Chapter 22
Sperm DNA and Pregnancy Loss After IVF and ICSI

Nirlipta Swain, Gayatri Mohanty, and Luna Samanta

22.1 Introduction

The relative rate of success of reproduction in humans is extremely low with only 30% of all conceptions resulting in live birth [1]. Assisted reproductive technologies (ART) are the treatment of choice for many couples facing infertility issues, be it due to male or female factor or idiopathic [2, 3]. Every year there is an increase by 4% in the number of couples seeking ART for conception. ART involves procedures like fertility medication, artificial insemination, in vitro fertilization (IVF), micromanipulation of gametes, and surrogacy as well. It is well established that high-quality gametes are required to produce high-quality embryos and that both the sperm and oocyte genomes contribute to the embryonic genome [4]. In contrast to natural selection of the male gametes that occurs during transit in the female genital tracts, in the ART laboratory healthy spermatozoa are selected with routine separation techniques. Despite the advancement in ART during the last 30 years, the rate of pregnancy failures post ART being high (about 70%), thus warrants further improvements [5, 6].

ART bypass the natural selection barrier which would compromise the quality of the fittest sperm selected for fertilization. There is a chance that a normal-looking sperm with abnormal genomic material, which naturally may be incompetent for

N. Swain
School of Life Sciences, Redox Biology Laboratory, Department of Zoology, Ravenshaw University, Cuttack, India

G. Mohanty
Redox Biology Laboratory, Department of Zoology, Ravenshaw University, Cuttack 753003, India

L. Samanta (✉)
Redox Biology Laboratory, Department of Zoology, School of Life Sciences, Ravenshaw University, Cuttack, India
e-mail: lsamanta@ravenshawuniversity.ac.in

A. Zini, A. Agarwal (eds.), *A Clinician's Guide to Sperm DNA and Chromatin Damage*, https://doi.org/10.1007/978-3-319-71815-6_22

impregnation, may still be utilized for ART [7]. In such a scenario, concern over the use of the damaged DNA for artificial fertilization is growing. With the success rate of pregnancy using ART remains comparatively low and somewhat unpredictable, the identification of perfect sperm is an area of active research. Procedures for detection of suitable spermatozoa having strong prognostic power in predicting successful IVF and ICSI outcomes are mostly aimed at techniques for determination of sperm DNA quality. However, the true clinical significance of sperm DNA damage assays remains to be established since the available studies are few and heterogeneous.

Several studies have shown the role of paternal genomic alterations in predicting the success rates of ART [8, 9]. Not only fertilization rates and embryo quality but also subsequent 'embryo viability' and progression of pregnancy would be affected by the status of sperm DNA integrity [10]. When spermatozoa with extensive DNA damage are used, the embryo may fail to develop or implant in the uterus or it may be naturally aborted at a later stage [11]. It could be suggested that functional spermatozoon with intact DNA may have higher chances of successfully delivering a healthy progeny. In this chapter the primary focus is on the role of a fertilizing spermatozoon carrying DNA damage on pregnancy outcome. The ability of DNA tests assessing different aspects of DNA damage, in predicting IVF or ICSI outcome, is discussed with consistent proofs and meta-analysis studies. Moreover, the chapter gives an insight into the late paternal effect and repair capability of oocytes of damaged sperm DNA. In an era where ART are frequently used, study of the influence of sperm DNA damage on embryonic development holds a pivotal role for improvement of success rate.

22.2 DNA Damage and Sperm

DNA damage refers to alterations in the chemical structure of DNA, namely, DNA strand breaks, a base missing from the backbone of DNA (depurination or depyrimidination), and a chemically changed base such as 8-oxoguanine (8-oxoGua), 5-hydroxymethyluracil (5-hmUra), 6-methylguanine, and deaminated cytosine. Most of these changes are attributed to oxidative stress since despite extensive DNA repair oxidatively, damaged DNA are abundant in many human tissues, and these modified bases are potential mutagens [12]. Thus, it will not be out of context to mention that spermatozoa, devoid of substantial cytoplasm, lack effective antioxidants within the cell making them more prone to oxidative DNA damage. When the oxidative DNA damage occurs in the germ cells of the testis, it will result in the production of spermatozoa laden with damaged DNA and/or mutated DNA, and if inseminated with these spermatozoa, the ART outcome will be severely affected.

The aetiology of DNA damage is multifactorial (Fig. 22.1) and categorized as (i) primary (i.e. testicular) or secondary (i.e. environmental) [13]. Single and double DNA strand breaks resulting in abnormal sperm chromatin/DNA structure are thought to arise from four potential sources, namely, (i) strand breaks during

