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Reproduction, Fertility and Development

Supplementary Material

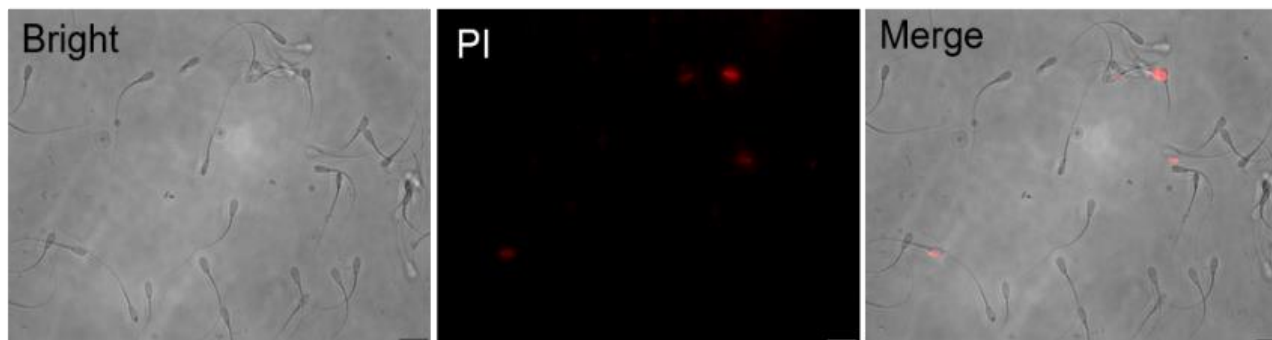
Tributyltin chloride exposure to post-ejaculatory sperm reduces motility, mitochondrial function and subsequent embryo development

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Supplemental Material 1



TBT 100nM is not toxic to sperm. Frozen thawed bull sperm were treated with TBT 100nM for 90 min and stained with propidium iodide (PI-14nM) to detect membrane-compromised sperm. A minimum of 100 sperm were evaluated by brightfield to enumerate total sperm and then by immunofluorescence to determine percent PI positive (red) staining. TBT exposed sperm at 100nM resulted in greater than 90% viability. Scale bar = 10 μ M.

Supplemental Table 1

Acute exposure to TBT (± 10 min) does not decrease bovine sperm kinematics. Frozen-thawed bull sperm from two different bulls were pooled and exposed to TBT (0.1, 1.0, 10, 100 nM) and vehicle control (VC – 0.1% MeOH) for 10 minutes at 20×10^6 total sperm/mL at 37°C in non-capacitating conditions. Sperm exposed to 100nM of TBT immediately after exposure (± 10 min) did not affect sperm kinematics N= 2 bulls, 5 replicates.

Variables	VC	TBT 100nM	TBT 10nM	TBT 1nM	TBT 0.1nM
<i>Mitochondrial function</i>					
High MMP (%)	78.80 \pm 1.02	77.00 \pm 0.95	76.80 \pm 1.50	76.60 \pm 1.63	77.80 \pm 1.16
Intermediate MMP (%)	12.00 \pm 0.77	12.00 \pm 0.89	11.20 \pm 1.02	13.60 \pm 1.78	11.40 \pm 1.12
Low MMP (%)	9.20 \pm 0.37	11.00 \pm 0.63	12.00 \pm 0.63	10.80 \pm 0.37	10.80 \pm 0.86
<i>Sperm kinematics</i>					
ALH (μ m)	6.16 \pm 0.54	6.02 \pm 0.57	6.16 \pm 0.43	6.35 \pm 0.46	6.20 \pm 0.46
BCF (Hz)	29.50 \pm 1.23	29.33 \pm 1.19	29.34 \pm 1.04	29.78 \pm 1.13	29.10 \pm 0.78
LIN (%)	65.22 \pm 2.37	64.54 \pm 2.30	65.36 \pm 2.37	63.77 \pm 2.29	64.33 \pm 1.73
STR (%)	92.29 \pm 1.09	92.08 \pm 1.07	92.57 \pm 1.04	91.44 \pm 0.74	91.62 \pm 0.81
VAP (μ m/s)	104.99 \pm 2.35	101.51 \pm 2.59	104.58 \pm 1.00	103.35 \pm 1.34	103.36 \pm 1.80
VCL (μ m/s)	152.32 \pm 6.58	148.86 \pm 7.73	151.16 \pm 5.31	152.55 \pm 5.82	150.71 \pm 5.75
VSL (μ m/s)	97.70 \pm 1.80	94.01 \pm 1.78	97.35 \pm 1.05	95.48 \pm 0.98	95.53 \pm 1.24
Total motility (%)	88.64 \pm 1.65	86.00 \pm 1.34	86.30 \pm 1.65	86.02 \pm 1.91	86.98 \pm 1.23
Progressive (%)	76.78 \pm 1.12	72.00 \pm 1.55	73.52 \pm 1.24	71.98 \pm 0.93	73.14 \pm 1.19

