We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Sperm DNA Fragmentation and Its Relation With Fertility

Javier García-Ferreyra

Abstract

Sperm DNA integrity is vital for successful fertilization, embryo development, pregnancy, and transmission of genetic material to the offspring. DNA fragmentation is the most frequent DNA anomaly present in the male gamete that has been associated to poor semen quality, low fertilization rates, impaired embryo quality, and preimplantation development and reduced clinical outcomes in assisted reproduction procedures. This work summarizes the causes of fragmentation in the spermatic DNA, and its relation with seminal parameters, male aging, and results in assisted reproduction procedures.

Keywords: Spermatozoa, DNA fragmentation, seminal parameters, ROS, IVF, ICSI

1. Introduction

Semen quality is frequently used as an indirect measure of male infertility. Ejaculate volume, sperm concentration, motility, and morphology determined according to the World Health Organisation (WHO) are the most important parameters evaluated in infertility centers as part of routine semen analysis. The genetic composition in a newborn is the results of oocyte and sperm DNA information, and it should be intact for further embryo and fetal development that will result in a healthy offspring. Any type of damage present in the DNA of male or female gametes can lead to an interruption of the reproductive process. Sperm DNA fragmentation might be the most frequent cause of paternal DNA anomaly transmission to progeny and is found in a high percentage of spermatozoa from subfertile and infertile men.

Several hypotheses have been proposed as to the molecular mechanism of sperm DNA fragmentation, the most important ones being: apoptosis, abnormal chromatin packaging, and reactive oxygen species [1]. Several studies show that spermatozoa with DNA fragmentation are able to fertilize an oocyte [2-4], but are related to abnormal quality embryo, block in the blastocyst development, and lower pregnancy rates either natural or using IUI, IVF, or ICSI procedures [5-10]. Various studies demonstrate that the oocytes and the embryo retain the ability to repair DNA damage that may be present in the paternal genome; however, it is not yet clear if all types of damage can be repaired. For instance, double-stranded DNA breaks appear to be less repairable than single-stranded breaks and, therefore, have a greater impact on embryo quality and/or embryo development. Additionally, the capacity of oocyte to repair DNA damage will depend on factors like maturity, maternal age, and external factors. This review summarizes the causes that produce sperm DNA fragmentation, its relation to seminal parameters, paternal age, and effect on assisted reproduction procedures.

2. Human sperm chromatin structure

Germ cells mediate the transfer of genetic information from generation to generation and are thus pivotal for the maintenance of life. Spermatogenesis is a continuous and precisely controlled process that involves extremely marked cellular, genetic and chromatin changes resulting in a generation of highly specialized sperm cells (Figure 1). Spermatogonial stem cells replicate and differentiate into primary spermatocytes that undergo genetic recombination to give rise to round haploid spermatids [11]. Round spermatids then undergo a differentiation process called spermiogenesis where marked cellular, epigenetic, and chromatin remodeling takes place [12, 13]. The nucleosomes are disassembled and the histones are removed and replaced by the high positively charged protamines forming tight toroidal complexes, organizing 85—95% of the human sperm DNA [14]. Human spermatozoa have two types of protamine (P1 and P2). P2 has fewer thiol groups for disulfide bonding and this makes human sperm chromatin less stable [15]. Finally, during the transit in the epididymis the cysteines become progressively oxidized forming inter- and intraprotamine disulfide bonds that, along with zinc bridges, stabilize and compact completely the chromatin [16, 17]. All these interactions make mammalian DNA the most condensed eukaryotic DNA [18], adjusting to the extremely limited volume of the sperm nucleus [19].