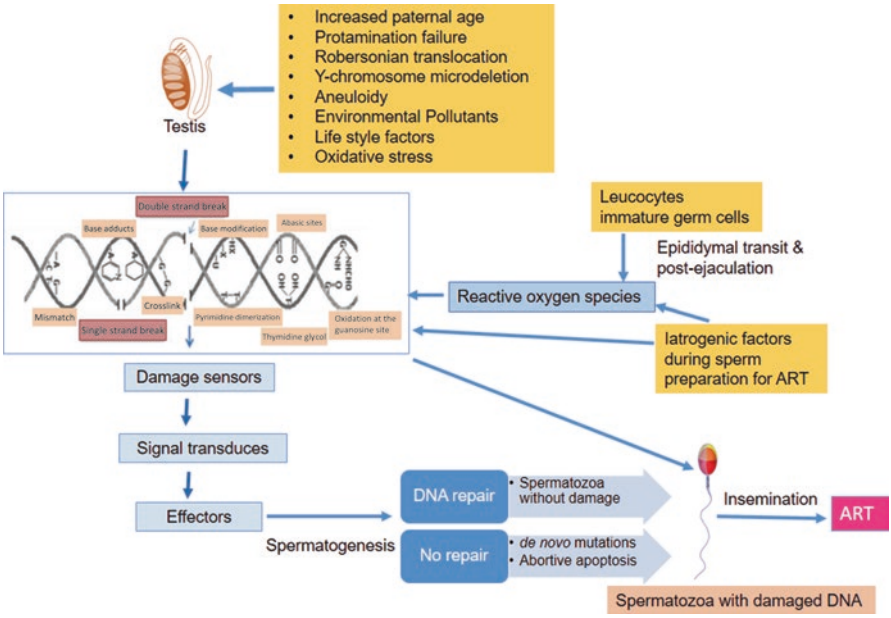

Fig. 22.1 Sources of DNA damage in spermatozoa used in assisted reproductive technologies

chromatin remodelling in the processes of spermiogenesis, (ii) abnormal spermatid maturation (disturbances in protamination), (iii) abortive apoptosis during spermatogenesis, and (iv) oxidative stress [14–16]. Moreover, the damage can be induced and aggravated by exogenous factors like environmental conditions, toxins, pathological diseases [17], and iatrogenic (e.g. ART preparation protocols) [9].

Damaged DNA has been observed in testicular, epididymal, and ejaculated human spermatozoa. Single-strand breaks are a direct result of oxidative damage on sperm DNA, while double-strand breaks may arise from exposure to 4-hydroxyl-2-nonenal, a major product of lipid peroxidation [18]. Two types of DNA adducts, namely, 8-hydroxy-2-deoxyguanosine and two ethenonucleosides (1, N6-ethenoadenosine and 1, N6-ethenoguanosine), are found in human spermatozoa, both of which have been considered key biomarkers of DNA damage caused by oxidative stress [19].

22.3 Effect of Sperm DNA Damage on Fertilization Rate and Embryogenesis

Depending on the level of DNA fragmentation, a sperm may lose its fertilizing ability and developmental potential. Analysis of 170 non-fertilized oocytes from couples attending an IVF programme showed that sperm with a high degree of defective

chromatin packaging would lead to probable sperm DNA decondensation failure in oocytes [20]. Similarly, Sakkas et al. [21] have demonstrated that spermatozoon with a high level of abnormalities in the chromatin when used for ICSI apparently would impede the initiation or completion of decondensation, therefore leading to a failed fertilization. It can be postulated that DNA fragmentation, improper chromatin packaging (protamine deficiency), epigenetic defects, or sperm chromosomal aneuploidies might impair fertilization [22]. However, studies on animal models have shown the association of sperm DNA damage with abnormal embryo development and subsequent impaired implantation. Ahmadi and Ng [23, 24] showed that high sperm DNA fragmentation did not impair fertilization but prohibited the blastocyst formation. They artificially created different levels of DNA damage in sperm by exposing the sample to different doses (5, 10, 50, and 100 GY) of gamma radiation prior to insemination. Fertilization rates (FR) of 64.3, 59.9, 58.5, and 61.1% for the different dosages were seen as compared to 53.2% in the control group, implying that DNA-damaged sperm can fertilize the oocytes at a rate comparable to that of sperm having intact DNA. However, the blastocyst development was decreased from 49.8% in the control group to 20.3, 7.8, 3.4, and 2.3%. Of the transferred blastocysts in the control group, 69.8% were implanted and 33.9% developed into live foetuses. The rates of implantation (57.1 and 21.4%) and live foetuses (20 and 0%) were decreased significantly when spermatozoa were exposed to doses of 5 and 10 GY, respectively. Higher dosages of gamma radiation, resulting in severe DNA damage, reduced blastocyst formation to less than 5%. Furthermore, none of these could reach full term.

