8. THE IDENTIFICATION OF MUTATIONS AFFECTING MALE FERTILITY USING DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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For ~40% of infertile men the underlying cause of their infertility remains unidentified, but it is expected that many will be genetic in origin. It is essential that these mutations are identified as with the rising popularity in vitro fertilization and intra-cytoplasmic sperm injection, the genetic alterations will be passed onto the next generation thus perpetuating the problem with potentially serious additional consequences. We are using denaturing high performance liquid chromatography (DHPLC) for the high throughput detection of base-pair changes (mutations or single nucleotide polymorphisms (SNPs)), insertions or deletions within genes thought to be involved in spermatogenesis. Two types of gDNA samples are currently being screened, human infertile males and mice from an ethylnitrosourea (ENU) mutagenesis program. Human samples chosen for screening are selected based on gene and protein expression. Genomic DNA samples are taken from the Andrology Australia DNA repository and compared to known fertile controls. Patients displaying asthenozoospermia are currently being screened for *TPX-1* changes. TPX-1 is a member of the CRISP family of proteins and a component of the sperm head and tail. To date, 7 SNPs have been found in equal proportions in normal and infertile men. Additionally, 1 mutation was identified in a patient with severe teratospermia and resulted in the change of a highly conserved cysteine to an arginine in the cysteine rich carboxyl end of *TPX-1*. A kinase anchor protein 4 (AKAP4) is the major protein of the fibrous sheath of the sperm tail. The gene for this protein is also being screened for mutations and thus far alterations have been found in both the translated and untranslated regions. Further we have refined the DHPLC technology to allow the high throughput screening of gDNA from ENU-mutagenized founder mice, showing that a single heterozygous base pair change is reliably detected within pooled gDNA of 3 mice. This technology coupled with the high mutation load of the founder mice will allow for the rapid screening and subsequent generation of mouse models of altered gene function at a greatly reduced cost compared to traditional knockout and transgenic technologies.

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