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Role of Sperm DNA Integrity in Fertility

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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; Hammadeh 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 (Neuwinger 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 (Irvine 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≥13) 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; Hammadeh 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 el 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 ICSI 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

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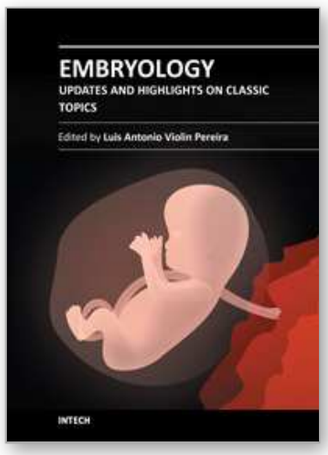

Embryology - Updates and Highlights on Classic Topics
Edited by Prof. Luis Violin Pereira

ISBN 978-953-51-0465-0
Hard cover, 222 pages
Publisher InTech
Published online 30, March, 2012
Published in print edition March, 2012

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.

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