Recently, Wdowiak et al. [25] reported that higher sperm genomic damage can also slow down embryo morphokinetic parameters such as attaining the blastocyst stage much later, thus affecting ICSI outcome. Tesarik et al. [26] have reported that with pre-damaged paternal genome, high proportions of zygotes would be formed with abnormal pronuclear morphology. These zygotes would cleave slowly and show extensive fragmentation and blastomere irregularities resulting in arrested growth even before blastocyst formation. An early transcriptional activity of human male pronucleus is essential for early embryonic development. A weak transcriptional activity detected in defective male pronucleus would lead to retarded male pronuclear development in comparison to female pronucleus, thus impairing amphimixis. Furthermore, Speyer et al. [27] postulated that strand breaks in the sperm DNA may not affect early embryo growth but begin to have an effect at the stage of blastocyst development and then have a very marked effect on implantation of the embryo. A late paternal effect [11] has been mainly attributed to anomalies in the organization of the sperm chromatin (i.e. reduced chromatin condensation, chromosome anomalies, and increased DNA strand breaks or fragmentation). The embryonic genome is demonstrated to be activated on day 3 [28], and blastocyst shows the earliest expression of an ‘errant paternal genome’ [29]. A negative effect of high DNA fragmentation index (DFI) on the formation of blastocysts has been reported [30–32]. If critical genes are damaged when the paternal genome is activated at day 3 (four- to eight-cell stage), then sensitive developmental programme of embryo is badly affected [33]. Tesarik et al. [11] have demonstrated that a (late) adverse

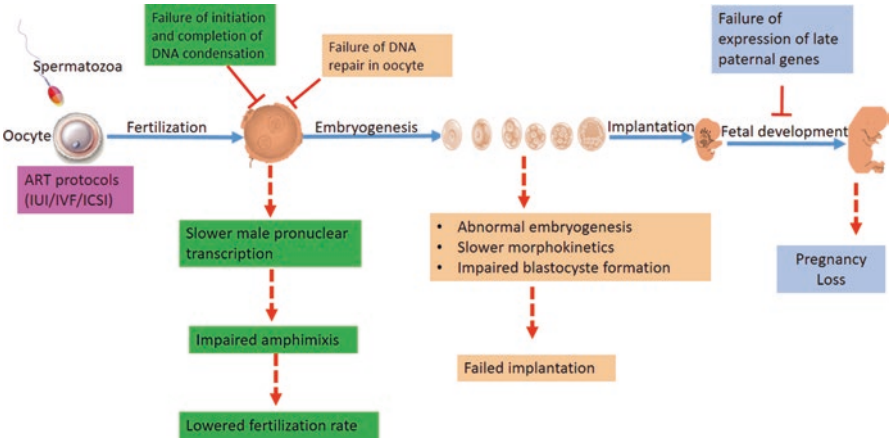

Fig. 22.2 Effect of sperm DNA damage and failure of its repair on different stages of development from fertilization to live birth. Blue solid arrows: normal development; red broken arrows: impaired development leading to pregnancy loss

paternal effect on embryo development can be existent even in the absence of any morphological abnormalities at the zygote stage. Thus, embryos with extensive paternal DNA damage may reach the blastocyst stage. Nevertheless, only those embryos without extensively compromised parental genetic material can progress to full term (Fig. 22.2).

22.4 Failure of DNA Damage Repair by Oocyte: A Confounding Factor

One of the limiting factors in analysing the adverse effect of sperm DNA fragmentation on pregnancy rates following ART is its dependency on both male factors (extent of DNA damage) and female factors (capacity to repair DNA) [34]. This might be the rationale underlying the disparity between the correlations of sperm DNA damage and fertility reported by different studies (Fig. 22.2). Experimental evidence in a number of in vivo and in vitro systems demonstrated the repair ability of vertebrate oocyte of both endogenous and exogenous DNA damage [19]. DNA repair can occur either during or post-fertilization in the oocyte and the developing zygote. Expression of genes and maternal mRNA in human oocytes and blastocysts involved in DNA repair have been detected suggesting the existence of potentially functional DNA repair systems [35, 36]. Ahmadi and Ng [23] suggested that the oocyte repair machinery may not be sufficient to repair DNA damage of sperm >8%. Studies have indicated that implantation of embryos with a normal karyotype may be impaired if there is the presence of unrepaired DNA damage above a critical threshold. Therefore, the varying quality of the oocyte would represent a major

potential confounding variable when making fertility predictions based solely on sperm DNA damage. This favours the use of high-quality oocytes from proven donors as a useful strategy for controlling female factor contribution [37]. The quality and competence of the oocyte especially depends on female age, as the innate capacity to repair sperm DNA damage may be weaker in eggs from older women (>35 years) [38]. Moreover, when DNA damage is extensive, some lesions remain unrepaired or are mis-repaired, and the embryo may fail to develop or implant in the uterus or may be aborted naturally at a later stage (uncompensable damage) [19]. The factors affecting this inadequate repair are female age, ovarian environment, and level of fertility as evident from donated oocytes [34].Therefore, several studies have used young healthy egg donors to obtain embryos and to acknowledge the effect of sperm DNA damage on implantation and pregnancy rates which reduces the variability of associated oocyte quality [37, 39–41].