Chromatin organization plays an important role during the fertilization process and early embryo development. The sperm chromatin is a crystalline, insoluble, compact, and well-organized structure in DNA loop domains with an average length of 27 kilobytes. These loops, which can be visualized by using fluorescent in situ hybridization (FISH), are attached at their bases to the nuclear matrix. During sperm decondensation the DNA remains anchored to the base of the tail, suggesting the presence of a nuclear annulus-like structure in human sperm [20]. This DNA organization permits the transfer of the very tightly packaged genetic information to the egg and ensures that the DNA will be delivered in a physical and chemical form that allows the developing embryo to access the genetic information [1].
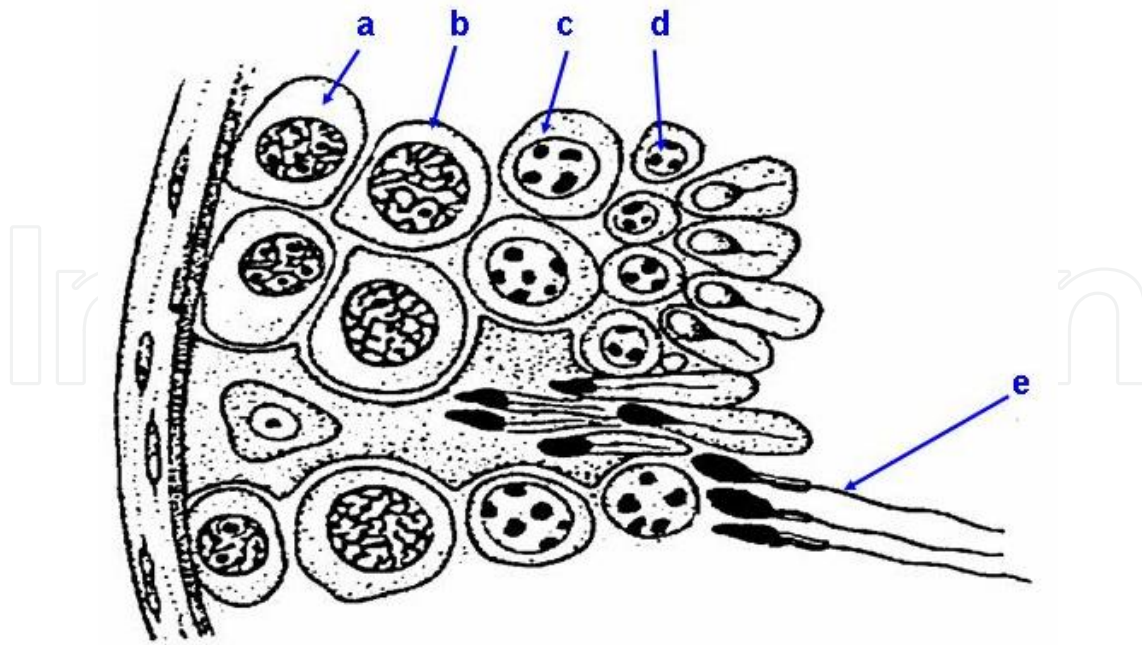
Figure 1. Espermatogenesis. **a:** Spermatogonia (2n); **b:** primary spermatocyte (2n); **c:** secondary spermatocyte (n); **d:** spermatid (n); **e:** spermatozoa

3. Causes of DNA fragmentation

Sperm DNA fragmentation can be caused by apoptosis, defects in chromatin remodeling during the process of spermiogenesis, and oxygen radical-induced DNA damage.

3.1. Apoptosis

During spermiogenesis, apoptosis allows the monitoring of the germ cell population that will be sustained by Sertoli cells [21], to regulate the overproduction of sperm cell and the elimination of abnormal cells [22]. Sperm apoptosis is mediated by type Fas proteins [23], and their concentration is above 50% in males with abnormal seminal parameters [24]. Generally, cells marked with Fas proteins are phagocytized and eliminated by Sertoli cells to which these are associated [25, 26]. However, a percentage of defective germ cells undergo sperm remodeling during spermiogenesis, appearing later on the ejaculate, showing normal morphology but are genetically altered [27]. Apoptosis entails cell membrane disruption, cytoskeletal rearrangement, nuclear condensation and intranucleosomal DNA fragmentation in numerous fragments ≥185 bp [28].

3.2. Damage during chromatin packing in the spermiogenesis

Sperm chromatin structure has a complex arrangement of DNA and sperm nuclear protein with different levels of compaction to shrink the nuclear volume and head size [29]. Then, DNA fragmentation may be the result of unresolved strand breaks created during the normal process spermiogenesis in order to relieve the torsional stresses involved in packaging a very

large amount of DNA into the very small sperm head. These physiological strand breaks are corrected through H2Ax phosphorylation and activation of nuclear poly (ADP-ribose) polymerase and topoisomerase [30].

3.3. Oxygen radical-induced DNA damage by reactive oxygen species

ROS or free radicals are oxidizing agents that are generated as byproducts of the metabolism of oxygen. Due to the presence of at least one unpaired electron, they form highly reactive molecules (e.g. hydroxyl ion [OH], superoxide ion [O_2^-], nitric oxide [NO], peroxyl [RO_2], lipid peroxyl [LOO], and Thyl [RS]) and non-radical molecules (singlet oxygen [O_2], hydrogen peroxide [H_2O_2], hypochloric acid [HOCl], lipid peroxide [LOOH], and ozone [O_3]) [31].

It has been reported that the chromatin in the sperm nucleus is vulnerable to oxidative damage, leading to base modifications and DNA fragmentation [32]. De luliis *et al.* [33] showed that electromagnetic radiation induces ROS production, resulting in DNA damage and decreased motility and vitality in human spermatozoa. Moreover, several toxins released from structural materials or industrial products (e.g., benzene, methylene chloride, hexane, toluene, trichloro-ethane, styrene, heptane, and phthalates) and toxins in the form of metals (e.g. cadmium, chromium, lead, manganese, and mercury) increase ROS production in the testes, impairing the spermatogenesis and inducing sperm DNA fragmentation [34-36]. Additionally, consumption of tobacco and alcohol leads to higher rates of ROS production and high levels of DNA strand breaks [37], decreasing in sperm motility [38] and apoptosis.

