1. Introduction

The traditional semen analysis is a cornerstone in diagnosis and treatment of male fertility (World Health Organisation (WHO), 1999, 2010). The sperm parameters concentration, motility and morphology are, however, claimed to be poor predictors of a male’s fertility status, this in natural conception (Bonde et al., 1998; Guzick et al., 2001) as well as in assisted reproductive techniques (ART) (Wolf et al., 1996).

As a consequence of the limited predictive role of the traditional semen analysis there have for long been searched for better parameters, For the last decade an increased focus on sperm DNA is seen.

Infertile men are shown to have significantly more sperm DNA damage compared to fertile men (Evenson et al., 1999; Gandini et al., 2000; Irvine et al., 2000; Larson et al., 2000; Spanò et al., 2000; Carrell and Liu 2001; Hammadéh et al., 2001; Zini et al., 2001a; Zini et al., 2002) and time to spontaneous pregnancy is proved to be longer in couples where the male partner have an increased amount of sperm with DNA damage (Evenson et al., 1999; Spanò et al., 2000). Methods assessing sperm DNA integrity have shown a better predictivity of both in vivo and in vitro fertility than the WHO sperm parameters (Bungum et al., 2007). Moreover, studies have shown that sperm DNA integrity assessment can be applied in ART in order to find the most effective treatment in a given couple (Bungum et al., 2004, 2007; Boe-Hansen et al., 2006).

Semen quality is known to be influenced by a variety of lifestyle, environmental, and occupational factors. Although still much is unknown, the origins of sperm DNA damage are believed to be multi-factorial where defects during spermatogenesis, abortive apoptosis and oxidative stress may be possible causes of a defective sperm DNA (reviewed in (Erenpreiss et al., 2006a)).

During the last decades a variety of techniques to assess sperm DNA integrity have been developed. In the context of fertility the COMET, TUNEL, and Sperm Chromatin Structure assays (SCSA) as well as the sperm chromatin dispersion (SCD) test are the most frequently used (reviewed in (Erenpreiss et al., 2006a)). So far, SCSA is the test that is found to have the most stable threshold values in regard to fertility and therefore of best clinical value (Bungum et al., 2007).

Accordingly, this chapter will first of all refer to available SCSA data in regard to fertility.
2. Male infertility

Infertility is a health problem affecting approximately 15% of all couples trying to conceive. It is now evident that in at least 50% of all cases, reduced semen quality is a factor contributing to the problem of the couple. In 20% of the couples, the main cause is solely male related, and in another 27%, both partners contribute to the problem (WHO 2000).

Male infertility can be the result of congenital and acquired urogenital abnormalities, infections of the genital tract, varicocele, endocrine disturbances, genetic or immunological factors (WHO, 2000). However, in at least 50% of the infertile men, no explanation to their reduced semen quality can be found (Seli and Sakkas, 2005; Matzuk and Lamb, 2008; O’Flynn O’Brien et al., 2010).

Recent studies have shown that also sperm factors at a molecular level can cause infertility. One example of this is DNA breaks (Evenson et al., 2002; Sharma et al., 2004; Lewis and Aitken, 2005; Lewis 2007; Aitken, 2006; Erenpreiss et al., 2006a; Evenson and Wixon, 2006; Muratori et al., 2006; Collins et al., 2008; Lewis and Agbaje, 2008; Lewis et al., 2008; Bungum et al., 2007; Zini and Sigman, 2009; Aitken and De Iuliis, 2010; Sakkas and Alvarez, 2010).

2.1 Diagnosis and treatment of male infertility

Traditionally, diagnosis of male infertility is based on the conventional sperm analysis where World Health Organisation (WHO) has set criteria for normality in regard to semen volume, sperm concentration, motility and morphology (WHO, 2010). The traditional semen analysis has, however, been criticized (Bonde et al., 1998; Giwercman et al., 1999; Auger et al., 2001; Guzick et al., 2001; Nallella et al., 2006; Swan 2006), in particular because of lack of power in regard to predict fertility. Human semen is a highly fluctuable fluid and all WHO parameters vary significantly between individuals, seasons, countries and regions and even between consecutive samples from one individual (Chia et al., 1998; WHO 1999; Auger et al., 2000; Jorgensen et al., 2001; Chen et al., 2003; Jorgensen et al., 2006). The traditional analysis is performed by light microscopy of 1-200 spermatozoa and this means a high grade of intra- and interlaboratory variation (Neuwingen et al., 1990; Cooper et al., 1992) and a considerable overlap in all three parameters; sperm concentration, motility and morphology between fertile and infertile is shown (Bonde et al., 1998; Guzick et al., 2001). One of the reasons behind the low status as fertility predictor may be that the WHO analysis only takes few sperm characteristics into consideration. Generally overlooked has been the fact that sperm carry DNA and that the DNA can be of a different quality. Such parameters describing sperm nuclear potential are not routinely assessed. However, there are ongoing debates whether sperm DNA integrity assessment should be introduced as a routine test in all or selected groups of infertile men (Evenson et al., 2000; Giwercman et al., 2010; ASRM, 2008, Makhlouf and Niederberger, 2006; Erenpreiss et al., 2006; Zini and Sigman, 2009).

Until the 1990s, the majority of cases of severe male factor subfertility were virtually untreatable, however, the introduction of ICSI revolutionized the treatment of male infertility (Palermo et al., 1992). However, ICSI is a subject of an ongoing debate regarding its indications and safety (Govaerts et al., 1996; Griffin et al., 2003; Kurinczuk 2003; Verpoest and Tournaye 2006; Varghese et al., 2007). One of the causes to this is that ICSI must be seen as a symptomatic treatment, not taking the underlying causes of infertility into account.
3. Sperm DNA structure

With a volume 40 times less than that of a somatic cell nucleus the genetic material of a spermatozoon is more compact packaged than in the nucleus of a somatic cell (Ward et al., 1991). During spermiogenesis histones are replaced by the more basic and small protamines (Fuentes-Mascorro et al. 2000). Each unit of mammalian sperm chromatin is a toroid containing 50–60 kb of DNA and individual toroids represent DNA loop-domains highly condensed by protamines and fixed at the nuclear matrix. The toroids are bound by disulfide crosslinks, formed by oxidation of sulfhydryl groups of cysteine present in the protamines and each chromosome represents a garland of toroids (Fuentes-Mascorro et al., 2000; Ward et al., 1993). While in most other species the protamines comprise as much as 95%, human protamines comprise 85% of the spermatozoal nucleoproteins. (Fuentes-Mascorro et al., 2000). This may explain why human sperm chromatin is less compacted and more frequently contains DNA breaks (Bench et al., 1993) compared to other species.