22.5 Iceberg Effect

The discrepancies between the studies to support the predictive value of sperm DNA damage in ART can also be explained by the 'iceberg effect' [9, 42]. The first level of iceberg corresponded to easily detectable sperm cells with high sperm DNA fragmentation (SDF), using current available technologies, while the second level includes the sperm with undetectable, cryptic SDF within the population with a high possibility that this cryptic population would contain sufficient DNA damage to have a detrimental effect on embryonic development, especially if the oocyte is not capable of DNA repair. The bottom layer of the 'iceberg' model represents the spermatozoa with minimal damage; however, current methodologies are difficult to isolate them from the rest. Gosalvez [37] proposed a strong correlation between spermatozoa found in the tip of the 'iceberg' and the proportion of spermatozoa in level 2 under the surface. For example, it is possible that a patient may have a high underlying undetectable population of sperm with a predisposition for DNA damage but has a low detectable level of DFI. On the other hand, a patient might have a low underlying subpopulation of sperm with a predisposition for DNA damage but may have a high detectable level of DFI. The situation may also exist where a similar detectable level of SDF is present in two individuals, but differences in the underlying undetectable population are present. Therefore, the variability in the amount, quality, and distribution of DNA damage among the different spermatozoa in the ejaculate explains the possibility of successful pregnancies despite a high DFI in sperm [43]. Besides DNA damage, protamination failure is another compounding factor leading to defective chromatin condensation affecting FR and embryo development. In spontaneous recurrent pregnancy loss, the number of spermatozoa having intermediate acidic aniline blue staining were significantly higher than their fertile counterparts [44], suggesting that spermatozoa with intermediate defect are equally responsible for successful pregnancy.

22.6 The ART Protocol and Sperm DNA Damage

The ART procedures involve extensive sperm handling and processing that increase the potential risk of damaging paternal DNA material (Table 22.1). These procedures utilize sperm sorting methods (swim-up and density-gradient centrifugation) to select viable sperm from the semen. These methods use multiple centrifugation steps, which have been shown to generate reactive oxygen species (ROS) affecting DNA integrity. Exposure to artificial media and light during ART protocols are also examples of non-natural environment that has no equivalent when fertilization is natural. As spermatozoa get exposed to conditions that are contrary to physiological state, it can be hypothesized that all these procedures could damage sperm DNA. However, studies have found that the percentage of spermatozoa with fragmented DNA and the degree of fragmentation within these cells in prepared spermatozoa are significantly less than in neat semen [45–48]. Sperm preparation can enrich the sperm population by eliminating defective sperm with nicked DNA and poorly condensed chromatin, which is likely to improve the chances of achieving a viable pregnancy [49–51]. Moreover Zini et al. [52] reported that in comparison to density-gradient centrifugation, spermatozoa recovered after swim-up possess higher DNA integrity. But Hammadeh et al. [53] observed that the fertilization, implantation, and pregnancy rates were similar in both semen preparation methods. Moreover, arguments were put forth to justify the effectiveness of DFI in neat semen as better predictors of pregnancy outcome post ART as compared to DFI in processed semen [54]. Tomlinson et al. [55] propose the 'normalizing' effect of density-gradient preparations as the reason for the little prognostic value of DFI in processed semen. Nevertheless, if the DFI is high in both neat and processed semen, both fertilization rate and embryo quality are adversely affected [48, 56]. It is presumed that advanced techniques (motile sperm organelle morphology examination: MSOME) [57], electrophoresis [58], microfluidics [59], zeta potential [60, 61], and birefringence [62]) that eliminate the centrifugation steps of conventional sperm preparation (Table 22.1) may improve the selection of sperm with higher DNA integrity, normal morphology, and motility resulting in improved ART outcomes (as reviewed by Rappa et al. [63]).

22.7 In Vitro Fertilization and Pregnancy Loss

Conventional IVF involves ovarian hyperstimulation to generate and collect multiple eggs, preparation and co-incubation of gametes, and fertilization, culture, and selection of resultant embryos before embryo transfer into a uterus. In congruence to natural conception, IVF allows naturally selected best sperm to compete and reach the oocyte in artificial media unlike ICSI [64]. Studies have reported a significant adverse effect of defective DNA structural integrity and breakage on different parameters of reproductive outcome post IVF (Table 22.2).

Table 22.1 Effect of sperm processing in ART on DNA integrity

Sperm processing technique	Outcome	Limitations	References
Conventional swim-up Direct swim-up/ density-gradient centrifugation	Mean numbers and percentage of structurally normal spermatozoa with less DNA damage can be selected by swim-up	Immature sperm with elliptical or roundish nuclei, distorted acrosomes, and uncondensed chromatin remain part of fertilizing pool	[50]
	Density-gradient-prepared spermatozoa have significantly less DNA damage than in neat semen ($P < 0.005$)	Generated ROS during processing may cause sperm DNA damage	[45, 46]
	Both PureSperm® and Percoll® density-gradient-prepared spermatozoa have less nicked DNA than swim-up preparation	–	[49]
Zeta potential	Larger percentages of mature sperm, intact DNA, strict normal morphology, hyperactivation, and progressive motility	Recovery rate only 8.8% Not suitable for oligozoospermic samples	[60, 61]
Magnetic-assisted cell sorting (MACS) system	Selects higher proportion of sperm with normal protamine content and lesser DNA fragmentation	–	[61]
Electrophoresis (microflow)	Less oxidative DNA damage due to decrease in exposure to ROS	–	[58]
Motile sperm organellar morphology examination (MSOME)	Sperm with more than 50% vacuolated nuclei are associated with DNA fragmentation	Incubation of sperm for longer time compromises quality	[57]
Microfluidics	In comparison to swim-up, a microfluidic device resulted in a significantly lower rate of DNA damage (16.4% swim-up vs. 8.4% MF)	–	[59]
Birefringence	Partial birefringence had a significant lower proportion of DNA fragmentation compared to total birefringence (7.3% vs. 19.5%)	–	[62]