Furthermore, the activation of sperm caspases and endonucleases by ROS induce sperm DNA fragmentation. Studies by Cui *et al.* [39] and Banks *et al.* [40] showed that *in vivo* exposure of mouse testis at 40º—42ºC results in a significant increase in DNA fragmentation, occurring in the epididymis by activation of caspases and endonucleases. The potential damage that sperm may experience during passage through the epididymis could be limited by removing them before that passage. Patients with high levels of DNA fragmentation in semen and repeated IVF failure can increase their clinical outcomes using testicular sperm obtained by testicular sperm extraction (TESE or TESA) [41].

Human sperm chromatin becomes cross-linked under conditions of oxidative stress and exhibits increased DNA strand breakage [42]. When DNA is minimally damaged, spermatozoa can undergo self-repair and potentially regain the ability to fertilize the oocyte and proceed with development [43]. In fact, the oocyte is also capable of repairing damaged sperm DNA; but when the oocyte machinery is not sufficient to repair DNA damage the embryo may fail to develop or implant in the uterus.

4. Age and DNA fragmentation

The increase in life expectancy, women's entry into the labor market and the popular use of contraception has contributed to the social phenomena of delaying family planning and parenthood to the mid or late thirties. This has also had a significant impact on males. In

Germany, the median age of married fathers has increased from 31.3 years in 1991 to 33.1 years in 1999 [44]. The same trend has also been seen in England. In 1993, fathers aged 35—54 years accounted for 25% of live births. Ten years later, these percentages grew to 40% [45]. Among couples seeking pregnancy through assisted reproduction technologies (ART), fathers are significantly older compared with those not needing ART (36.6 vs. 33.5 years) [46].

In Western societies, advanced paternal age is a phenomenon that parallels advanced maternal age and is associated with various reproductive hazards including decrease of testicular volume, alterations in testicular histomorphology, and a decrease in the inhibin B/FSH ratio consistent with a reduced Sertoli cell mass [47]. Other observable patterns include risk of chromosomal disorders, decline in semen volume, progressive motility, and daily sperm production with advanced age [48].

On the other hand, García-Ferreyra *et al.* [49] evaluated the effect of age on fertility and showed that the sperm DNA fragmentation, progressive motility, and spermatozoa morphology are associated with advanced paternal age. They analyzed seminal samples of 217 infertile patients between 21 and 68 years, which were distributed into four groups: <30 years, 30—39 years, 40—49 years and ≥50 years. The results showed an age-dependent increase in sperm DNA fragmentation, which was statistically significant starting at 40 years old (Table 1). Patients ≥ 50 years old had morphologically normal spermatozoa, significantly lower compared to those men <40 years (Figure 2).

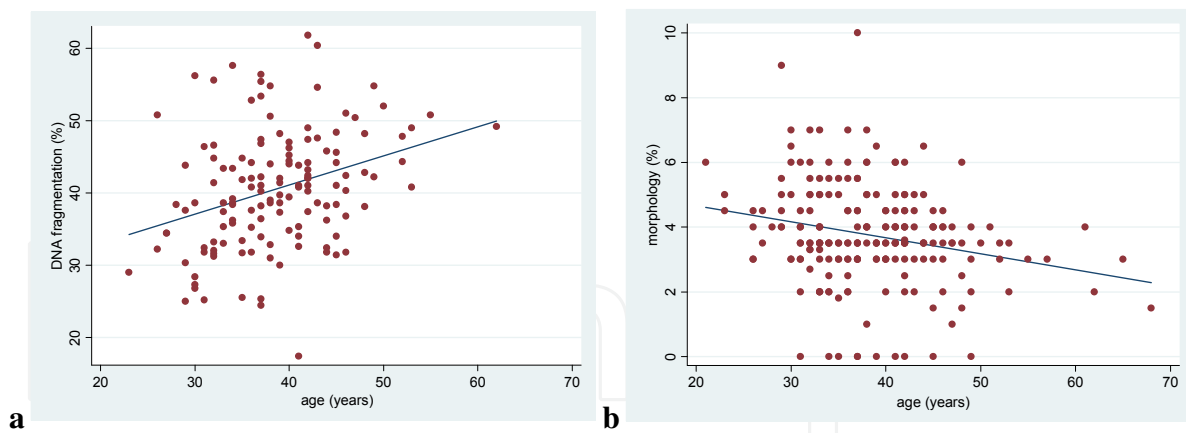| Age	N	DNA Fragmentation (%)
<30 years	16	35.56±7.52
30—39 years	111	39.37±8.39
40—49 years	78	41.99±7.65 [a,b]
≥50 years	12	47.70±3.89 [a,b,c]