4. Sperm DNA damage

Human sperm DNA is often not so well packaged as meant to be (Sakkas et al., 1999a) and is therefore susceptible to DNA damage (Irvin et al., 2000). Whilst the mature sperm is a repair-deficient cell (Sega et al., 1978), oocytes and embryos are, to a certain degree, able to repair DNA damage (Matsuda and Tobari 1988; Ahmadi and Ng 1999b).

Sperm possess a variety of abnormalities at the nuclear level and that these anomalies can have an impact on fertility (Evenson et al., 1980; Hewitson 1999; Huszar 1999). The most common types of DNA damage include chemical modification of a base, inter- and intra-strand crosslinks, and single or double DNA strand breaks (Marchetti and Wyrobek 2005).

The origin of human sperm DNA damage is involving both testicular and post-testicular mechanisms. Testicular mechanisms include a) alterations in chromatin modelling during the process of spermiogenesis, and b) abortive apoptosis, whereas post-testicular factors are mostly related to the action of c) reactive oxygen species (ROS), and d) activation of caspases and endonucleases( Reviewed in (Aitken and De Iuliis, 2010 and Sakkas and Alvarez, 2010)).

Oxidative stress during sperm transport through the male reproductive tract is likely the most frequent cause of sperm DNA damage (Aitken and De Iuliis, 2010; Sakkas and Alvarez, 2010). Under normal conditions ROS are necessary for the functioning of sperm (reviewed by Aitken 2006), however, oxidative stress resulting from an over production or reduced antioxidant protection are thought to cause DNA damage (Aitken et al. 1998).

The risk of having ROS induced DNA damaged sperm increases by advanced age, abstinence time, influence of cancer treatment, varicocele and obesity (reviewed in Erenpreiss et al., 2006a; Aitken and De Iulius 2007). Moreover, several studies have reported a negative effect of cigarette smoking on sperm DNA, but data are not conclusive (Robbins et al., 1997; Sun et al., 1997; Rubes et al., 1998; Potts et al., 1999; Saleh et al., 2002b; Sepaniak et al., 2006). Other sources of ROS include organophosphorous pesticides (Sanchez-Pena et al., 2004) and other types of air pollution (Rubes et al., 1998; Selevan et al., 2000; Evenson and Wixon 2005). These agents possess estrogenic properties that are capable of inducing ROS production (Sanchez-Pena et al., 2004; Baker and Aitken 2005; Bennetts et al., 2008).
5. Sperm DNA integrity testing

Several tests developed to assess sperm DNA damage are available. The most frequently used is the Sperm Chromatin Structure assay (SCSA), the single-cell gel electrophoresis assay (COMET assay) in its alkaline, neutral, 2-tailed versions (Singh et al., 1997; Lewis and Agbaje, 2008; Enciso et al., 2009), the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay (Gorczyka et al., 1993) and the sperm chromatin dispersion (SCD) test (Fernandez et al., 2003). Although these tests correlate to each other (Aravindan et al. 1997; Zini et al. 2001; Perera et al. 2002; Erenpreiss et al. 2004, Donelly et al., 2000) and all measure single and double strand breaks, the methodologies are based on different principles and different aspects of sperm DNA damage (Makhlouf and Niederberger, 2006).

5.1 Sperm Chromatin Structure Assay (SCSA)

The SCSA® is a flow-cytrometic test based on the fact that damaged sperm chromatin denatures when exposed to a low pH-buffer, whereas normal chromatin remains stable (Evenson et al., 1980). The SCSA measures the denaturation of sperm DNA stained with acridine orange, which differentially stains double- and single stranded DNA. Five to ten thousand cells are analysed. Thereafter, the flow cytometric data is further analyzed using dedicated software (SCSASoft; SCSA Diagnostics, Brookings, SD, USA). Data appears in histo- and cytograms and results given as DAN fragmentation index (DFI) and High DNA stainability (HDS). It is still unclear which mechanisms and types of DNA damage that are lying behind DFI and HDS, however, it is believed that DFI are related to the percentage of sperm with both single strand breaks (SSB) and double strand breaks (DSB) or problems in the histone to protamine exchange. HDS is thought to represent immature sperm. The clear advantage of SCSA is the objectivity of the test as well as the high reproducibility (Giwercman et al., 2003) when ran after the standardised protocol (Evenson et al., 2002). Moreover, the clear cut-off levels in relation to fertility is maybe the most obvious benefit compared to other sperm DNA integrity tests (Bungum et al., 2007). A disadvantage is that an expensive flow cytometer is required to run the analysis. Moreover, the test irreversibly damage spermatozoa; after analysis they cannot be used for fertilisation purposes.

5.2 COMET assay

The COMET assay (Singh et al., 1997) is a single cell gel electrophoresis of immobilised sperm, which involves their encapsulation in agarose, lysis and electrophoresis. When the electric field is applied the negatively charged DNA will be drawn towards the positively charged anode. While undamaged DNA are too large and will remain in nucleus, the smaller broken DNA fragments move in a given period of time. The amount of DNA that leaves the nucleus is a measure of the DNA damage in the cell. The sperm are stained with a DNA-binding dye and the intensity of the fluorescence is measured by image analysis. The overall structure resembles a COMET with a circular head corresponding to the undamaged DNA that remains in the nucleus and a tail of damaged DNA. COMET assay can be ran under neutral or alkaline conditions. Under neutral conditions (pH 8–9), mainly DSB are detected (Collins et al. 2004). Under alkaline conditions, DSB and SSB (at pH 12.3) and additionally alkali labile sites (at pH e13) can be visualised resulting in increased DNA migration in the electrophoretic field (Fairbairn et al., 1994). In COMET assay, normally only around 100 cells are analysed.
5.3 TUNEL assay

The terminal deoxynucleotidyl transferase (TdT)-mediated 2’-deoxyuridine 5’-triphosphate- nick end labelling (TUNEL) assay can be applied for both light microscopy and flow cytometry. The assay uses TdT to label the 3’-OH ends of double-stranded DNA breaks, but also works on the single strand 3’-OH (Gorczyka et al., 1993). The assay detects the DNA breaks directly, without any initial step of denaturation as in SCSA or by introducing acid or alkaline pH as in COMET assay. Whilst TUNEL assay based on light microscopy normally assess 2-500 cells the flow cytometry TUNEL assess 5-10 000 cells.

5.4 Sperm Chromatin Dispersion (SCD) test

The Sperm chromatin dispersion (SCD) test is a relatively simple test that can be applied either by fluorescence microscopy or by bright field microscopy. The test is based on the principle that sperm with fragmented DNA fails to produce the characteristic halo of dispersed DNA loops that are observed on sperm with non-fragmented DNA, when mixed with aqueous agarose following acid denaturation and removal of chromatin nuclear proteins (Fernandez et al., 2003). In the SCD test normally 500 spermatozoa are analysed.