Table 22.2 Effect of sperm DNA damage on IVF outcome

DFI assay undertaken	Study population	Sample size	Results after IVF	References
TUNEL	Canada	298	Fertilization failure	[65]
	France	111		[64]
	Italy	82		[71]
	Denmark	50		[56]
	Australia	45		[66]
Alkaline COMET	Ireland	73		[48]
SCD	China	136		[70]
	Slovenia(Europe)	113		[69]
AOT	China	302		[65]
	South Africa	76		[74]
CA3	South Africa	72		[20]
TUNEL	USA	49	Impaired blastocyst formation	[31]
SCSA	South Dakota	63		[30]
TUNEL	Germany	249	Lower pregnancy rates	[77]
COMET	Ireland	203		[79]
	England	40		[80]
SCD	Spain	152		[78]
	Croatia (Europe)	88		[64]
NT and CA3	England	140		[55]

22.7.1 Fertilization Rate

Studies have shown that high DFI may impair FR in IVF procedures, resulting in poor embryo quality (EQ) and higher pregnancy loss (PL). A negative correlation between the percentage of sperm with high DFI (TUNEL assay) and FR in couples undergoing IVF was reported [65–67]. It was proposed that FR was more likely to be adversely affected by high DNA damage in a sample with abnormal chromatin packaging [68, 69]. Studies have shown a negative correlation between FR and sperm chromatin defects (as detected by staining methods such as chromomycin A3 [70, 71] or ethidium bromide [72]). Moreover, underprotamination would also adversely affect the FR [73]. A higher level of intact DNA with an acridine orange test (AOT) score of >24% results in a better FR [74]. Similarly Liu et al. [75] reported that the percentage of sperm bound to zona pellucida had low amounts of DNA damage and good IVF rates.

22.7.2 Pregnancy and Live-Birth Rates

Several studies have shown that a compromised sperm DNA would reduce the chances of positive pregnancy outcome. The blastocyst formation rate is significantly lower in couples with severely impaired sperm DNA [76]. Cut-off scores as determined by different DFI analysis assays could be associated with higher probability of IVF failure. Cut-off DFI values as measured by microscopy-based TUNEL were reported to be 20% [31], FACS-based TUNEL to be 36.5% [77], SCSA to be 30% [30], and SCD to be 25.5% [78]. Similarly, couples with sperm DFI >50% (Comet assay) had 13% live-birth rate, while sperm DFI <25% had a live-birth rate of 33% [79]. Another study has shown that both Comet head DNA damage and tail damage can be used as good predictors of successful pregnancy or failure [80]. Recently Tandara et al. [64] argued the suitability of measuring the percentage of spermatozoa with undamaged DNA as better prognostic parameter of embryo quality and pregnancy achieved by conventional IVF rather than DFI. Samples with AOT score of ≥12% [77] and big halo % of >38% have lower blastocyst rates and pregnancy failure.

22.8 ICSI and Pregnancy Loss

In intra-cytoplasmic sperm injection (ICSI), a single sperm is selected and injected directly into oocyte. The results of a meta-analysis support the use of ICSI over IVF in men with high sperm DNA fragmentation [81]. The rationale advocated that ICSI involves selection of morphologically normal motile sperm which is believed to have lower DNA fragmentation. The stratified analysis by type of procedure (IVF vs. ICSI) revealed that sperm with high DNA damage have higher pregnancy rate in ICSI, while the rate of miscarriage is similar in both IVF and ICSI [38]. Table 22.3 summarizes the studies correlating sperm DNA integrity and ICSI outcome.

Table 22.3 Effect of sperm DNA damage on ICSI outcome at different stages

DFI assay	Study population	Sample size	Results after ICSI	References
TUNEL	Canada	150	Lowered fertilization rate	[82]
	France	54		[32]
SCD	Iran	92		[22]
AOT	Turkey	56		[83]
TUNEL	Italy	50	Lowered pregnancy rate	[84]
	Virginia	36		[33]
SCSA	Poland	60		[85]
AOT	Italy	50		[86]
FISH	Italy	48		[89]
	Spain	19		[87]
	Italy	18		[88]
	USA	9		[90]

22.8.1 Pregnancy Rate

Paternal genomic alterations may compromise not only fertilization and embryo quality [82, 83] but also 'embryo viability' and progression of pregnancy, resulting in spontaneous miscarriage. Avendano et al. [33] investigated the percentage of morphologically normal sperm with fragmented DNA and observed a negative association with mean embryo score. The study showed that when the percentage of normal sperm DNA fragmentation was ≤17.6%, the likelihood of pregnancy was 3.5 times higher. Another study reported a threshold TUNEL score of 20% as cut-off for miscarriage [84]. Similarly, a reduction in pregnancy rates was observed with samples having DFI of 23% as determined by SCSA [85]. Dar et al. [86] found a close relationship between DNA fragmentation and post-implantation development in ICSI by comparing the miscarriage rates between two groups with low DFI (<15%) and with high DFI (>50%). The study detected a trend toward a higher miscarriage rate in high DFI group.

