Chapter 1

Introduction

Fertilization, which is commonly defined as the physical union of a competent sperm and ovum to yield a zygote (Wassarman et al., 2001; Cooke and Saunders, 2002; Dadoune et al., 2004) is the preliminary step which dictates the hundreds of different cell types and the positional information for the ~10^{12} cells that constitute a human being (Mattick, 2004). Germ cells perform a unique and critical biological function in this regard: they propagate the DNA that will be used to direct development of the next generation (Menez, 2006).

While the molecular contribution of the oocyte at fertilization is well understood, (Schultz, 2002), knowledge and awareness of the role of sperm is still evolving (Krawetz, 2005; Yanagimachi, 2005; Carrell, 2008; Lalancette et al., 2008; Matzuk and Lamb, 2008; Barroso et al., 2009). The contribution of the male gamete to fertilization and embryogenesis has been ignored or regarded unimportant. This view has however been challenged lately, with accumulating evidence on various spermatozoal components suspected of actively participating in early human development (Kumar et al., 1993; Siffroi and Dadoune, 2001; Krawetz, 2005; Carrell, 2008; Matzuk and Lamb, 2008; Barroso et al., 2009). The spermatozoon is now no longer merely a simple carrier or vector that transfers DNA to the egg. The contributions of the fertilizing spermatozoon to the oocyte at a minimum, include, the delivery of the paternal haploid genome, a putative oocyte-activating factor (OAF) that acts as signal to initiate metabolic activation of the oocyte and a centriole which directs microtubule assembly leading to the
formation of the mitotic spindles during the initial zygote development (Sutovsky and Schatten, 2000). The presence of an extensive cross-talk between the fertilizing sperm and the egg (known to activate the egg and leading to sperm head decondensation) has now been established, which is followed by female and male pronuclear formation, syngamy, and the first cleavage divisions (Barroso et al., 2009).

Several structures/organelles and molecules present in the spermatozoon appear to be critical for the accomplishment of the milestones resulting in normal fertilization and early embryo development (Barroso et al., 2009). Sperm have now been known to deliver their entire structure on fertilization, including a host of novel RNAs. Relatively newer studies in this regard indicate that some of this unique suite of paternal mRNAs provided by the spermatozoa to the zygote might be crucial for early and late embryonic development; deficient delivery, or aberrant transcription of which might contribute to abnormal development and arrest (Ostermeier et al., 2002, 2004, 2005; Krawetz, 2005). In addition, clinical evidence derived from assisted reproduction suggests that failure to complete the fertilization process, syngamy, or early cleavage might be the result of an early paternal effect (Tesarik et al., 2004; Tesarik, 2005). It is speculated that an abnormal release of a putative OAF and/or dysfunctions of the centrosome and cytoskeletal apparatus may mediate these effects (Asch et al., 1995; Hewitson et al., 2000, 2003; Yoon et al., 2008). On the other hand, a late paternal effect (Tesarik et al., 2004) resulting in embryonic failure to achieve implantation, pregnancy loss, and/or developmental abnormalities resulting from “carried over” sub lethal effects may be associated with sperm nuclear/chromatin defects, including the presence of aneuploidy, genetic anomalies, DNA damage, and possibly other causes (Barroso et al., 2009).
Interestingly, as mammalian germ cells spend protracted periods of time in various stages of meiosis during the course of a given species reproductive lifespan, these cells are particularly susceptible to age-related DNA damage that is further exacerbated by lifestyle and environmental factors. Although both male and female germ cells can transmit genetic defects that lead to pregnancy loss, birth defects, and genetic diseases in offspring (McFadden and Friedman, 1999), the parental origins of transmitted defects are not random (Hassold and Hunt, 2001), with de novo mutations and chromosomal structural aberrations transmitted predominantly by sperm (Crow, 2000; Marchetti et al., 2007). Male gametes, compared with female gametes have a greater possibility of damage to nuclear DNA of Y chromosomes because of the lack of a recombination repair, as there is only one Y chromosome during meiosis (Singh et al., 2003). Furthermore, male germ cells are particularly susceptible to the accumulation of DNA lesions because their DNA repair capacity declines during the latter part of spermatogenesis (Olsen et al, 2005).

Recently, much concern has been expressed about the influence of sperm DNA integrity on abnormal reproductive outcome (Zini et al., 2005b; Borini et al., 2006). DNA damage is a common feature observed in human spermatozoa with purported links to poor rates of conception, impaired embryonic development, increased incidence of miscarriage and the appearance of various kinds of morbidity in the offspring including childhood cancer (Evenson et al., 1999; Seli et al., 2004; Borini et al., 2006; Zini et al., 2008). There is now clinical evidence to show that damage to human sperm DNA may adversely affect reproductive outcomes (Lopes et al., 1998b; Evenson et al., 2002; Morris et al., 2002) and that spermatozoa of infertile men possess substantially more DNA damage than do spermatozoa of fertile men.
(Evenson et al., 1980; Spano et al., 2000; Zini et al., 2002). This is clinically relevant given that infertile men (especially those with severe male-factor infertility and with poor sperm DNA integrity) will be seeking treatment with Assisted Reproductive Technology (ART) (Zini et al., 2008).