6. Intra-individual variation of DFI

The WHO semen analysis is a golden standard in diagnosis of male infertility, this despite an intra-individual variation reported to be as high as up to 54% (Keel 2006). The first SCSA studies demonstrated that sperm chromatin parameters varied less within a man (Evenson et al., 1991; Spanò et al., 1998). In a study of 45 men who delivered monthly semen samples Evenson reported an average within-donor CV of the SCSA-parameter DFI around 23% (Evenson et al., 1991). These results were confirmed by another SCSA study of 277 men whose semen was measured two times during a period of six months (Spanò et al., 1998). Also Smit and co-workers in 100 men from an outpatient andrology clinic demonstrated a lower biological variation of sperm DNA fragmentation than the classical WHO sperm parameters (Smit et al., 2007). However, conflicting results were obtained in 282 patients undergoing ART with repeated (between 2 and 5) SCSA measurements. In this study, CV of DFI was as high as 29% (Erenpreiss et al., 2006b). In a more recent study these results were reproduced by Olechuk et al., (2011) who also found the mean CV for DFI to be around 30%. In this study 85% of the men that repeated their SCSA analysis remained in the same DFI category (DFI <30% or DFI >30%) from sample one to sample two.

7. Sperm chromatin damage and male infertility

It is evident that infertile men possess more sperm with DNA damage than fertile men (Evenson et al., 1999; Gandini et al., 2000; Host et al., 2000b; Irvine et al., 2000; Larson et al., 2000; Spanò et al., 2000; Carrell and Liu 2001; Hammad et al., 2001; Zini et al., 2001a; Sakkas et al., 2002; Saleh et al., 2002b; Zini et al., 2002; Erenpreisa et al., 2003; Muratori et al., 2003; Saleh et al., 2003a). However, very few infertile men are offered a sperm DNA integrity analysis during their fertility work-up, and are therefore not aware of the problem. This despite the fact that in 10-25% of men diagnosed with unexplained infertility sperm DNA damage can, at least partly explain their childlessness (Bungum et al., 2007; Erenpreiss et al., 2008; Smit et al., 2010; Giwercman et al., 2010).
8. Predictive role of DFI in spontaneous pregnancy

Only few studies have studied the role of sperm DNA integrity in relation to fertility in an unselected population. The US Georgetown study included 165 couples (Evenson et al., 1991) and the Danish first pregnancy planners study (Spanò et al., 2000) included 215 couples who tried to obtain pregnancy. Both studies analysed sperm DNA damage by the use of SCSA and demonstrated that the chance of spontaneous pregnancy, measured by the time-to-pregnancy (TTP), decreased when DFI exceeded 20–30% and became infinite when DFI was more than 30%.

These results have been confirmed in a more recent SCSA case-control study of 127 men from infertile couples where female factors were excluded and 137 men with proven fertility. The risk of being infertile was increased when DFI rised above 20% in men with normal standard semen parameters (OR 5.1), whereas if one of the WHO parameters were abnormal, the OR for infertility was increased already at DFI above 10% (OR 16) (Giwercman et al., 2010). This above mentioned study demonstrated that SCSA can be used in prediction of the chance of spontaneous pregnancy, independently of the standard sperm parameters but also that combining WHO parameters with DFI can be beneficial. Giwercman and co-workers claimed that since a DFI >20% was found in 40% of men with otherwise normal standard sperm parameters, in almost half of the cases of unexplained infertility, sperm DNA defects are a contributing factor to the problem.

9. Predictive role of DFI in intrauterine insemination (IUI)

Several reports have studied DFI in prediction of fertility following intrauterine insemination (IUI). The first report used the TUNEL assay on prepared semen for sperm DNA integrity analysis (Duran et al., 2002). In 154 couples they found lack of pregnancy when DFI was above 12%. Other smaller SCSA studies have confirmed this (Saleh et al., 2003; Boe-Hanssen et al., 2006). In 2007, our group published a study based on 387 IUI cycles where DFI was assessed by SCSA (Bungum et al., 2007). DFI was shown to be a predictor of fertility independent of other sperm parameters. In men having a DFI level below 30% the proportion of children born per cycle was 19.0%. This was in contrast to those having a DFI value above 30% who only had a take-home-baby rate of 1.5 %. The chance of IUI pregnancy started to decrease already when the DFI value exceeded the 20%-level, but became close to zero when exceeding 30%.

10. Predictive role of DFI in IVF and ICSI

More contrasting data exist regarding role of sperm DNA damage in relation to fertilisation, embryo development and pregnancy outcome in IVF and ICSI.

10.1 IVF and ICSI pregnancy

The first SCSA studies based on a relatively limited number of couples indicated that DFI above 27% could be used as a cut-off value for infertility (Larsson et al., 2000; Larsson-Cook et al., 2003). However, in 2004 three independent SCSA reports demonstrated that one through the use of IVF and ICSI were able to compensate for poor sperm chromatin quality (Bungum et al., 2004; Virro et al., 2004; Gandini et al., 2004). Then in 2007 data based on 388
IVF and 223 ICSI cycles were published (Bungum et al., 2007). We observed no statistically significant differences between the outcomes of ICSI and IVF when a DFI level of 30% was used. However, in the DFI >30% group, the results of ICSI were significantly better than those of IVF. When comparing ICSI to IVF the odds ratios (ORs) for biochemical pregnancy, clinical pregnancy and delivery were 3.0 (95% CI: 1.4–6.2), 2.3 (5% CI: 1.1–4.6) and 2.2 (95% CI: 1.0–4.5), respectively. Also smaller reports using TUNEL or COMET assays shows that sperm DNA damage is more predictive in IVF than in ICSI (Hammadeh et al., 1998; Host et al., 2000; Simon et al., 2011). In contrast, one single SCSA study has reported that DFI threshold did not predict IVF outcome (Payne et al., 2005). However in this study the authors did not discriminate between IVF and ICSI.

10.2 Fertilisation and embryo development

In a mouse model Ahmadi and Ng demonstrated that spermatozoa with DNA damage were able to fertilise oocytes (Ahmadi and Ng 1999a). They also reported sperm DNA damage to be related to poor embryo development (Ahmadi and Ng 1999b), although the oocyte, to a certain degree, was able to repair the sperm DNA damage. The human data from ART populations regarding fertilisation and embryo development in relation to DNA damage is, however, conflicting.