[a]$P<0.05$ in relation to the group <30 years

[b]$P<0.05$ in relation to the group 30—39 years

[c]$P<0.05$ in relation to the group 40—49 years

García-Ferreyra et al. Sperm DNA fragmentation. JFIV Reprod Med Genet 2012

Table 1. Sperm DNA fragmentation according to male age

In males, germ cells divide continuously. It has been estimated that 30 spermatogonial stem cell divisions take place before puberty, when they begin to undergo meiotic divisions. From then on, 23 meiotic divisions per year occur, resulting in 150 replications by the age of 20 and 840 replications by the age of 50 [50]. Because of these numerous divisions of stem cells, older men may have an increased risk of errors in DNA transcription. Furthermore, germ cells are continuously under attack from endogenous and exogenous factors that can induce a wide range of DNA lesions, thereby affecting normal cellular processes such as transcription, recombination and replication [51]. One of the main theories of aging states that aging results

García-Ferreyra et al. Sperm DNA fragmentation. JFIV Reprod Med Genet 2012

Figure 2. Scatter graph illustrating the associations between age and DNA fragmentation (a; *r*=0.106; *p*=0.0001) and morphology (b; *r*=0.054; *p*=0.0017)

from an accumulation of unrepaired DNA lesions; such lesions have been routinely linked to aging in many tissues including the brain, the liver, and the testis [52, 53]. Paul *et al*. [53] showed that there is an age-related accumulation of DNA damage in the testis, particularly caused by oxidative stress in the form of 8-oxodG lesions. Furthermore, aging seems to lower the capacity of germ cells to repair such DNA damage, resulting in the production of spermatozoa with increased DNA damage. This is likely to lead to a decline in genome quality that may be passed on to future generations, specifically the offspring of older males.

5. Spermatozoa morphology and DNA fragmentation

Teratozoospermia is defined as ≤ 4% normal sperm morphology at semen analysis with normal sperm count and normal progressive motility [54], and has been associated with infertility and low fertilization rates in conventional IVF procedures [55, 56].

Several studies indicate that DNA damage is associated with abnormalities in conventional semen parameters [24, 57-59]. Irvine *et al*. [57] found a stronger inverse correlation between DNA damage with concentration (-0.54) and Saleh *et al*. [60] showed an inverse correlation with the motility (-0.47). Larson-Cook *et al*. [61] demonstrated that only three of the 10 men with high levels of DNA damage had asthenozoospermia and/or oligozoospermia. In the study of García-Ferreyra *et al*. [49] evaluating the effect of age on semen parameters in infertile men, it was shown that the advanced paternal age was related to high percentages of fragmented DNA and low values of spermatic concentration, motility and morphology. Recently, García-Ferreyra *et al*. [62] assessed the quality of spermatic DNA according to spermatozoa morphology in 196 men, concluding that high levels of DNA damage were related to abnormal sperm morphology (Figure 3). Besides, when splitting the patients into a group of normozoospermic men and a group of men with at least one impaired conventional semen parameter or infertile men, the two groups were significantly different from each other in DNA fragmentation,

motility, and morphology percentages (Table 2). Similar results were reported by Levitas *et al*. [63], Cardona *et al*. [64], Molina *et al*. [65], and Brahem *et al*. [66] while Winkle *et al*. [67] only reported a decrease in sperm motility.

Group	DNA fragmentation (%)	Motility (%)	Morphology (%)
Normozoospermic	34.92±5.89	61.57±11.61	5.02±1.12
Infertile men	44.41±7.47*	35.40±20.45*	2.78±1.09*