Supplemental Table 2

Short-term exposure to TBT decreases bovine sperm kinematics. Frozen-thawed bull sperm from two different bulls were pooled and exposed to TBT (0.1, 1.0, 10, 100 nM) and vehicle control (VC – 0.1% MeOH) for 6 hours at 20×10^6 total sperm/mL at 25°C in non-capacitating conditions and warmed to 37°C for evaluation. Sperm exposed to 100nM of TBT at 6 hours displayed decreased sperm kinematics parameters. Different superscripts indicate $P \leq 0.05$ between treatments. N= 2 bulls, 5 replicates.

Variables	VC	TBT 100nM	TBT 10nM	TBT 1nM	TBT 0.1nM
<i>Mitochondrial function</i>					
High MMP (%)	69.00 ± 1.76 ^a	60.00 ± 0.55 ^c	63.60 ± 2.40 ^{bc}	68.20 ± 1.36 ^{ab}	70.60 ± 1.33 ^a
Intermediate MMP (%)	16.80 ± 2.60	20.00 ± 1.38	19.60 ± 2.60	15.60 ± 2.16	14.00 ± 1.48
Low MMP (%)	14.20 ± 0.97 ^a	20.00 ± 1.38 ^b	17.00 ± 1.82 ^{ab}	16.20 ± 0.97 ^{ab}	15.40 ± 0.68 ^a
<i>Sperm kinematics</i>					
ALH (µm)	5.50 ± 0.20 ^{ab}	4.71 ± 0.44 ^c	5.05 ± 0.23 ^{bc}	5.56 ± 0.14 ^{ab}	5.81 ± 0.16 ^a
BCF (Hz)	30.59 ± 1.46	31.63 ± 2.26	31.11 ± 1.54	28.93 ± 1.14	28.21 ± 0.99
LIN (%)	64.85 ± 1.61	67.07 ± 1.85	64.95 ± 2.24	63.67 ± 1.76	63.47 ± 1.38
STR (%)	91.95 ± 0.58	92.98 ± 0.47	91.59 ± 1.04	90.93 ± 0.91	91.52 ± 0.75
VAP (µm/s)	98.24 ± 4.28	92.38 ± 6.04	90.79 ± 6.05	96.00 ± 5.51	98.04 ± 5.93
VCL (µm/s)	140.57 ± 4.64	128.03 ± 8.28	128.75 ± 6.81	137.73 ± 6.07	141.43 ± 7.19
VSL (µm/s)	91.70 ± 4.33	87.29 ± 5.63	84.81 ± 6.20	88.63 ± 5.57	91.26 ± 5.82
Total motility (%)	77.30 ± 1.91 ^a	64.40 ± 2.32 ^b	74.15 ± 0.94 ^a	75.12 ± 2.81 ^a	74.32 ± 1.13 ^a
Progressive (%)	64.92 ± 1.73 ^a	53.43 ± 2.34 ^b	58.8 ± 2.72 ^{ab}	60.60 ± 3.00 ^{ab}	60.42 ± 1.45 ^a