In our large study of 611 IVF and ICSI couples we compared fertilisation rates between those having a DFI>30% and those having a DFI≤30%, however, no statistically significant differences were seen, neither for IVF nor for ICSI patients (Bungum et al., 2007). Our findings are in accordance with most other reports using the SCSA analysis (Larson et al., 2000; Larson-Cook et al., 2003; Gandini et al., 2004; Virro et al., 2004; Li et al., 2006) as well as other sperm DNA integrity testing methods (Sakkas et al., 1996; Hammadeh et al., 2001; Tomlinson et al., 2001; Morris et al., 2002; Tomsu et al., 2002; Henkel et al., 2004; Lewis et al., 2004; Huang et al., 2005; Nasr-Esfahani et al., 2005). In contrast, a negative correlation between sperm DNA fragmentation and IVF and ICSI fertilisation rates were reported by others (Sun et al., 1997; Lopes et al., 1998a; Saleh et al., 2003a; Payne et al., 2005). A Danish study (Host et al., 2000a) assessed sperm DNA breaks with the TUNEL- assay in infertile couples and found negative correlations between the proportion of spermatozoa with DNA damage and fertilisation in all groups except for those treated with ICSI.

In our ART-study from 2007 (Bungum et al., 2007), embryo development was compared between those having a SCSA-DFI>30% and those having a DFI≤30%, however, no statistically significant differences between the groups were seen. Several other authors have reported identical results (Larson et al., 2000; Larson-Cook et al., 2003; Gandini et al., 2004; Boe-Hansen et al., 2006). This, however, is contrasted by the findings of others (Sun et al., 1997; Morris et al., 2002; Saleh et al., 2003a), who showed that DFI levels were negatively correlated with embryo quality after IVF and ICSI. Seli et al. (2004) and Virro et al. (2004) reported that men with a high DFI had higher risk of low blastocyst formation rate compared to those with a low DFI.

10.3 DFI and risk of pregnancy loss

Traditionally, pregnancy loss has been explained by either genetic, structural, infective, endocrine or unexplained causes (reviewed in (Rai and Regan 2006)). However, recently
also sperm DNA damage has been associated to recurrent pregnancy loss (Evenson et al., 1999; Carrell et al., 2003). In a population of first pregnancy planners Evenson et al. (1999) reported a miscarriage rate higher in fertile couples where the partner had a high SCSA-DFI compared to those with a low DFI. Also Carrell et al. (2003) by using the TUNEL assay on sperm from 24 couples with unexplained recurrent pregnancy loss showed that pregnancy loss was associated with DNA damage. As control group they used donors of known fertility and unscreened men from the general population.

Several studies have reported an increased risk of pregnancy loss after IVF and ICSI (Check et al., 2005; Zini et al. 2005; Lin et al., 2008), however data are conflicting (Bungum et al., 2007). In a recent meta-analysis Zini and co-workers (Zini et al., 2008) collected data from 11 studies involving 1549 IVF/ICSI treatments and 640 pregnancies. They demonstrated a combined odds ratio (OR) of 2.48 (95% CI; 1.52, 4.04, p<0.0001). The conclusions from this analysis should, however, be interpreted with care since different types of sperm DNA integrity tests and different sperm sources were included. We have demonstrated that washed semen is not predictive for the outcome of ART (Bungum et al., 2008). Further large-scale studies should be performed to get firm conclusions.

11. Prevention or treatment of sperm DNA damage

A more precise diagnosing would enable clinicians to better counsel the infertile couple and may also result in improvement and further development of cause-related therapy, which is very little used in today’s clinical practice (Skakkebaek et al., 1994). The effects of therapy based on antioxidants on sperm DNA quality has been evaluated, however, the studies have been small and conflicting (Greco et al., 2005a; Greco et al., 2005b; Silver et al., 2005; Song et al., 2006; Menezo et al., 2007; Kefer et al., 2009) and so far no standardized, clinically well-proven treatment of sperm oxidative stress and DNA damage are established.

Data on how to best advise couples in what they self can do to prevent against sperm DNA damage are lacking. Several life-style factors are suggested to negatively influence sperm DNA, however, data are conflicting. Examples are smoking and obesity (Reviewed in (DuPlessis et al., 2010 and Agarwal et al., 2008)). Interestingly, the first published intervention study in severe obese men demonstrated improvement in WHO parameters but not in sperm DNA integrity after weight reduction (Hakonsen et al., 2011).

12. Conclusion and future perspectives

Sperm DNA damage is a frequent problem in infertile men. Although not yet routine, sperm DNA integrity is a good tool in investigation and treatment of infertility. Sperm DNA integrity is a marker of male fertility, alone or in combination with the WHO semen parameters, this in natural conception as well as in ART.

The role of DFI as a predictor of fertilisation, embryo development and pregnancy in IVF and ICSI is still discussed. Although not conclusive, also a relation between sperm DNA damage and risk of pregnancy loss have been suggested.

Among the different sperm DNA integrity assays available SCSA is currently the only method that has provided clear and stable clinical cut-off levels and therefore can be
recommended for a clinical sperm DNA damage evaluation. The normality ranges and thresholds for male fertility potential of the other assays still need to be clarified.

It seems clear that ART, especially ICSI, are able to overcome the natural barriers of sperm DNA damage levels not compatible with fertilisation under natural circumstances. The consequences of this for the progeny are still not clear, however, it is also reason to concern regarding possible consequences of achieving a pregnancy using spermatozoa possessing DNA damage. So far no proven treatment of sperm DNA damage is available. Adequately powered, placebo-controlled trials of antioxidants in the prevention of sperm oxidative stress should be performed.