Sperm DNA fragmentation analysis is a potentially valuable tool to reveal the paternal origin of some unexplained repeated intracytoplasmic sperm injection (ICSI) failures in term of fertilization and implantation failures (Tesarik et al., 2004). It may even help to decide the choice of the most efficient ART procedure to reduce the paternal negative contribution (Bungum et al., 2007). These data provide a clinical indication for the evaluation of sperm DNA damage prior to infertility treatment and a rationale for further investigating the association between sperm DNA damage and pregnancy loss (Zini et al., 2008).

Sperm DNA damage has been clearly observed leading to delayed chromosomal instability in blastocysts and post implantation developmental abnormalities (Shimura et al., 2002a, 2002b; Toyoshima et al., 2005; Adiga et al., 2007a, 2007b). However, currently, very little is known about the nature of germ line DNA damage, and the extent to which germ cells are capable of engaging in and completing the process of DNA damage repair. Although it is known that both the oocyte and the embryo are equipped with mechanisms to possibly cope with some paternal DNA anomalies, our knowledge regarding the ability of the human oocyte and embryo to repair DNA damage is limited, owing to the relatively small number of gene expression studies in this regard. Interestingly, the ability of the oocyte to initiate repair is, to a large extent, going to depend on the cytoplasmic and genomic quality of the oocyte, which is known to be impaired
dramatically by increasing age. Second, the quality of sperm DNA is increasingly being linked to paternal age (Wyrobek et al., 2006), accounting for an attenuation in pregnancy rate (PR) observed in women of advanced age (Bellocc et al., 2008).

Based on above observations and an earlier study conducted in our laboratory in murine model which demonstrated transgenerational changes in somatic and germ line genetic integrity of first-generation offspring derived from the DNA damaged sperm (Adiga et al., 2010), it is hypothesized that DNA damage in human sperm will be carried over to the embryo, thereby affecting the reproductive outcome.

While a number of studies have been conducted worldwide to understand the effect of sperm DNA integrity on fertilization and embryogenesis; association of life style factors and sperm quality, studies on Indian men are lacking. India has a high heterogeneous human population density contributing to approximately 25% of the global population. In view of substantial geographical and ethnic variation in semen quality reported by several investigators (Jorgensen et al., 2001; Richthoff et al., 2002; Iwamoto et al., 2006; Pal et al., 2006) it is necessary to investigate population based trends in semen quality and sperm DNA damage with reference to the Indian scenario.

Indeed, the results of a study conducted by our own group in 7770 subjects (from 1993 to 2005) provided the first evidence that the quality of human semen evaluated for infertility is deteriorating in the southern part of India over the years (Adiga et al., 2008). Another subsequent large-scale study which analysed the semen quality and age-specific changes of 3729 male
partners of couples attending an andrology laboratory for infertility-related problems also confirmed a significant decline in the sperm motility parameters and seminal volume in the present decade (Mukhopadhyay et al., 2010). Clearly, the above data, taken together with increased focus on career planning and delayed parenting, a feature now commonly observed in urban India, demands further attention.

In India, although male factor infertility remains a significant problem, contributing to nearly half of all cases of infertility, it is still regarded a women’s issue and has far-reaching societal implications. Although there are no detailed figures of the extent of infertility prevalent in India, a multinational study carried out by World Health Organization (WHO) that included India, places the incidence of infertility approximately between 10 and 15% (Cates et al., 1988). By extrapolation of the WHO estimate, nearly 13 to 19 million couples are likely to be infertile in our country at any given time (Indian Council of Medical Research, 2005). About 8% of these infertile couples, need serious medical intervention involving the use of advanced ART procedures such as IVF (In vitro fertilization) or ICSI, the success of which is still below 30% in most clinics even under the best of circumstances (Indian Council of Medical Research, 2005).

Infertility research is a rapidly emerging area of biomedical research in India with enormous therapeutic potential. As both the academic and research base broaden, with health care industry beginning to adapt the new technologies, the demand for research in this area is increasing. Most importantly, as the assessment of a male partner presenting for infertility evaluation still relies around standard semen analysis in our country, which does not address the integrity of the male genome, this project is timely and needs to be studied urgently.
It has 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).

In current clinical practice, 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 IUI 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 DNA damage in an ejaculate and how different sperm processing techniques can remove DNA-damaged sperm has to be better understood (Sakkas and Alvarez, 2010).
Furthermore, as all of the existing methods for the detection of DNA damage in sperm lead to destruction of the same, rendering them unusable for therapeutic approach (Stanger et al., 2010), it has been suggested that the onus must now be shifted to identification of the DNA damaged sperm and how to select individual or populations of “normal” sperm for use in ART (Sakkas and Alvarez, 2010). As advanced treatment of infertility through ART is expensive; not easily affordable to the majority of Indians, there is also an urgent need to develop a simpler and cost-effective sperm selection technique with minimal DNA fragmentation for routine use in ART. The present study has therefore been designed, considering the above-mentioned concerns. The aim of the study is to assess the influence of paternal effect, specifically, sperm DNA damage on the overall reproductive outcome using a combination of genetic and metabolomic approaches. The study is expected to contribute information on the level of sperm DNA integrity in infertile men seeking assisted conception with the hope of identifying some clues on possible association between sperm DNA damage with failed conception, repeated miscarriage and fetal abnormalities. Additionally, as good sperm preparation is essential for ensuring success in both IUI and ART, the objectives proposed in the study will help us to decide which of the methods will be best suited to eliminate sperms with DNA damage, and hence optimize success.