Summary

It has been widely recognized that all fathers essentially contribute half their genome to the next generation. However, recent progress towards understanding biological processes such as sperm maturation and fertilization now indicates that the paternal contribution has been underestimated (Krawetz, 2005). Integrity of the germ cell DNA has been established to be crucial in the production of healthy and reproductively fit offspring. There is accumulating evidence that such genetic damage, which can originate from various endogenous and exogenous factors, may be transmitted via sperm to offspring. Paternal effect on embryonic development occurs as early as fertilization. Incorrect formation of the spermatozoon due to centrosome defects and abnormal concentrations of any components involved in the activation process lead to failure immediately or in the subsequent cell cycles. Sperm chromosomal abnormalities have been known to result in early embryo developmental arrests and poor blastocyst formation. Sperm DNA fragmentation may impair even late post-implantation development (Menezo, 2006).

The present study was carried out to assess the influence of this paternal effect, specifically, sperm DNA damage on the overall reproductive outcome. It has recently been illustrated that a high DNA fragmentation index (DFI %) only among spermatozoa in raw semen was related to low success after intrauterine insemination (IUI) (Bungum et al., 2007). A subsequent follow-up study conducted later identified that the observed effect was because the prepared sperm populations that were actually used for the insemination all had low (4–6%) and normal DFI% (Bungum et al., 2008). Thus the ‘negative impact’ of sperm DNA damage originating from an ejaculate with 30% DFI is associated with the selected sperm population but hidden to the investigator as a ‘falsely’ normal value for DFI. For the same reason, it has been suggested that
to improve the diagnostic capability of sperm DNA damage, further methodological work is needed to distinguish whether elevated levels of DNA observed in an ejaculate can be truly transmitted to future generations (Sakkas and Alvarez, 2010).

Currently, the results of most studies on fertility and sperm DNA damage point to a greater utility of sperm DNA tests in relation to natural conception and intrauterine insemination rather than ART treatments (Spano et al., 2005). This could be because in techniques, such as IVF or ICSI, although a high percentage of sperm in a sample may have damaged DNA, as a consequence of sperm processing, sperm with minimal amount of DNA damage may still be selected (Sakkas and Alvarez, 2010). On the contrary, DNA damage may even arise during sperm processing. Previous studies have shown that incubation of semen at room temperature (Gosalvez et al., 2008) or at 37°C (Dalzell et al., 2004) after their isolation by density gradient centrifugation may lead to an increase in the levels of sperm DNA fragmentation. Hence the extent of extent of DNA damage in an ejaculate and how different sperm processing techniques can remove DNA-damaged sperm need to be better understood (Sakkas and Alvarez, 2010).

The present study addresses the above-mentioned concerns. Using a systematic approach, in Chapter 3 of the study, employing alkaline comet assay, it has been identified that infertile men, specifically those with idiopathic infertility and oligozoospermia possess substantially elevated levels of DNA damage compared to fertile controls. In a subsequent large scale study, (Chapter 4) the average incidence of sperm DNA damage in patients with idiopathic male infertility has been observed to be <10%. Significantly higher level of DNA damaged sperm was observed in patients with oligospermia, severe oligospermia, necro spermia and multiple sperm abnormalities
(oligoasthenoteratospermia) suggesting that poor semen quality is associated with compromised sperm genomic integrity. In addition, age-dependent increase in sperm DNA damage, elevated level of damage in subjects with varicocele and history of alcohol consumption was also observed among the large cohort of infertile men studied. Owing to the ambiguous results observed in relation to sperm head morphology and DNA damage, attempts were made to understand the association between the two, employing alkaline comet assay. The results have been presented in Chapter 5 where the lack of association between abnormal sperm head morphology and DNA damage has been observed. Chapter 6 identifies that following induction of damage by hydrogen peroxide, viable sperm selected by hypoosmotic swelling possess superior DNA integrity. The observation supports the view that sperm selection based on HOS may be utilized as a cost-effective strategy for non-destructive, selective identification of spermatozoa with minimal DNA fragmentation for use in ART. Most importantly, Chapter 7 emphasizes that although high levels of DNA damage may be seen in ejaculates of infertile men, currently used sperm selection methods effectively eliminate sperm with DNA damage. As a consequence of sperm processing, the risk of using a genetically incompetent sperm for medically assisted conception and assisted reproduction seems to be low. The above argument can be used to explain the findings of Chapter 8 where sperm DNA damage does not appear to influence either the establishment or maintenance of a viable pregnancy in medically assisted conception (IUI) and assisted reproduction. Moreover, neither the TUNEL assay which measures DNA strand breaks nor the sperm chromatin structure assay (SCSA), which measures susceptibility to DNA denaturation in situ, was predictive of adverse reproductive outcome when IUI or ART is employed.
Unless the diagnostic and prognostic value of this one measure of sperm quality is refined, currently, it cannot be recommended as a routine investigation to improve treatment of the infertile couple. Owing to the same reason, there is a need for new signature markers that offer rapid, non-invasive, sensitive, accurate and unambiguous method for the diagnosis and treatment of infertility. As a first step forward, using $^1$H-NMR spectroscopy, biochemical differences between different forms of male infertility, specifically idiopathic infertility has been identified in Chapter 9 of the study. It is hoped that future initiatives to achieve a complete assignment of the $^1$H NMR metabolic profile for seminal fluid may enhance the information obtainable from metabolomic studies and aid as a valuable tool useful in the diagnosis and management of infertility.