13. References
Agarwal, A., Desai,. NR., Ruffoli, R. & Carpi A. (2008). Lifestyle and testicular dysfunction: a brief update. Biomed Pharmacother 62(8):550-553.
Ahmadi, A. & Ng, S. C. (1999a). Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 284, 696-704.
Ahmadi, A. & Ng, S. C. (1999b). Developmental capacity of damaged spermatozoa. Hum Reprod 14, 2279-2285.
Aitken, J. & De Iulius, G. N. (2007). Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online 14, 727-733
Aitken, R. J. & De Iulius, G.N. (2010). On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod 16:3-13.
Aitken, R. J. (2006). Sperm function tests and fertility. Int J Androl 29, 69-75; discussion 105-108.
Aitken, R. J., De Iulius, G.N. & McLachlan R.I. (2009). Biological and clinical significance of DNA damage in the male germ line. Int J Androl 32:46-56.
Aravindan, G. R., Bjordahl, J., Jost, L. K. & Evenson, D. P. (1997). Susceptibility of human sperm to in situ DNA denaturation is strongly correlated with DNA strand breaks identified by single-cell electrophoresis. Exp Cell Res 236, 231-237
Auger, J., Eustache, F., Andersen, A. G., Irvine, D. S., Jorgensen, N., Skakkebaek, N. E., Suominen, J., Toppari, J., Vierula, M. & Jouannet, P. (2001). Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities. Hum Reprod 16, 2710-2717.
Baker, M. A. & Aitken, R. J. (2005). Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. Reprod Biol Endocrinol 3, 67.
Bench, G.S., Friz, A.M., Corzett, M.H., Morse, D.H. & Balhorn R. (1996). DNA and total protamine masses in individual sperm from fertile mammalian subjects. Cytometry 23(4):263-271.
Bennetts, L. E., De Iulius, G. N., Nixon, B., Kime, M., Zelski, K., McVicar, C. M., Lewis, S. E. & Aitken, R. J. (2008). Impact of estrogenic compounds on DNA integrity in human spermatozoa: Evidence for cross-linking and redox cycling activities. Mutat Res 641, 1-11.
Boe-Hansen, G. B., Fedder, J., Ersboll, A. K. & Christensen, P. (2006) The sperm chromatin structure assay as a diagnostic tool in the human fertility clinic. Hum Reprod 21, 1576-1582.

Bonde, J. P., Ernst, E., Jensen, T. K., Hjollund, N. H., Kolstad, H., Henriksen, T. B., Scheike, T., Giwercman, A., Olsen, J. & Skakkebaek, N. E. (1998). Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 352, 1172-1177.

Bungum, M., Humaidan, P., Axmon, A., Spanò, M., Bungum, L., Erenpreiss, J. & Giwercman A. (2007) Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Human Reproduction 22:174-179.

Bungum, M., Humaidan, P., Spanò, M., Jepson, K., Bungum, L. & Giwercman, A. (2004). The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod 19:1401-1408.

Bungum, M., Spanò, M., Humaidan, P., Eleuteri, P., Rescia, M. & Giwercman, A. (2008). Sperm Chromatin Structure Assay (SCSA) parameters measured after density gradient centrifugation are not predictive for the outcome of ART. Hum Reprod 23:4-10.

Carrell, D. T. & Liu, L. (2001) Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. J Androl 22, 604-610.

Carrell, D. T., Liu, L., Peterson, C. M., Jones, K. P., Hatasaka, H. H., Erickson, L. & Campbell, B. (2003). Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 49, 49-55.

Check, J. H., Graziano, V., Cohen, R., Krotec, J. & Check, M. L. (2005). Effect of an abnormal sperm chromatin structural assay (SCSA) on pregnancy outcome following (IVF) with ICSI in previous IVF failures. Arch Androl 51, 121-124.

Chen, Z., Toth, T., Godfrey-Bailey, L., Mercedat, N., Schiff, I. & Hauser, R. (2003) Seasonal variation and age-related changes in human semen parameters. J Androl 24, 226-231.

Chia, S. E., Tay, S. K. & Lim, S. T. (1998) What constitutes a normal seminal analysis? Semen parameters of 243 fertile men. Hum Reprod 13, 3394-3398.

Collins, A.R. (2004). The COMET assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26(3):249-261.

Collins, J. A., Barnhart, K. T. & Schlegel, P. N. (2008). Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 89, 823-831.

Cooper, T. G., Neuwinger, J., Bahrs, S. & Nieschlag, E. (1992). Internal quality control of semen analysis. Fertil Steril 58, 172-178.

Donnelly, E. T., O’Connell, M., McClure, N. & Lewis, S. E. (2000). Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. Hum Reprod 15:1552-1561.

Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. (2010). The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 7(3):153-161
Role of Sperm DNA Integrity in Fertility

Duran, E. H., Morshedi, M., Taylor, S. and Oehninger, S. (2002) Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 17, 3122-3128.

Enciso, M., Muriel, L., Fernández, J.L., Goyanes, V., Segrelles, E., Marcos, M., Montejo, J.M., Ardon, M., Pacheco, A. & Gosálvez J. (2006). Infertile men with varicocele show a high relative proportion of sperm cells with intense nuclear damage level, evidenced by the sperm chromatin dispersion test. J Androl 27:106-111.

Erenpreisa, J., Erenpreiss, J., Freivalds, T., Slaidina, M., Krampe, R., Butikova, J., Ivanov, A. & Pjanova, D. (2003). Toluidine blue test for sperm DNA integrity and elaboration of image cytometry algorithm. Cytometry A 52, 19-27.

Erenpreiss, J., Bungum, M., Spano, M., Elzanaty, S., Orbidans, J. & Giwercman, A. (2006). Intra-individual variation in Sperm Chromatin Structure Assay parameters in men from infertile couples: clinical implications. Human Reproduction 21:2061-2064.

Erenpreiss, J., Elzanaty, S. & Giwercman, A. (2008). Sperm DNA damage in men from infertile couples. Asian J Androl 10:786-790.

Erenpreiss, J., Jepson, K., Giwercman, A., Tsarev, I., Erenpreisa, J. & Spanò, M. (2004). Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Hum Reprod 19:2277–2282.

Erenpreiss, J., Spano, M., Erenpreisa, J., Bungum, M. & Giwercman, A. (2006a). Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl 8, 11-29.

Evenson, D. & Jost, L. (2000). Sperm chromatin structure assay is useful for fertility assessment. Methods Cell Sci 22, 169-189.

Evenson, D. & Wixon, R. (2006). Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online 12, 466-472.

Evenson, D. P., Darzynkiewicz, Z. & Melamed, M. R. (1980). Relation of mammalian sperm chromatin heterogeneity to fertility. Science 210, 1131-1133.

Evenson, D. P., Jost, L. K., Baer, R. K., Turner, T. W. & Schrader, S. M. (1991). Individuality of DNA denaturation patterns in human sperm as measured by the sperm chromatin structure assay. Reprod Toxicol 5, 115-125.

Evenson, D. P., Jost, L. K., Marshall, D., Zinaman, M. J., Clegg, E., Purvis, K., de Angelis, P. & Claussen, O. P. (1999). Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 14, 1039-1049.

Evenson, D. P., Larson, K. L. & Jost, L. K. (2002). Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23, 25-43.

Fairbairn, D.W., Olive, P.L. & O’Neill K.L.. (1995). The COMET assay: a comprehensive review. Mutat Res 339(1):37-59.

Fernández, J.L., Muriel, L., Goyanes, V., Segrelles, E., Gosálvez, J., Enciso, M., LaFromboise, M. & De Jonge C. (2005). Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril;84:833-842.
Fernández, J.L., Muriel, L., Rivero, M.T., Goyanes, V., Vazquez, R., Alvarez, J.G. (2003). The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 24:59-66.