Couples with a clinical background of recurrent miscarriages of unknown aetiology or implantation failure after ICSI were also characterized for abnormal sperm aneuploidy by FISH [87]. Higher rates of miscarriage were obtained in patients with abnormal sperm FISH results. Calogero et al. [88] reported that unselected patients undergoing ICSI had an elevated sperm aneuploidy rate related to subsequent pregnancy failure. Similarly, Burrello et al. [89] focussed on role of sperm aneuploidy on ICSI outcome in patients with male factor infertility. Taking a cut-off value of aneuploidy as >1.55%, lower pregnancy and implantation rates were observed. Targeting the recurrent miscarriages post ICSI in oligoasthenozoospermic sample, FISH, using directly labelled (fluorochrome-dUTP) satellite or contig DNA probes specific for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X, and Y, was performed on decondensed spermatozoa [90]. Significantly elevated frequencies of diploidy, autosomal disomy and nullisomy, sex chromosome aneuploidy, and total aneuploidy in these patients suggest increased risk of abortion.

22.9 Effect of DNA Damage on Outcome of IVF and ICSI: A Comparison

In patients with poor spermiogram, ICSI is the treatment of choice, as it is assumed that DFI has a minimal effect on ICSI outcome [91]. However, the chances of selecting a normal sperm with fragmented DNA for oocyte injection put every ICSI cycle at high risk. Thus it warrants finding a threshold value of DFI which determines the type of ART treatment to be chosen. Nonetheless, the heterogeneity of the threshold value set among the various studies is due to difference in methods used for sperm preparation and assessment of DNA damage.

Sergerie et al. [92] proposed a pathological DFI threshold of 20% (TUNEL) for fertility status in vivo; however, a threshold for IVF and ICSI failures is controversial.

Taking 15% as TUNEL threshold score, the risk of non-transfer due to blocked embryo development increased, more so for ICSI (18.2%) than that for IVF (4.2%) with an odds ratio of 5.05 [93]. Similarly, the miscarriage risk increased fourfold (37.5% for ICSI vs. 8.8% for IVF) with ≥15% DFI. The threshold DFI (SCSA) for better pregnancy rates is higher in case of IVF (≤ 30%), in comparison to ICSI (≤19%) [27]. Bungum et al. [94] found that in the patients with the SCSA score >30%, the pregnancy results of ICSI were significantly better than those of IVF. It is advocated that in vitro ART is able to bypass the impairment of sperm chromatin, in particular if ICSI is chosen as a fertilization method. So, when DFI exceeded a level of 30%, ICSI is a more efficient treatment option. However, another study reported a DFI value of 30% (AOT) as threshold for decreasing FR, cleavage, implantation, and pregnancy in ICSI cycles but not significantly in IVF cycles [95]. The biological rationale explained is that in IVF mostly sperm with higher DNA integrity are naturally selected for oocyte penetration and fertilization [96].

Irrespective of ART technique used, FR were affected when TUNEL score was >10% [97]. The effect on FR was more pronounced in IVF than ICSI. Similarly, above a threshold SCD score of 18%, EQ and FR can also be adversely affected [98]. Muriel et al. [10] proposed that higher DNA fragmentation determined by SCD would produce an increased proportion of zygotes showing asynchrony between the nucleolar precursor bodies of zygote pronuclei (73.8% vs. 28.8% $P < 0.001$). Moreover, slower embryo development and reduced implantation rate in IVF/ICSI were also associated with higher sperm DNA fragmentation. Similarly, no patients achieved clinical pregnancy after ART, if SCSA values exceeded 27% ($P < 0.01$) [99]. Henkel et al. [100] observed a significantly reduced pregnancy rate in both IVF and ICSI patients inseminated with TUNEL-positive spermatozoa. It could be inferred here that although patients may be able to conceive via ART, sperm DNA damage might be a limiting factor, and severe damage would lead to increase in abortion rates [101].

22.10 Inference Drawn from Meta-analysis Studies

Evenson et al. [9] carried out comparative meta-analyses taking 17 studies to compare the effect of sperm DNA damage on pregnancy outcome after IVF and ICSI. Using the Cochran–Mantel–Haenszel (CMH) statistic, the meta-analysis results found that patients using in vivo fertilization were 7.0 times (CI 3.17, 17.7) more likely to achieve a pregnancy/delivery if the DFI was <30% (n = 362, P = 0.0001). In comparison, infertile couples were approximately 2.0 times (CI 1.02, 2.84) more likely to become pregnant with IVF treatment if their DFI was <30% (n = 381, P = 0.03). For ICSI, the results indicated 1.6 times (CI 0.92, 2.94) higher possibility to achieve a pregnancy/delivery if the DFI was <30% (n = 323, P = 0.06). A MEDLINE and bibliographic search (from Jan 1978 to Apr 2006) resulted in selection of eight articles based on inclusion/exclusion criteria [102]. RevMan software was used, and the relative likelihood of DNA damage effect on

