

Title: **Gestational exposure to an epidemiologically defined mixture of phthalates leads to gonadal dysfunction in mouse offspring of both sexes**

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SUPPLEMENTAL TABLES & FIGURES

Table S1. Body weights of male and female offspring on PND1, PDN21 and PND90

Body weight (grams)	Males			
	DMSO	10x	100x	500x
PND1	1.57±0.02 (34)	1.64±0.03 (14)	1.51±0.02 (19)	1.50±0.02 (26)
PND21	7.76±0.10 (30)	8.01±0.15 (13)	7.49±0.11 (19)	7.48±0.09 (26)
PND90	23.53±0.20 (21)	24.17±0.32 (8)	23.28±0.27 (12)	24.21±0.24 (13)
	Females			
PND1	1.52±0.03 (26)	1.59±0.04 (15)	1.47±0.03 (14)	1.42±0.03 (16)
PND21	8.14±0.09 (24)	8.21±0.11 (17)	7.31±0.11 [#] (14)	7.23±0.11 [#] (12)
PND90	18.52±0.22 (14)	18.01±0.27 (9)	17.82±0.25 (9)	18.8±0.25 (9)

Values represent estimated marginal means ± SE. The number of animals is given in parentheses.
p < 0.001 vs. DMSO.

Table S2. Number of litters used, litter size and % of male offspring per group.

	DMSO	10x	100x	500x
Number of litters	9	6	5	6
Litter size (mean ± SEM)	6.78 ± 0.66	5.33 ± 0.92	6.6 ± 0.51	7 ± 0.45
Male offspring (% of total offspring)	55.7	43.8	57.6	61.9

Table S3. qRT-PCR primers used in the study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	PrimerBank ID / citation
<i>β-actin</i>	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	6671509a1
<i>Star</i>	GTCATCAGAGCTGAACACGG	GGTGGTTGGCGAACTCTATC	designed using NCBI software
<i>Cyp17a1</i>	CTCCAGCCTGACAGACATTCTG	TCTCCCACCGTGACAAGGAT	6681099a1
<i>Cyp19a1</i>	ATGTTCTTGAAATGCTGAACCC	AGGACCTGGTATTGAAGACGAG	Ref.1
<i>Fshr</i>	TGCTCTAACAGGGTCTTCCTC	TCTCAGTTCAATGGCGTTCCG	242397445c1
<i>Lhcgr</i>	CGCCCGACTATCTCTCACCTA	GACAGATTGAGGAGTTGTCAAA	7305233a1
<i>Sox9</i>	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT	165932320c1
<i>Dmrt1</i>	CAGAGGGACGCATGGTCATC	TGTAGTAGGCGGGTCTGATA	158631231c1
<i>Foxl2</i>	AACACCGGAGAAACCAGACC	CGTAGAACGGGAACTTGGCT	designed using NCBI software
<i>HPRT1</i>	GCCCTGGCGTCGTGATTAGT	TGTCCTGACTTGAGAACGAGC	designed using NCBI software
<i>HSD3B2</i>	ATCTAAGTTACGCCCTCTTC	CTTCTCTTCTTGACTTCCT	KiCq Start Primers, Sigma-Aldrich
<i>STAR</i>	GACAAAGTGATGAGTAAAGTGG	GTAAGTAATGAGTGCTCGAC	KiCq Start Primers, Sigma-Aldrich
<i>CYP17A1</i>	GAAGTTATCATCAATCTGTGGG	GTCATTCGATAAACGGGAAG	KiCq Start Primers, Sigma-Aldrich
<i>CYP19A1</i>	ATGTGGACGTGTTGACCCTTCT	AACTACGTACCGTTCGAGAGGA	KiCq Start Primers, Sigma-Aldrich

References

1. Bouma, G.J., Hart, G.T., Washburn, L.L., Recknagel, A.K. & Eicher, E.M. Using real time RT-PCR analysis to determine multiple gene expression patterns during XX and XY mouse fetal gonad development. *Gene Expr. Patterns* **5**, 141-9 (2004).

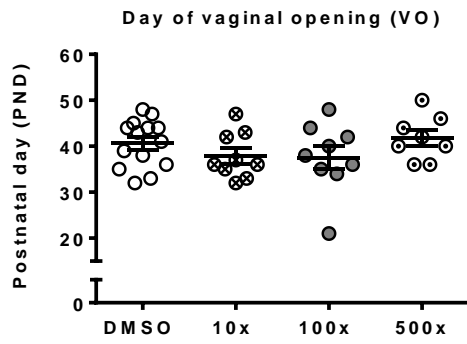


Figure S1. Day of vaginal opening (VO) for female offspring exposed *in utero* to DMSO (n=14) or to 10x (n=9), 100x (n=9) and 500x (n=8) the geometric mean of SELMA mothers' levels for the chemicals in Mixture S. Circles represent individual values, also means \pm SEM are given. No significant differences were detected vs. DMSO.

