Postejaculatory Sperm Exposure to Tributyltin Chloride Induces Novel Structural, Genomic and Epigenomic Alterations in Mammalian Sperm

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A global top priority for ameliorating male factor infertility includes identification of environmental factors and mechanisms that impact sperm function. Detection of endocrine disruptors (EDs) in seminal plasma and within the female reproductive tract has created an urgent need to understand how environmental stressors alter postejaculatory sperm function. Tributyltin chloride (TBT) is a model organotin, ED and epigenetic modifier that causes reproductive disorders in testicular sperm; however, consequences of TBT exposure on postejaculatory sperm prior to fertilization remain unknown. Our previous data indicate that TBT-exposed sperm does not affect fertilization but lowers embryo development potential. However, identifying reasons for idiopathic male factor subfertility is challenged by lack of routine clinical assessment for subtle phenotypes that compromise sperm function and therefore remain undetected with current diagnostic tools. The present study was aimed at identifying structural, genomic and epigenomic consequences of TBT exposure to postejaculatory sperm in a temporal manner reflective of sperm transport prior to fertilization within the female reproductive tract. Cryopreserved bovine sperm from two different bulls were independently exposed to TBT (0 ,1, 10, 100nM; n = 4) for 24 hr under non-capacitating conditions at 25°C followed by quantification of sperm kinematics at 37°C, DNA integrity, and methylation status of select loci. Samples of TBT-exposed sperm and vehicle control (0.1% DMSO) were collected in replicate for all analyses following immediate addition of TBT (0 hr) and again at 24 hr. Data were analyzed by logistic regression with a generalized linear mixed effect model. Computer-assisted sperm assessment of total motility, progressive motility and average-path velocity did not detect any differences in TBT-exposed sperm compared to vehicle-control after 24 hr of exposure. Capacitation status of TBT-exposed sperm also remained unaffected following anti-tyrosine phosphorylation staining. However, acrosome integrity was compromised in an acute and dose dependent manner as detected by FITC-PSA ($P \leq 0.05$). DNA isolation and recovery for genomic and epigenomic analyses was negatively affected following TBT exposure (10, 100nM) after 24 hr ($P < 0.05$). TUNEL assay did not reveal an acute effect on DNA integrity from TBT at 0 hr, but DNA integrity was negatively compromised in a dose-dependent manner after 24 hr ($P \leq 0.05$). For epigenetic studies, DNA

was isolated after 24 hr of TBT exposure followed by bisulfite conversion and PCR amplification of select loci (*PTK2B*, *HDAC11*, *PAK1*, *SNRPN*, *H19*, *HDAC11*, *KCNQ1*). The DNA methylation of CpG sites in these amplicons were measured by PacBio single-molecule real-time (SMRT) long-read high-fidelity sequencing. Preliminary findings indicate TBT-induced demethylation in the promoter region of *PTK2B* and the imprinted *H19* locus while increasing the methylation profile of *PAK1* and *HDAC11* loci. Confirmation of these findings will be determined by Enzymatic Methyl-Seq conversion and highly optimized PCR amplification following different exposure concentrations of TBT. In conclusion, postejaculatory mammalian sperm exposure to TBT affected acrosome integrity, DNA damage and the methylation status of open chromatin at regions important for early embryonic development suggesting a novel phenotype and paradigm shift to epigenetic modifications of transcriptionally quiescent and translationally repressed mature sperm. The consequences of TBT exposure to mature sperm are masked by unaffected motility but may affect gamete interaction and subsequent embryo development. These findings provided new insight towards environmental impacts on postejaculatory sperm function.