IVF/ICSI outcomes was expressed as a risk ratio (RR). About five studies which measured DFI by TUNEL assay revealed that there is 32% (CI 0.54–0.85, $P = 0.0006$) and 24% (CI 0.55–1.04, $P = 0.09$) reduction, respectively, in the odds of having pregnancy for IVF and ICSI patients ($n = 816$), with high degree of sperm DNA damage compared with those with low degree of sperm DNA damage. However, 3 studies that used the SCSA assay and took 299 subjects indicated there are no significant effects of sperm DNA damage on the clinical pregnancy rate after IVF (RR 0.58, 95% CI 0.25–1.31, $P = 0.19$) or ICSI (RR 1.18, 95% CI 0.81–1.74, $P = 0.38$). Thus, the above study favours TUNEL assay over SCSA as a better predictor of ART outcome.

Zini et al. [8] carried out meta-analysis looking at 11 studies that involve 1549 cycles of treatment (808 IVF and 741 ICSI) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses. Six (6) studies measured DFI using SCSA and estimated OR of 1.77 for pregnancy loss (95% CI, 1.01–3.13; $P = 0.05$); five studies measured DFI by TUNEL and estimated OR of 7.04 (95% CI, 2.81–17.67; $P = 0.001$). The fixed effects model combined OR of 2.48 (95% CI 1.52–4.04, $P < 0.0001$) indicates that sperm DNA damage is predictive of pregnancy loss after IVF and ICSI. Similarly another systemic review and meta-analysis were carried out on 16 cohort studies (2969 couples), 14 of which were prospective [103]. Searches were conducted on MEDLINE, EMBASE, and the Cochrane Library from database inception to January 2012. Meta-analysis of relative risks of miscarriage was performed with a random effects model. A cumulative risk ratio of 2.16 (1.54–3.03; $P = 0.00001$) indicates a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage. About six studies used TUNEL assay and had the strongest miscarriage association (RR = 3.94, 95% CI 2.45–6.32; $P < 0.00001$). While the summary RR estimate of studies using SCSA (six studies) was 3.94 (95% CI, 2.45–6.32; $P = 0.00001$), using the Comet assay (two studies) was 1.43 (95% CI, 0.4–5.14; $P = 0.58$), and using the AOT assay (one study) was 2.78 (95% CI, 0.59–13.11; $P = 0.20$). A subgroup analysis showed a strong association of the prepared semen with high DNA damage and miscarriage (RR = 3.47, 95% CI: 2.13 t–5.63; $P = 0.00001$) than the raw semen group (RR = 1.50, 95% CI: 1.11–2.01; $P = 0.007$).

An exhaustive electronic literature search from database inception to October 2013 included 16 cohort studies (3106 couples) and examined the influence of sperm DNA damage on pregnancy and miscarriage following IVF/ICSI [38]. A meta-analysis showed that high-level sperm DNA fragmentation is detrimental to IVF/ICSI outcome, with decreased pregnancy rate (OR = 0.81, 95% CI:0.70–0.95; $P = 0.008$) and increased miscarriage rate (OR = 2.28, 95%CI:1.55–3.35; $P < 0.0001$). The stratified analysis by type of procedure (IVF vs. ICSI) indicated that high sperm DNA damage was related to lower pregnancy rates in IVF with OR of 0.66 (95% CI: 0.48–0.90; $P = 0.008$) but not in ICSI cycles, whereas it was significantly associated with higher miscarriage rates in ICSI cycles (OR 2.68; 95% CI:1.40–5.14; $P = 0.003$). Furthermore, the study also observes significant OR when DFI was measured by TUNEL as compared to SCSA. Osman et al. [81] conducted a meta-analysis of six studies to evaluate the relationship between the extent

of sperm DNA damage and live-birth rate (LBR) per couple. Overall, they found a significant increase in LBR (RR 1.17, 95% CI 1.07–1.28; $P = 0.0005$) in couples with low sperm DNA fragmentation compared to those with high sperm DNA fragmentation. After IVF and ICSI, men with low sperm DNA fragmentation had significantly higher LBR (RR 1.27, 95% CI 1.05–1.52; $P = 0.01$) and (RR 1.11, 95% CI 1.00–1.23, $P = 0.04$), respectively. A sensitivity analysis observed no statistically significant difference in LBR between low and high sperm DNA fragmentation when ICSI treatment was used (RR 1.08, 95% CI 0.39–2.96; $P = 0.88$). High sperm DNA fragmentation in couples undergoing ART is associated with lower LBR. The most recent and extensive meta-analytical report [104] identified 41 articles (with a total of 56 studies) including 16 IVF studies, 24 ICSI studies, and 16 mixed (IVF + ICSI) studies. These studies measured DNA damage (by one of four assays: 23 SCSA, 18 TUNEL, 8 SCD, and 7 Comet) and included a total of 8068 treatment cycles (3734 IVF, 2282 ICSI, and 2052 mixed IVF + ICSI). The combined OR of 1.68 (95% CI: 1.49–1.89; $P < 0.0001$) indicates that sperm DNA damage affects clinical pregnancy following IVF and/or ICSI treatment. In addition, the combined OR estimates of IVF (16 estimates, OR = 1.65; 95% CI: 1.34–2.04; $P < 0.0001$), ICSI (24 estimates, OR = 1.31; 95% CI: 1.08–1.59; $P = 0.0068$), and mixed IVF + ICSI studies (16 estimates, OR = 2.37; 95% CI: 1.89–2.97; $P < 0.0001$) were also statistically significant. Moreover, a strong negative association was observed between sperm DNA damage and clinical pregnancy (with a statistically significant combined OR estimate) utilizing assays that measure sperm DNA damage directly (TUNEL and Comet assays) than those measured indirectly (SCSA and SCD assay).

