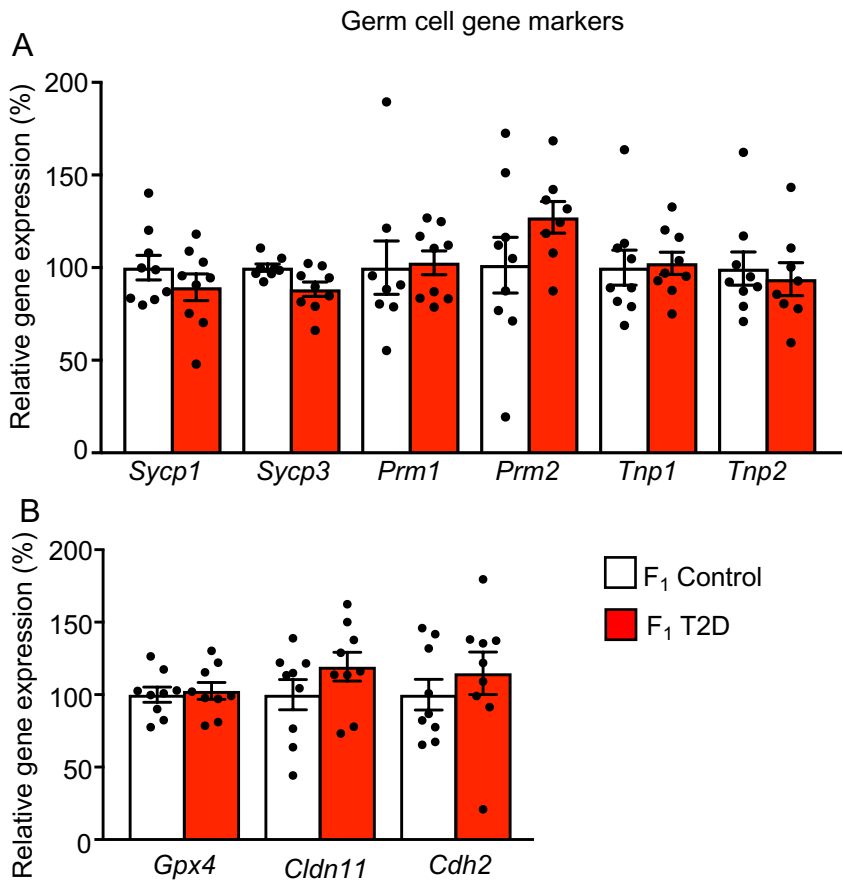
Data are presented as the mean ± SEM. Differences between groups were tested by ANCOVA with body weight as covariate (Ancova – STATISTICA 7.0). **P<0.01. AGD, anogenital distance.

Table S6. Sperm parameters (the offspring generations, F₁ and F₂)

Group	n	Concentration (1 * 10 ⁶ /ml)	Viability (%)	CMA 3 (%)	Protamine ratio (P1/P2)	Annexin V (%)
Control F ₁	10	14.53±1.90	80.98±0.81	97.63±0.53	0.50±0.01	54.69±2.06
T2D F ₁	9	12.94±1.15	83.43±1.09	98.31±0.35	0.50±0.02	44.38±1.84
Control F ₂	10	15.45±1.74	82.43±1.82	98.53±0.27	0.53±0.02	54.50±2.31
T2D F ₂	10	16.29±1.63	83.32±1.45	98.05±0.40	0.51±0.02	48.30±1.58

Data are presented as the mean ± SEM. Differences between groups were tested by *t*-test (GraphPad Prism 7.0). CMA3, chromomycin A3

Supplementary Figures



Supplementary Figure S1. Relative expression of selected genes in the testes of the F₁ generation. qPCR analysis of markers for (A) spermatogenesis and spermiogenesis: synaptonemal complex protein 1 (*Sycp1*), synaptonemal complex protein 3 (*Sycp3*), protamine 1 (*Prm1*), protamine 2 (*Prm1*), transition protein 1 (*Tnp1*) and transition protein 2 (*Tnp2*); (B) blood testis barrier: cadherin 2 (*Cdh2*), claudin 11 (*Cldn11*); and oxidative stress: glutathione peroxidase 4 (*Gpx4*). The control group represents 100% of relative gene expression. The values are means $\pm$ SEM, tested by *t*-test.