P<0.05 in relation to the Normozoospermic group

García-Ferreyra et al. Sperm DNA fragmentation JFIV Reprod Med Genet 2014

Table 2. Relation between DNA fragmentation, motility and morphology.
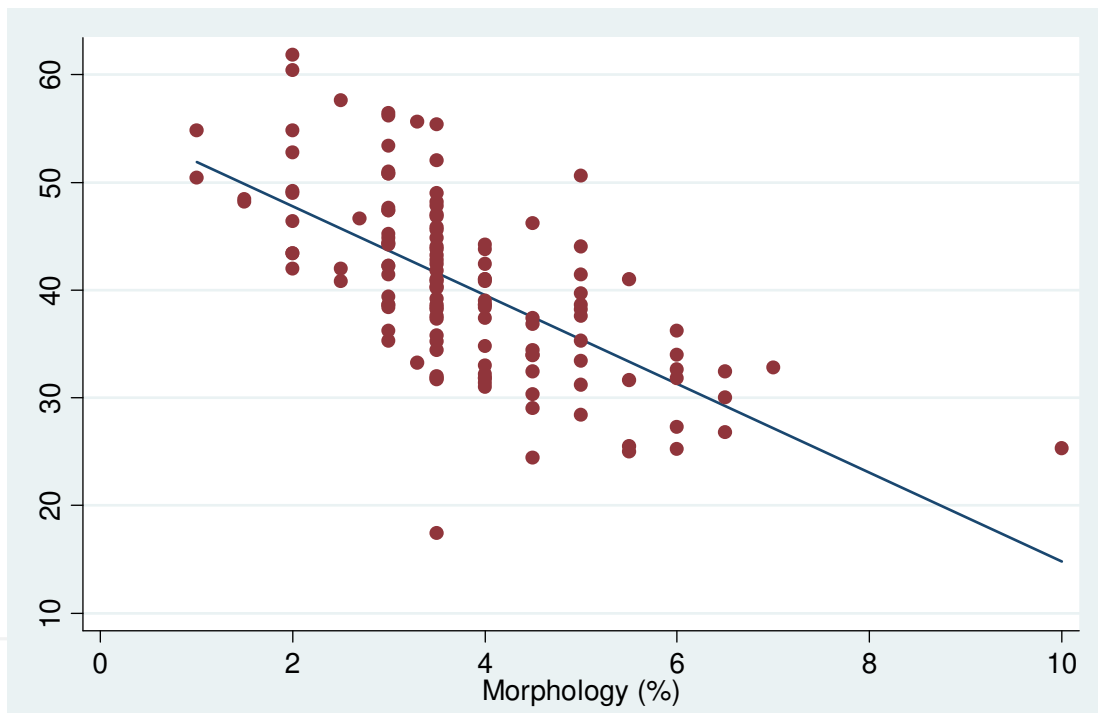
García-Ferreyra et al. Sperm DNA fragmentation JFIV Reprod Med Genet 2014

Figure 3. Scatter graph illustrating associations between DNA fragmentation and morphology (r=2.464; p=0.000)

6. IVF/ICSI procedures and sperm DNA fragmentation

Sperm DNA contributes half of the offspring's genomic material and abnormal DNA can lead to derangements in the reproductive process. Several studies provide good evidence that sperm DNA and chromatin damage are associated with male infertility and reduced natural conception rates [6, 68, 69]. In humans, high levels of sperm DNA damage have been related

to low fertility potential, failure to obtain blastocysts, blockage in embryo development after embryo implantation, increased risk of recurrent miscarriages, reduced chances of successful implantation, and negative effects on the health of the offspring [70-72].

Studies of Virro et al. [73], Huang et al. [59], and Borini et al. [76] showed a negative correlation between fertilization rates and high levels of sperm DNA fragmentation. However, if the type and extent of DNA damage can be balanced by the reparative ability of the oocyte, it is possible to achieve fertilization even in the presence of elevated sperm DNA fragmentation rates [74, 75]. Given that, excessive damage in sperm DNA may result in early reproductive failures and during the 4 to 8 cell stage, when the paternal genome is switched on, the development of the embryo will be affected by sperm DNA integrity causing apoptosis, fragmentation, and difficulty to reach the blastocyst stage [19, 76].

An inverse relationship has been reported between the likelihood of achieving pregnancy either by natural intercourse and intrauterine insemination (IUI), but there are conflicting results with IVF/ICSI procedures and the presence of high sperm DNA fragmentation levels [72, 74, 77, 78]. An extended study by Bungum et al. [79] performed on a total of 998 IUI cycles showed significantly lower odds ratios for clinical pregnancy and delivery when the male partner had a DNA fragmentation index >30% measured by SCSA. On the other hand, published studies suggest conflicting results of the influence of sperm DNA fragmentation on embryo quality and development capacity in the outcomes of IVF and ICSI [3, 5, 7, 60].

Two meta-analyses made by Evenson and Wixon [80] and Li et al. [81] evaluating the relation of sperm DNA fragmentation and assisted reproduction outcomes reported different results; the first one showed that the clinical outcomes in IIU, IVF, and ICSI were closely related to DNA fragmented; whereas the other one suggested only negative effect on IVF procedures. A possible explanation for these differences is the different methods used to detect DNA integrity and the lack of standardization of methods used to evaluate sperm DNA fragmentation. Recently, Zini et al. [82] performed a systematic review of 28 studies to examine the influence of sperm DNA fragmentation on embryo quality and/or embryo development at IVF and ICSI (8 IVF, 12 ICSI, and 8 mixed IVF-ICSI). In 11 of 28 studies there was a positive relation between DNA fragmented and poor embryo quality/development. Sperm DNA fragmentation was associated with poor embryo development in 7 of 11 positive studies, and with poor embryo quality in 5 of the 11 positive studies. Moreover, according to ART procedures the sperm DNA fragmentation was associated only with 1/8 IVF studies (poor embryo quality), and 5/12 ICSI studies (poor quality and/or delayed development). These data suggest that the effect of sperm DNA fragmentation on embryo quality/development is more dramatic in ICSI compared to IVF, probably because with ICSI the natural selection barriers are bypassed entirely and the fertilization with highly DNA fragmented sperm is possible, which does not occur in IVF where the integrity of sperm DNA is closely related to sperm motility and sperm membrane characteristics important during the natural selection process reducing the probability of fertilization with DNA-damage sperm at IVF [83, 84]. Finally, the majority of studies indicate that sperm DNA fragmentation has negative effects on pregnancy rate, embryo quality, live birth, and early pregnancy loss.