Fuentes-Mascorro, G., Vergara-Onofre, M., Mercado, E., Hernandez-Perez, O. & Rosado, A. (2000). Participation of DNA structure on sperm chromatin organization. Arch Androl 45, 61-71.

Gandini, L., Lombardo, F., Paoli, D., Caponecchia, L., Familiari, G., Verlengia, C., Dondero, F. & Lenzi, A. (2000). Study of apoptotic DNA fragmentation in human spermatozoa. Hum Reprod 15, 830-839.

Gandini, L., Lombardo, F., Paoli, D., Caruso, F., Eleuteri, P., Leter, G., Ciriminna, R., Culasso, F., Dondero, F. & Lenzi, A. et al. (2004). Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod 19, 1409-1417.

Giwercman, A., Lindstedt, L., Larsson, M., Bungum, M., Spanó, M., Levine, R.J. & Rylander, L. (2010). Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case-control study. Int J Androl 33:221-227.

Giwercman, A., Richthoff, J., Hjollund, H., Bonde, J. P., Jepson, K., Frohm, B. & Spano, M. (2003). Correlation between sperm motility and sperm chromatin structure assay parameters. Fertil Steril 80, 1404-1412.

Giwercman, A., Spano, M., Lahdetie, J. & Bonde, J. P. (1999). Quality assurance of semen analysis in multicenter studies. Asclepios. Scand J Work Environ Health 25 Suppl 1, 23-25; discussion 76-28.

Gorczyca, W., Gong, J. & Darzynkiewicz, Z. (1993). Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res 53, 1945-1951.

Govaerts, I., Koenig, I., Van den Bergh, M., Bertrand, E., Revelard, P. & Englert, Y. (1996) Is intracytoplasmic sperm injection (ICSI) a safe procedure? What do we learn from early pregnancy data about ICSI? Hum Reprod 11, 440-443.

Greco, E., Iacobelli, M., Rienzi, L., Ubaldi, F., Ferrero, S. & Tesarik, J. (2005). Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 26, 349-353.

Greco, E., Romano, S., Iacobelli, M-, Ferrero, S., Baroni, E., Minasi, M.G., Ubaldi, F., Rienzi L. & Tesarik J. (2005). ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment. Hum Reprod 20:2590-2594.

Griffin, D. K., Hyland, P., Tempest, H. G. & Homa, S. T. (2003). Safety issues in assisted reproduction technology: Should men undergoing ICSI be screened for chromosome abnormalities in their sperm? Hum Reprod 18, 229-235.

Guzick, D. S., Overstreet, J. W., Factor-Litvak, P., Brazil, C. K., Nakajima, S. T., Coutifaris, C., Carson, S. A., Cisneros, P., Steinkampf, M. P. & Hill, J. A. et al. (2001). Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 345, 1388-1393.

Håkonsen, L.B., Thulstrup, A.M., Aggerholm, A.S., Olsen, J., Bonde, J.P., Andersen, C.Y., Bungum, M., Ernst, E.H., Hansen, M.L., Ernst, E.H. & Ramlau-Hansen CH (2011).
Does weight loss improve semen quality and reproductive hormones? results from a cohort of severely obese men. Reprod Health 17;8:24.

Hammadeh, M. E., Zeginiadov, T., Rosenbaum, P., Georg, T., Schmidt, W. & Strehler, E. (2001). Predictive value of sperm chromatin condensation (aniline blue staining) in the assessment of male fertility. Arch Androl 46, 99-104.

Hammadeh, M.E., Stieber, M., Haidl, G. & Schmidt W. (1998). Association between sperm cell chromatin condensation, morphology based on strict criteria, and fertilization, cleavage and pregnancy rates in an IVF program. Andrologia 30(1):29-35.

Henkel, R., Hajimohammad, M., Stalf, T., Hoogendijk, C., Mehnert, C., Menkveld, R., Gips, H., Schill, W. B. & Kruger, T. F. (2004). Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril 81, 965-972.

Hewitson, L., Simerly, C, Sutovsky, P., Dominko, T., Takahashi, D. & Schatten, G. (1999). The fate of sperm components within the egg during fertilization: implications for infertility. In C, G. (ed) The Male Gamete: From Basic Science to Clinical Applications Cache River Press, Vienna.

Host, E., Lindenberg, S. & Smidt-Jensen, S. (2000). The role of DNA strand breaks in human spermatozoa used for IVF and ICSI. Acta Obstet Gynecol Scand 79(7):559-563.

Huang, C. C., Lin, D. P., Tsao, H. M., Cheng, T. C., Liu, C. H. & Lee, M. S. (2005). Sperm DNA fragmentation negatively correlates with velocity and fertilization rates but might not affect pregnancy rates. Fertil Steril 84, 130-140.

Huszar, G., Zeyneloglu, H. B. & Vigue, L. (1999). Cellular maturity and fertilizing potential of sperm populations in natural and assisted reproduction. In C, G. (ed) The Male Gamete: From Basic Science to Clinical Applications. Cache River Press, Vienna, pp. 385–396.

Irvine, D. S., Twigg, J. P., Gordon, E. L., Fulton, N., Milne, P. A. & Aitken, R. J. (2000). DNA integrity in human spermatozoa: relationships with semen quality. J Androl 21, 33-44.

Jorgensen, N., Andersen, A. G., Eustache, F., Irvine, D. S., Suominen, J., Petersen, J. H., Andersen, A. N., Auger, J., Cawood, E. H. & Horte, A. et al. (2001). Regional differences in semen quality in Europe. Hum Reprod 16, 1012-1019.

Jorgensen, N., Asklund, C., Carllsen, E. & Skakkebaek, N. E. (2006). Coordinated European investigations of semen quality: results from studies of Scandinavian young men is a matter of concern. Int J Androl 29, 54-61; discussion 105-108.

Keel, B. A. (2006). Within- and between-subject variation in semen parameters in infertile men and normal semen donors. Fertil Steril 85, 128-134.

Kefer, J.C., Agarwal, A., & Sabanegh, E. (2009), Role of antioxidants in the treatment of male infertility. Int J Urol 2009;16:449-457.

Kurinczuk, J. J. (2003) Safety issues in assisted reproduction technology. From theory to reality--just what are the data telling us about ICSI offspring health and future fertility and should we be concerned? Hum Reprod 18, 925-931.

Larson, K. L., DeJonge, C. J., Barnes, A. M., Jost, L. K & Evenson, D. P. (2000). Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. Hum Reprod 15, 1717-1722.
Larson-Cook, K. L., Brannian, J. D., Hansen, K. A., Kaspersion, K. M., Aamold, E. T. & Evenson, D. P. (2003). Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 80, 895-902.