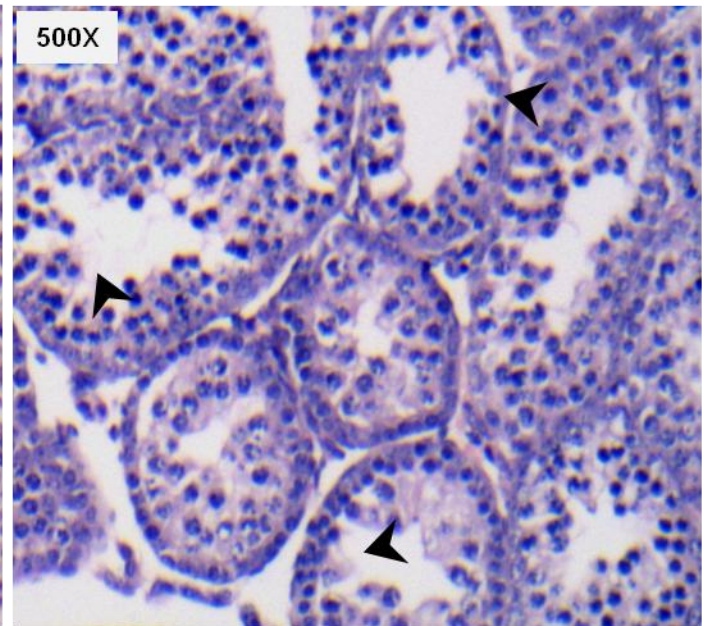
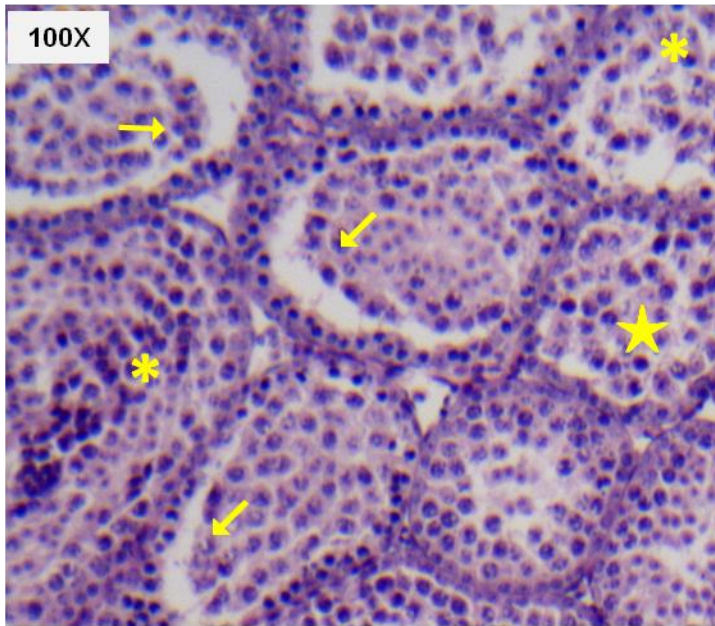
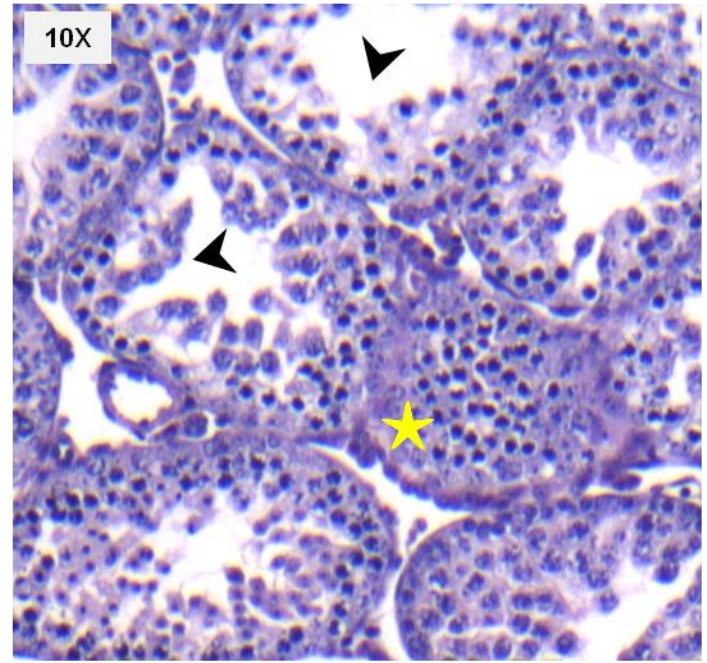
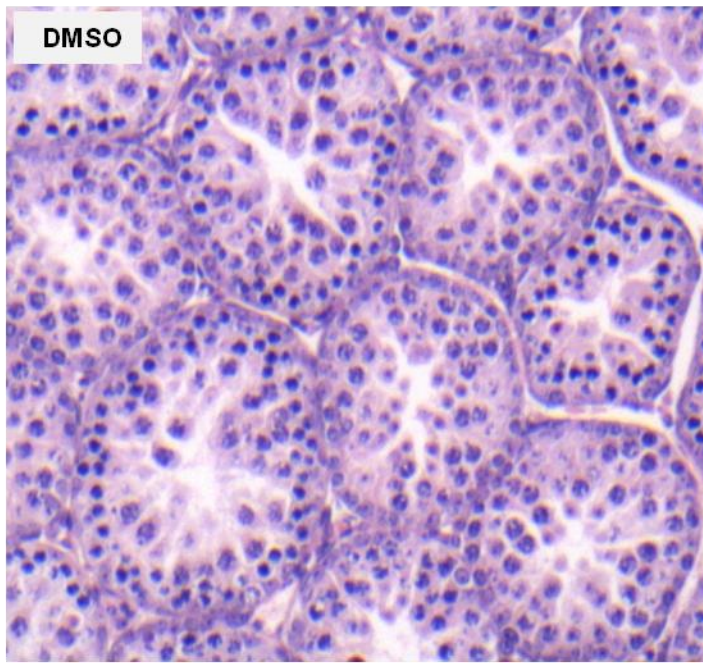