7. Conclusions

Sperm DNA fragmentation is an important factor that should be evaluated in subfertile and infertile men because several studies have shown that it has an important impact, independent of the parameters of classic semen analysis, on the reproductive process in both natural and assisted reproduction. Particularly, it affects the embryo quality and/or embryo development that decrease the implantation rates and increase the rates of early miscarriage in ART. Finally, it is important to obtain a clear diagnosis and the application of adequate methods of sperm selection pre—ART when high levels of sperm DNA fragmentation are observed to increase the possibilities to achieve the pregnancy in couples with high sperm DNA fragmentation and repeated assisted reproduction failures.

Author details

Javier García-Ferreyra

Address all correspondence to: jgarciaf@fertilab.pe

FERTILAB Laboratory of Assisted Reproduction, Lima, Perú

References

[1] Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG, Bianchi U (1999) Origin of DNA damage in ejaculated human spermatozoa. Rev Reprod. 4:31-37.

[2] Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, Irvine DS (1998) Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod. 59:1037-1046.

[3] Lopes S, Jurisicova A, Sun JG, Casper RF (1998) Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa. Hum Reprod. 13:896-900.

[4] Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, Criminna R, Culasso F, Dondero F, Lenzi A, Spano M (2004) Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod. 19:1409-1417.

[5] Sun JG, Jurisicova A, Casper RF (1997) Detection of deoxyribonucleic acid in human sperm: correlation with fertilization in vitro. Biol Reprod. 56:602-607.

[6] Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, De Angelis P, Claussen OP (1999) Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 14:1039-1049.

[7] Larson KL, DeJonge CJ, Barnes AM, Jost LK, Evenson DP (2000) Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproduction techniques. Hum Reprod 15:1717-1722.

[8] Duran EH, Morshedi M, Taylor S, Oehninger S (2002) Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod. 17:3122:3128.

[9] Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, Gips H, Schill WB, Kruger TF (2004) Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril. 81:965-972.

[10] Muriel L, Garrido N, Fernández JL, Remohí J, Pellicer A, De los Santos MJ, Meseguer M (2006) Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril. 85:371-383.

[11] Baccetti B, Afzelius BA (1976) The biology of sperm cell. Monogr Dev Biol. 10:1-254.

[12] Oliva R (2006) Protamines and male infertility. Hum Reprod Update. 12:417-435.

[13] Shaman JA, Yamauchi Y, Ward WS (2007) Function of the sperm nuclear matrix. Arch Androl. 53:135-140.

[14] Ward WS, Coffey DS (1991) DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod. 44:569-574.

[15] Jager S (1990) Sperm nuclear stability and male infertility. Arch Androl. 25:253-259.

[16] Aitken RJ, Nixon B, Lin M, Koppers AJ, Lee YH, Baker MA (2007) Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian J Androl 9:554-564.

[17] Björndahl L, Kvist U (2010) Human sperm chromatin stabilization: a proposed model including zinc bridges. Mol Hum Reprod. 16:23-29.

[18] Ward WS, Coffey DS (1990) Specific organization of genes in relation to the sperm nuclear matrix. Biochem Biophys Res Commun. 173:20-25.

[19] Acharyya S, Kanjilal S, Bhattacharyya AK (2005) Does human sperm nuclear DNA integrity affect embryo quality?. Indian J Exp Biol. 43:1016-1022.

[20] Berone JG, De Lara J, Cummings KB, Ward WS (1994) DNA organization in human spermatozoa. J Androl. 15:139-144.

[21] Sinha Hikim AP, Swerdloff RS (1999) Hormonal and genetic control of germ cell apoptosis in the testis. Rev Reprod. 4:38-47.

[22] Sakkas D, Seli E, Bizzaro D, Tarozzi N, Manicardi GC (2003) Abnormal spermatozoa in the ejaculate: abortive apoptosis and faulty nuclear remodeling during spermatogenesis. Reprod Biomed Online. 7:428-432.

[23] Spadafora C (1998) Sperm cells and foreing DNA: a controversial relation. Bioessays. 20:955-964.

[24] McVicar CM, McClure N, Williamson K, Dalzell LH, Lewis SEM (2004) Incidence of Fas positivity and deoxyribonucleic acid double-stranded breaks in human ejaculated sperm. Fertil Steril. 81:767-774.

[25] Billig H, Chun SY, Eisenhauer K, Hsueh AJ (1996) Gonadal cell apoptosis: hormone-regulated demise. Hum Reprod Update. 2:103-117.

[26] Pentikainen V, Erkkila K, Dunkel L (1999) Fas regulates germ cell apoptosis in the human testis in vitro. Am J Physiol. 276:310-316.