Lewis, S. E & Agbaje, I. M. (2008). Using the alkaline comet assay in prognostic tests for male infertility and assisted reproductive technology outcomes. Mutagenesis 23:163-170.

Lewis, S. E. (2007). Is sperm evaluation useful in predicting human fertility? Reproduction 134, 31-40.

Lewis, S. E. & Aitken, R. J. (2005) DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 322, 33-41.

Lewis, S. E., O’Connell, M., Stevenson, M., Thompson-Cree, L. & McClure, N. (2004). An algorithm to predict pregnancy in assisted reproduction. Hum Reprod 19, 1385-1394.

Li, Z., Wang, L., Cai, J. & Huang, H. (2006). Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. J Assist Reprod Genet 23, 367-376.

Lin, M. H., Kuo-Kuang Lee, R., Li, S. H., Lu, C. H., Sun, F. J. and Hwu, Y. M. (2007). Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 90(2):352-359.

Lopes, S., Sun, J. G., Jurisicova, A., Meriano, J. & Casper, R. F. (1998). Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertil Steril 69, 528-532.

Makhlouf, A. A. and Niederberger, C. (2006) DNA integrity tests in clinical practice: it is not a simple matter of black and white (or red and green). J Androl 27, 316-323.

Marchetti, F. and Wyrobek, A. J. (2005) Mechanisms and consequences of paternally-transmitted chromosomal abnormalities. Birth Defects Res C Embryo Today 75, 112-129.

Matsuda, Y. and Tobari, I. (1988) Chromosomal analysis in mouse eggs fertilized in vitro with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). Mutat Res 198, 131-144.

Matzuk., MM. & Lamb, D.J. (2008), The biology of infertility: research advances and clinical challenges. Nat Med 14:1197–1213.

Ménézo, Y.J., Hazout, A., Panteix, G., Robert, F., Rollet, J., Cohen-Bacrie, P., Chapuis, F., Clément, P. & Benkhalfa, M. (2007). Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. Reprod Biomed Online 14:418-421.

Morris, I. D., Ilott, S., Dixon, L. & Brison, D. R. (2002). The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. Hum Reprod 17, 990-998.
Muratori, M., Maggi, M., Spinelli, S., Filimberti, E., Forti, G. and Baldi, E. (2003) Spontaneous DNA fragmentation in swim-up selected human spermatozoa during long term incubation. J Androl 24, 253-262.

Nallella, K. P., Sharma, R. K., Aziz, N. and Agarwal, A. (2006) Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril 85, 629-634.

Nasr-Esfahani, M. H., Salehi, M., Razavi, S., Anjomshoa, M., Rozbahan, S., Moulavi, F. & Mardani, M. (2005). Effect of sperm DNA damage and sperm protamine deficiency on fertilization and embryo development post-ICSI. Reprod Biomed Online 11, 198-205.

Neuwinger, J., Behre, H. M. and Nieschlag, E. (1990) External quality control in the andrology laboratory: an experimental multicenter trial. Fertil Steril 54, 308-314.

O’Flynn O’Brien KL, Varghese AC, Agarwal A. The genetic cause of male factor infertility: a review. Fertil Steril 2010;93:1–12.

Olechuk, K., Giwercman, A & Bungum M. (2011). Intra-individual variation of the sperm chromatin structure assay DNA fragmentation index in men from infertile couples. Hum. Reprod. doi: 10.1093/humrep/der328Palamero, G., Joris, H., Devroey, P. and Van Steirteghem, A. C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340, 17.

Payne, J. F., Raburn, D. J., Couchman, G. M., Price, T. M., Jamison, M. G. and Walmer, D. K. (2005) Redefining the relationship between sperm deoxyribonucleic acid fragmentation as measured by the sperm chromatin structure assay and outcomes of assisted reproductive techniques. Fertil Steril 84, 356-364.

Perera, D, Pizzey, A., Campbell, A., Katz, M., Porter, J., Petrov, M., Irvine, D.S. & Chatterjee R. (2002). Sperm DNA damage in potentially fertile homozygous beta-thalassaemia patients with iron overload. Hum Reprod 17:1820-1825.

Potts, R. J., Newbury, C. J., Smith, G., Notarianni, L. J. & Jefferies, T. M. (1999). Sperm chromatin damage associated with male smoking. Mutat Res 423, 103-111.

Rai, R. & Regan, L. (2006). Recurrent miscarriage. Lancet 368, 601-611

Richthoff, J., Spano, M., Giwercman, Y. L., Frohm, B., Jepson, K., Malm, J., Elzanaty, S., Stridsberg, M. and Giwercman, A. (2002) The impact of testicular and accessory sex gland function on sperm chromatin integrity as assessed by the sperm chromatin structure assay (SCSA). Hum Reprod 17, 3162-3169.

Robbins, W. A., Vine, M. F., Truong, K. Y. & Everson, R. B. (1997). Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ Mol Mutagen 30, 175-183.

Rubes, J., Lowe, X., Moore, D., 2nd, Perreault, S., Slott, V., Evenson, D., Selevan, S. G. & Wyrobek, A. J. (1998). Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril 70, 715-723.

Sakkas, D. & Alvarez J.G. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 93:1027-1036.

Sakkas, D. & Alvarez, J.G. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 93:1027-1036.

Sakkas, D., Mariethoz, E., Manicardi, G., Bizzaro, D., Bianchi, P. G. & Bianchi, U. (1999). Origin of DNA damage in ejaculated human spermatozoa. Rev Reprod 4, 31-37.
Sakkas, D., Moffatt, O., Manicardi, G. C., Mariethoz, E., Tarozzi, N. and Bizzaro, D. (2002) Nature of DNA damage in ejaculated human spermatozoa and the possible involvement of apoptosis. Biol Reprod 66, 1061-1067.

Sakkas, D., Urner, F., Bianchi, P. G., Bizzaro, D., Wagner, I., Jaquenoud, N., Manicardi, G. & Campana, A. (1996). Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. Hum Reprod 11, 837-843.

Saleh, R. A., Agarwal, A., Nada, E. A., El-Tonsy, M. H., Sharma, R. K., Meyer, A., Nelson, D. R. and Thomas, A. J. (2003a) Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 79 Suppl 3, 1597-1605.

Saleh, R. A., Agarwal, A., Nelson, D. R., Nada, E. A., El-Tonsy, M. H., Alvarez, J. G., Thomas, A. J., Jr. and Sharma, R. K. (2002b) Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study. Fertil Steril 78, 313-318.