Figure S2A. Supplementary photomicrographs of testicular histology of PND21 offspring exposed to 0x (DMSO), 10x, 100x and 500x SELMA mothers' levels. ★ indicate tubules without lumen; * Indicate tubules with germ cells in the lumen; arrows indicate detached layers; arrowheads indicate tubules with abnormal layers.

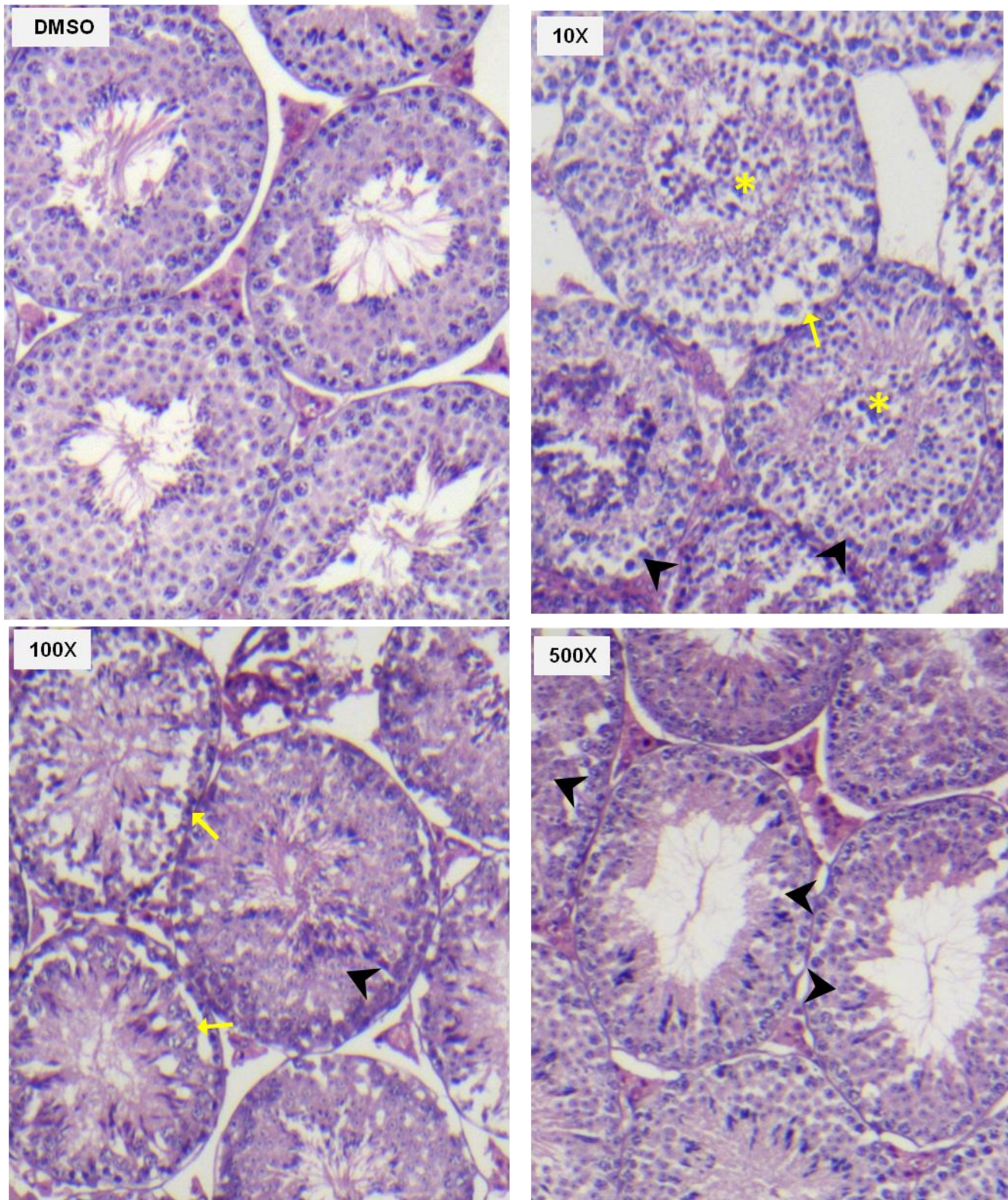


Figure S2B. Supplementary photomicrographs of testicular histology of PND90 offspring exposed to 0x (DMSO), 10x, 100x and 500x SELMA mothers' levels. * Indicate tubules with germ cells in the lumen; arrows indicate detached layers; arrowheads indicate tubules with abnormal layers.

