

Supplementary Material

Long-term maternal exposure to atrazine in the drinking water reduces penis length in the tammar wallaby *Macropus eugenii*

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Figure S1. Localisation of SOX9 protein (pink) in day 5 pp pouch young testis treated with (a) control, (b) ATZ, in drinking water. Cell nuclei are counterstained with DAPI (blue), white dotted line distinguishes the gonad from the mesonephros (c) Boxplot of average Mander's overlapping coefficient for pouch young treated with control (n = 4) and ATZ (n = 4).

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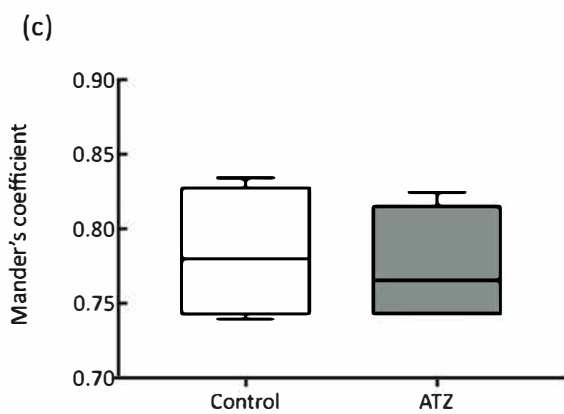
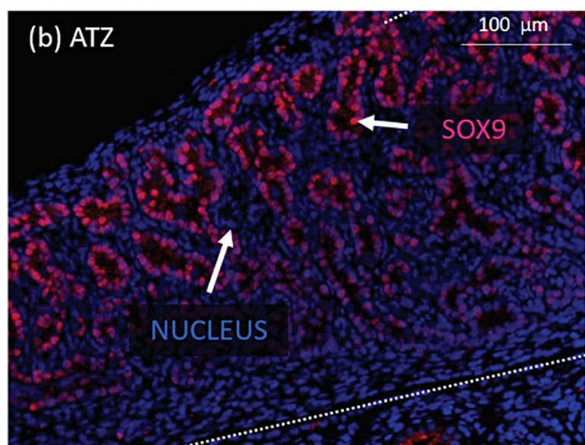
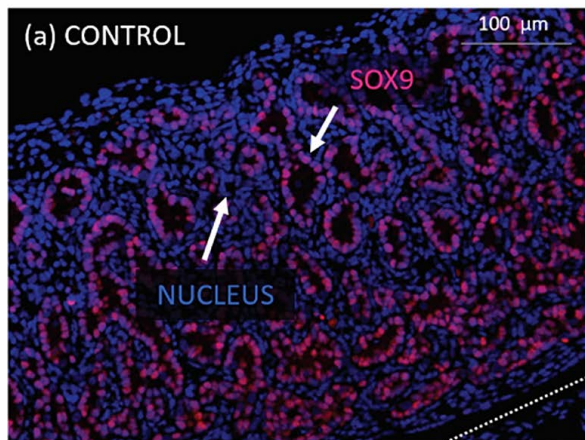


Table S1. RT-qPCR primer sequences in 5' to 3' orientation

Gene	Forward primer	Reverse primer
TATA box binding protein (TBP)	GGACAACTGAAGCAAAGGGACC	AGGGCATCATTGGGCTAAAGATAG
Sex-determining region Y (SRY)	ATGATATGGTCTCGGAGTCA	TGGTCAGGCAGCATCTTC
Forkhead box L2 (FOXL2)	AGAACAGCATCCGCCACAACC	CAGCGTCCAGTAGTTGCCCTTC
Anti-Müllerian hormone (AMH)	TCGGTCACAGTCAGCCCAAGA	CCCTCAGCACAGCAGACATCA
SRY-box 9 (SRY)	GAGGAAGTCAGTCAAGAACGGG	CCAGAGGAAGAATGCGGAGAG
Steroidogenic factor-1 gene (SF-1)	AAGCGCACGGTACAGAATAACAAACAC	TAAGGCACGGTCTCTTTGTACATGG

Table S2. Total number of females used, pouch young born and sex ratios of pouch young for the control and treatment group

	Atrazine		Control	
	<i>Male</i>	<i>Female</i>	<i>Male</i>	<i>Female</i>
Total Females (mothers, not euthanised)	20		20	
Total pouch young born	16		16	
Pouch young collected at D5 pp	5	2	4	4
Pouch young collected at D211 pp	5	4	4	4

Table S3. Mean head lengths (mm) of male pouch young. Student's t-test used to compare means. Significant differences are where $P < 0.05$

Age of pouch young	Control	ATZ	<i>t-value</i>	<i>p-value</i>
Day 148 pp	51.86	52.48	$t_7 = 0.70$	0.51
Day 162 pp	55.80	56.02	$t_7 = 0.23$	0.82
Day 176 pp	59.9	59.37	$t_7 = 0.62$	0.55
Day 190 pp	63.75	64.49	$t_6 = 0.41$	0.69

Table S4.

SAMPLE cDNA	TARGET GENE	Ct CONTROL - Ct TREATED FOR TARGET GENE	FOLD CHANGE IN TARGET GENE	Ct CONTROL - Ct TREATED FOR REFERENCE GENE	FOLD CHANGE IN REFERENCE GENE	RATIO TARGET GENE IN TREATED/CONTROL	log2(fold change)
7027 ATZ	SRY	0.61	1.53	0.02	1.01	1.51	0.60
6852 ATZ	SRY	0.07	1.05	-0.62	0.65	1.61	0.69
6871 ATZ	SRY	1.33	2.51	0.53	1.44	1.74	0.80
6672 ATZ	SRY	0.38	1.30	0.39	1.31	0.99	-0.01
7027 ATZ	SOX9	0.18	1.14	0.02	1.01	1.12	0.17
6852 ATZ	SOX9	-0.14	0.91	-0.62	0.65	1.39	0.48
6871 ATZ	SOX9	0.49	1.40	0.53	1.44	0.97	-0.04
6672 ATZ	SOX9	0.56	1.48	0.39	1.31	1.13	0.17
7027 ATZ	AMH	0.30	1.23	0.02	1.01	1.22	0.29
6852 ATZ	AMH	-0.36	0.78	-0.62	0.65	1.19	0.26
6871 ATZ	AMH	0.81	1.75	0.53	1.44	1.21	0.28
6672 ATZ	AMH	0.55	1.47	0.39	1.31	1.12	0.16
7027 ATZ	FOXL2	-0.99	0.50	0.02	1.01	0.50	-1.00
6852 ATZ	FOXL2	-0.05	0.97	-0.62	0.65	1.48	0.57
6871 ATZ	FOXL2	0.33	1.26	0.53	1.44	0.87	-0.19
6672 ATZ	FOXL2	-0.41	0.75	0.39	1.31	0.58	-0.80
7027 ATZ	SF1	-0.43	0.74	0.34	1.26	0.59	-0.76
6852 ATZ	SF1	-0.36	0.78	-0.19	0.88	0.89	-0.17
6871 ATZ	SF1	-0.12	0.92	0.46	1.37	0.67	-0.57
6672 ATZ	SF1	-0.31	0.81	0.49	1.40	0.57	-0.80