Sanchez-Pena, L. C., Reyes, B. E., Lopez-Carrillo, L., Recio, R., Moran-Martinez, J., Cebrian, M. E. & Quintanilla-Vega, B. (2004). Organophosphorous pesticide exposure alters sperm chromatin structure in Mexican agricultural workers. Toxicol Appl Pharmacol 196, 108-113.

Sega, G. A., Sotomayor, R. E. & Owens, J. G. (1978). A study of unscheduled DNA synthesis induced by X-rays in the germ cells of male mice. Mutat Res 49, 239-257.

Selevan, S. G., Borkovec, L., Slott, V. L., Zudova, Z., Rubes, J., Evenson, D. P. & Perreault, S. D. (2000). Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 108, 887-894.

Seli, E., Gardner, D.K., Schoolcraft, W.B, Moffatt, O. & Sakkas, D. (2004). Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 82:378-383.

Silver, E. W., Eskenazi, B., Evenson, D. P., Block, G., Young, S. & Wyrobek, A. J. (2005). Effect of antioxidant intake on sperm chromatin stability in healthy nonsmoking men. J Androl 26, 550-556.

Simon, L., Brunborg, G., Stevenson, M., Lutton, D., McManus, J. & Lewis, S.E. (2010). Clinical significance of sperm DNA damage in assisted reproduction outcome. Hum Reprod 25(7):1594-1608.

Simon, L., Lutton, D., McManus, J. & Lewis SE. (2011). Sperm DNA damage measured by the alkaline Comet assay as an independent predictor of male infertility and in vitro fertilization success. Fertil Steril 95(2):652-657.

Singh, N. P., Muller, C. H. and Berger, R. E. (2003) Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril 80, 1420-1430.

Skakkebaek, N. E., Giwercman, A. and de Kretser, D. (1994) Pathogenesis and management of male infertility. Lancet 343, 1473-1479.

Smit, M., Dohle, G. R., Hop, W. C., Wildhagen, M. F., Weber, R. F. and Romijn, J. C. (2007) Clinical correlates of the biological variation of sperm DNA fragmentation in infertile men attending an andrology outpatient clinic. Int J Androl 30, 48-55.

Song, G.J., Norkus, E.P.& Lewis, V. (2006). Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. Int J Androl. 2006;29:569-75.
Spano, M., Bonde, J. P., Hjollund, H. I., Kolstad, H. A., Cordelli, E. and Leter, G. (2000) Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 73, 43-50.

Spano, M., Kolstad, A. H., Larsen, S. B., Cordelli, E., Leter, G., Giwercman, A. and Bonde, J. P. (1998) The applicability of the flow cytometric sperm chromatin structure assay in epidemiological studies. Asclepios. Hum Reprod 13, 2495-2505.

Sun, J. G., Jurisicova, A. & Casper, R. F. (1997). Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 56, 602-607.

Swan, S. H. (2006) Semen quality in fertile US men in relation to geographical area and pesticide exposure. Int J Androl 29, 62-68; discussion 105-108.

The Practice Committee of the American Society of Reproductive Medicine (2008). The clinical utility of sperm DNA integrity testing. Fertil Steril 90 (Suppl 5):178-180.

Tomlinson, M. J., Moffatt, O., Manicardi, G. C., Bizzaro, D., Afnan, M. & Sakkas, D. (2001). Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod 16, 2160-2165.

Tomsu, M., Sharma, V. & Miller, D. (2002). Embryo quality and IVF treatment outcomes may correlate with different sperm comet assay parameters. Hum Reprod 17, 1856-1862.

Varghese, A. C., Goldberg, E. and Agarwal, A. (2007) Current and future perspectives on intracytoplasmic sperm injection: a critical commentary. Reprod Biomed Online 15, 719-727.

Verpoest, W. & Tournaye, H. (2006). ICSI: hype or hazard? Hum Fertil (Camb) 9, 81-92.

Virro, M. R., Larson-Cook, K. L. & Evenson, D. P. (2004). Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 81, 1289-1295.

Ward, W. S. (1993). Deoxyribonucleic acid loop domain tertiary structure in mammalian spermatozoa. Biol Reprod 48, 1193-1201.

Ward, W. S. and Coffey, D. S. (1991). DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod 44, 569-574.

WHO (2000). WHO Manual for the Standardised Investigation, Diagnosis and Management of the Infertile Male. Cambridge University Press, Cambridge.

Wolf, J.P., Bulwa, S., Ducot, B., Rodrigues, D. & Jouannet, P. (1996). Fertilizing ability of sperm with unexplained in vitro fertilization failures, as assessed by the zona-free hamster egg penetration assay: its prognostic value for sperm-oolemma interaction. Fertil Steril 65(6):1196-1201.

World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge: Cambridge University Press; 1999.

World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5 ed: World Health Organization; 2010.
Zini A, Sigman M. Are tests of sperm DNA damage clinically useful? Pros and cons. J Androl 2009;30:219-229.

Zini, A., Bielecki, R., Phang, D. and Zenzes, M. T. (2001a) Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 75, 674-677.

Zini, A., Boman, J.M., Belzile, E. & Ciampi, A. (2008). Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 23 (1),4-10.

Zini, A., Fischer, M. A., Sharir, S., Shayegan, B., Phang, D. and Jarvi, K. (2002). Prevalence of abnormal sperm DNA denaturation in fertile and infertile men. Urology 60, 1069-1072.

Zini, A., Kamal, K., Phang, D., Willis, J. and Jarvi, K. (2001b) Biologic variability of sperm DNA denaturation in infertile men. Urology 58, 258-261.
Embryology is a branch of science concerned with the morphological aspects of organismal development. The genomic and molecular revolution of the second half of the 20th century, together with the classic descriptive aspects of this science have allowed greater integration in our understanding of many developmental events. Through such integration, modern embryology seeks to provide practical knowledge that can be applied to assisted reproduction, stem cell therapy, birth defects, fetal surgery and other fields. This book focuses on human embryology and aims to provide an up-to-date source of information on a variety of selected topics. The book consists of nine chapters organized into three sections, namely: 1) gametes and infertility, 2) implantation, placentation and early development, and 3) perspectives in embryology. The contents of this book should be of interest to biology and medical students, clinical embryologists, laboratory researchers, obstetricians and urologists, developmental biologists, molecular geneticists and anyone who wishes to know more about recent advances in human development.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mona Bungum (2012). Role of Sperm DNA Integrity in Fertility, Embryology - Updates and Highlights on Classic Topics, Prof. Luis Violin Pereira (Ed.), ISBN: 978-953-51-0465-0, InTech, Available from: http://www.intechopen.com/books/embryology-updates-and-highlights-on-classic-topics/role-of-sperm-dna-integrity-in-fertility