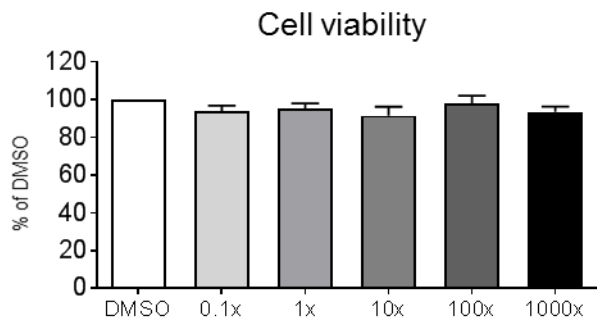


Figure S3. Cytotoxicity assessment of Mixture S on H295R cells after 48 h of Mixture exposure. N = 3 experiments with 3 technical replicates; data represent mean \pm SEM.

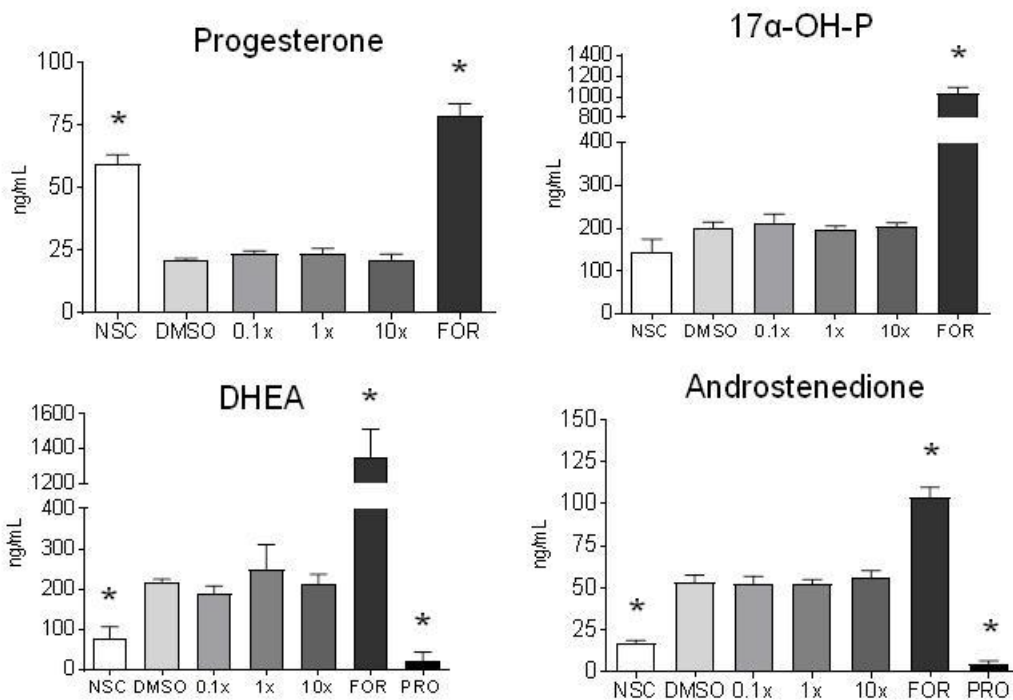


Figure S4. Production of steroid hormones by H295R cells after 24 h Mixture S exposure. NSC – non-stimulated, non-treated control; FOR – forskolin; PRO – Prochloraz. N = 3 experiments with 2 technical replicates; data represent mean \pm SEM; * $p < 0.05$ vs. DMSO control; One-Way ANOVA with Dunnett's multiple comparison test.