ANALYSIS OF DNA DAMAGE INDUCED BY PRO-OXIDANT TREATMENT OF MAMMALIAN SPERMATOZOA *IN VITRO*

***L. E. Bennetts*[1], *R. J. Aitken*[1,2,3]**

[1]Discipline of Biological Sciences, The University of Newcastle, Callaghan, NSW, Australia; [2]ARC Centre of Excellence for Biotechnology and Development, Australia; [3]Hunter Medical Research Institute, Australia

Defects in the male genome produced as a consequence of oxidative insult have been associated with decreased fertility levels, an elevated incidence of childhood cancer and dominant genetic disease in the offspring (1). The objective of this study was to determine the relative susceptibility of sperm DNA of different mammalian species to oxidative injury. We applied a highly sensitive quantitative PCR assay (QPCR) to measure gene-specific DNA damage in nuclear and mitochondrial compartments of spermatozoa treated with H_2O_2. Human, murine and tammar wallaby (*Macropus eugenii*) spermatozoa were treated with H_2O_2 (0–5 mM) over a 1 h period. After DNA purification, DNA damage was assessed in a nuclear and a mitochondrial fragment of DNA by quantitative polymerase chain reaction assay (QPCR). DNA damage was detected as a decrease amplification of the target sequences. In murine and human spermatozoa, mitochondrial DNA exhibited greater sensitivity to oxidative damage than nuclear DNA. Doses ranging from 0.25–5 mM H_2O_2 induced DNA damage of up to 0.65 lesions/10 kb in the mouse, and 1.42 lesions /10 kb in the human. No significant effect on DNA damage was observed over this dose range in the nuclear DNA fragments investigated in these species. In contrast, tammar wallaby spermatozoa were susceptible to DNA damage at the 5 mM H_2O_2 dose in both nuclear (0.51 lesions/10 kb) and mitochondrial (0.55 lesions/10 kb) genomes. This study is the first to compare DNA damage in specific DNA sequences in spermatozoa of different mammalian species. Nuclear DNA of the metatherian species, the tammar wallaby, was more susceptible to oxidative damage than that of the eutherian species. A major difference between metatherian and eutherian spermatozoa is that, in general, the former possess protamines that are not stabilised by disulfide cross-linkage. These findings therefore suggest that sperm chromatin packaging affects the susceptibility of sperm DNA to oxidative damage.

(1) Sawyer and Aitken (2000) *Reprod. Med. Rev.* **8**, 107–126.

10.1071/SRB04Ab215