[27] Burrello N, Arcidiacono G, Vicari E, Asero P, Di Benedetto D, De Palma A, Romero R, D'Agata R, Calogero AE (2004) Morphologically normal spermatozoa of patients with secretory oligo-astheno-teratozoospermia have an increased aneuploidy rate. Hum Reprod. 19:2298-2302.

[28] Kaufmann SH, Hengartner MO (2001) Programmed cell death: alive and well in the new millennium. Trends Cell Biol. 11:526-534.

[29] Getzenberg RH, Pienta KJ, Ward WS, Coffey DS (2004) Nuclear structure and three-dimensional organization of DNA. J Cell Biochem. 47:289-299.

[30] Meyer-Ficca ML, Lonchar J, Credidio C, Ihara M, Li Y, Wang ZQ, Meyer RG (2009) Disruption of poly(ADP-ribose) homeostasis affects spermiogenesis and sperm chromatin integrity in mice. Biol Reprod. 81:46-55.

[31] Bansal AK, Bilaspuri GS (2011) Impacts of oxidative stress an antioxidants on semen functions. Vet Med Int. 9:e686137.

[32] Zribi N, Chakroun NF, Elleuch H, Abdallah FB, Ben Hamida AS, Gargouri J, Fakhfakh F, Keskes LA (2011) Sperm DNA fragmentation and oxidation are independent of malondialdheyde. Reprod Biol Endocrinol. 9:47.

[33] De luliis GN, Newey RJ, King BV, Aitken RJ (2009) Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446.

[34] Esfandiari N, Saleh RA, Blaut AP, Sharma RK, Nelson DR, Thomas AJ Jr, Falcone T, Agarwal A (2002) Effects of temperature on sperm motion characteristics and reactive oxygen species. Int J Fertil Womens Med. 47:227-233.

[35] Jurasović J, Cvitković P, Pizent A, Colak B, Telisman S (2004) Semen quality and reproductive endocrine function with regard to blood cadmium in Croatian male subjects. Biometals. 17:735-743.

[36] Pant N, Shukla M, Kumar Patel D, Shukla Y, Mathur N, Kumar Gupta Y, Saxena DK (2008) Correlation of phthalate exposures with semen quality. Toxicol Appl Pharmacol. 231:112-116.

[37] Jarow JP (2003) Semen quality of male smokers and nonsmokers in infertile couples. J Urol. 170:675-676.

[38] Kiziler AR, Aydemir B, Onaran I, Alici B, Ozkara H, Gulyasar T, Akyolcu MC (2007) High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. Biol Trace Elem Res. 120:82-91.

[39] Cui J, Holmes EH, Greene TG, Liu PK (2000) Oxidative DNA damage precedes DNA fragmentation after experimental stroke in rat brain. FASEB J. 14:955-967.

[40] Banks S, King SA, Irvine DS, Saunders PT (2005) Impact of a mild scrotal heat stress on DNA integrity in murine spermatozoa. Reproduction. 129:505-514.

[41] Greco E, Scarselli F, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Franco G, Anniballo N, Mendoza C, Tesarik J (2005) Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod. 20:226-230.

[42] Twigg J, Fulton N, Gomez E, Irvine D, Aitken RJ (1998) Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. Hum Reprod. 13:1429-1436.

[43] Aitken RJ, Koppers AJ (2011) Apoptosis and DNA damage in human spermatozoa. Asian J Androl. 13:36-42.

[44] Kühnert B, Nieschlag E (2004) Reproductive functions of the ageing male. Hum Reprod Update. 10:327-339.

[45] Bray I, Gunnell D, Davey Smith G (2006) Advanced paternal age: how old is too old? J Epidemiol Community Health. 60:851-853.

[46] Engel W, Sancken U, Laccone F (2004) Paternal age from a genetic point of view. J Reproduktionsmed Endokrinol. 1:263-267.

[47] Mahmoud AM, Goemaere S, El-Garem Y, Van Potterlberrgh I, Camhaire FH, Kaufman JM (2003) Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. J Clin Endocrinol Metab. 88:179-184.

[48] Handelsman DJ, Staraj S (1985) Testicular size: the effect of aging, malnutrition, and illness. J Androl 6:144-151.

[49] García-Ferreyra J, Romero R, Hilario R, Dueñas-Chacón J (2012) High levels of DNA fragmentation observed in an infertile population attending a fertility center are related to advanced paternal age. J Fert In Vitro. 2:1-5.

[50] Crow JF (2000) The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet. 1:40-47.

[51] Baarends VW, Van der Laan R, Grootegoed JA (2001) DNA repair mechanisms and gametogenesis. Reproduction. 121:31-39.

[52] Moller P, Lohr M, Folkmann JK, Mikkelsen L, Loft S (2010) Aging and oxidatively damaged nuclear DNA in animal organs. Free Radic Biol Med. 48:1275-1285.

[53] Paul C, Nagano M, Robaire B (2011) Aging results in differential regulation of DNA repair pathways in pachytene spermatocytes in the Brown Norway Rat. Biol Reprod. 85:1269-1278.

[54] Younan D, Sorour A, Genedy R (2014) Aneuploidy frequency in spermatozoa of Egyptian men with normal and abnormal semen parameters using fluorescence in situ hybridization. Andrologia 47:228-235.

[55] Vawda AI, Gunby J, Younglai EV (1996) Semen parameters as predictors of in-vitro fertilization: the importance of strict criteria sperm morphology. Hum Reprod. 11:1445-1450.

[56] Lundin K, Soderlund B, Hamberger L (1997) The relationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme. Hum Reprod. 12:2676-2681.

[57] Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ (2000) DNA integrity in human spermatozoa: relationships with semen quality. J Androl. 21:33-44.

[58] Tomlinson MJ, Moffalt O, Manicardi GC, Bizzaro D, Afnan M, Sakkas D (2001) Inter-relationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod. 16:2160-2165.

[59] Huang CC, Lin DP, Tsao HM, Cheng TC, Liu CH, Lee MS (2005) Sperm DNA fragmentation negatively correlates with velocity and fertilization rates but might not affect pregnancy rates. Fertil Steril. 84:130-140.

[60] Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, Nelson DR, Thomas AJ (2003) Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril. 79:1593-1605.

[61] Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP (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.

[62] García-Ferreyra J, Villegas L, Romero R, Zavala P, Hilario R, Casafranca G, Dueñas-Chacón J (2014) Sperm DNA fragmentation is significantly increased in those men with morphologically abnormal spermatozoa. JFIV Reprod Genet. 2:1-5.

[63] Levitas E, Lunenfeld E, Weisz N, Potashnik G (2007) Relationship between age and semen parameters in men with normal sperm concentration: analysis of 6,022 semen samples. Andrologia. 39:45-50.

[64] Cardona Maya W, Berdugo J, Cadavid Jaramillo A (2009) The effects of male age on semen parameters: analysis 1364 men attending an Andrology center. Aging Male. 12:100-103.

[65] Molina RI, Martini AC, Tissera A, Olmedo J, Senestrari D, De Cuneo MF, Ruiz RD (2010) Semen quality and aging: analysis of 9.168 samples in Cordova. Argentina. Arch Esp Urol. 63:214-222.

[66] Brahem S, Mehdi M, elghezal H, Saad A (2011) The effects of male aging on semen quality, sperm DNA fragmentation and chromosomal abnormalities in an infertile population. J Assist Reprod Genet. 28:425-432.

[67] Winkle T, Rosenbusch B, Gagsteiger F, Paiss T, Zoller N (2009) The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility center. J Assist Reprod Genet. 26:41-46.

[68] Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G (2000) Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril. 73:43-50.

[69] Giwercman A, Lindstedt L, Larsson M, Bungum M, Spano M, Levine RJ, 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.

[70] Seli E, Gardner DK, Schoolcraft WB, Moffalt 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.

[71] Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, Coticchio G (2006) Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 21:2876-2881.

[72] Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, Giwercman A (2007) Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 22:174-179.

[73] Virro MR, Larson-Cook KL, Evenson DP (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.

[74] Collins JA, Bamhart KY, Schlegel PN (2008) Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?. Fertil Steril. 89:823-831.

[75] Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM (2008) 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. 90:352-359.

[76] Spano M, Seli E, Bizzaro D, Manicardi GC, Sakkas D (2005) The significance of sperm nuclear DNA strand break on reproductive outcome. Curr Opin Obstet Gynecol. 17:255-260.

[77] Zini A, Sigman M (2009) Are tests of sperm DNA damage clinically useful? Pros and cons. J Androl. 30:219-229.

[78] Tamburrino L, Marchiani S, Montoya M, Marino FE, Natali I, Cambi M, Forti G, Baldi E, Muratori M (2012) Mechanisms and clinical correlates of sperm DNA damage. Asian J Androl. 14:24-31.

[79] Bungum M, Humaidan P, Spano 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.

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

[81] 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.

[82] Zini A, Jamal W, Cowan L, Al-hathal N (2011) Is sperm DNA damage associated with IVF embryo quality? A systematic review. J Assist Reprod Genet. 28:391-397.

[83] Huszar G, Ozkavukcu S, Jakab A, Celik-Ozenci C, Sati GL, Cayli S (2006) Hyaluronic acid binding ability of human sperm reflects cellular maturity and fertilizing potential: selection of sperm for intracytoplasmic sperm injection. Curr Opin Obstet Gynecol. 18:260-267.

[84] Liu DY, Baker HW (2007) Human sperm bound to the zona pellucida have normal nuclear chromatin as assessed by acridine orange fluorescence. Hum Reprod. 22:1597-1602.