Contrary to the above studies, a systematic review and meta-analysis [105] evaluated 13 relevant studies with 18 estimates of the diagnostic test properties of sperm DNA integrity tests in 2162 cycles of treatment. The summary diagnostic OR was 1.44 (95% CI, 1.03, 2.03), but the likelihood ratios (LR) were not predictive of pregnancy outcome (LR+ = 1.23; 95% CI, 0.98, 1.54; LR− = 0.81; 95%CI, 0.67, 0.98). Thus, the above meta-analysis shows that neither SCSA, NT, nor TUNEL was predictive of IVF/ICSI outcome. Recently, Zhang et al. [106] included about 20 studies for a meta-analysis and proposed that infertile couples were more likely to get pregnant if DFI was less than threshold value (i.e. >27% and 15–27% group, combined overall OR (95% CI) = 1.437 (1.186–1.742), 1.639 (1.093–2.459) respectively). However, the predication value of DFI for IVF or ICSI outcome could not be confirmed.

22.11 Making the Right Choice

With a handful of DNA assessment assays with different levels of efficacy to identify sperm DNA damage, both the patient and physician can be frustrated. It is still noteworthy to mention that irrespective of the low predictive power of sperm DNA testing, clinicians counsel their patients depending upon the knowledge gained through several clinical trials. That is, for couples planning their first pregnancy, test

of sperm DNA damage (especially SCSA) is a good predictor of negative pregnancy outcome. The level of DNA fragmentation would help them to know their potential for natural fertility and opt for ART if needed [7]. Moreover, if evaluated in men before ART, sperm DNA abnormalities would likely identify the cause of infertility in a large percentage of patients. DNA tests like SCSA would help the infertile couples to go for intrauterine insemination (IUI) as the first-line treatment for unexplained infertility [96]. However, if the male partner has high levels of sperm DNA damage, the couples should consider advanced forms of assisted reproduction (IVF or ICSI) to achieve a pregnancy. Moreover, couples facing recurrent miscarriages post ART should be advised to check their sperm DNA integrity. When high DFI is detected (>30%), ICSI using testicular spermatozoa was an effective option particularly for those with repeated ART failures in terms of clinical, ongoing pregnancies and miscarriages even though conventional sperm parameters are within normal range. Recently, Pabuccu et al. [107] took normozoospermic subjects with high sperm DFI facing previous ART failures. They studied the pregnancy rates of testicular aspirated sperm (TESA sample) vs. ejaculated spermatozoa (EJ) in those subjects. They found that clinical (41.9% versus 20%) and ongoing pregnancy rates (38.7% versus 15%) were significantly better and miscarriages were lower in TESA group when compared to EJ group. The authors recommended sperm DFI to be a part of male partner's evaluation following unsuccessful ART attempts.

22.12 Conclusion

Conventional semen parameters remain the epitome for assessment of the fertility potential in males opting for ART. However, their utility in predicting reproductive success is questionable. In contrast, sperm DNA damage has been associated with a significantly increased risk of pregnancy loss post IVF and ICSI, as evidenced from the documented literature. Ambiguity over the influence of female factors can be minimized by ovum donation. Despite our limited knowledge about the possible mechanisms involved in miscarriage caused by DNA damage, the contribution of the paternal genome in miscarriages cannot be underestimated. Studies have shown that when the paternal genes are 'switched on', the deleterious consequences of fragmented paternal DNA became evident pausing further embryonic development.

Moreover, the ability of the oocyte to repair DNA damage in the fertilizing spermatozoon is going to depend not only on the severity but also on the type of damage. In general, single-stranded DNA damage is easier to repair than double-stranded DNA damage [19]. The failure of meta-analysis interpretations to address the controversial association between DFI and ART outcome could be based on the rationale that mostly the assays for sperm DNA fragmentation were performed on raw semen samples. These samples would contain a high percentage of immotile, nonviable, or degenerated sperm with abnormal chromatin. On the contrary, the procedures followed for sperm preparation may not directly affect the integrity of the DNA but increase the susceptibility of the DNA to damage. The significant

limitations (methodological and design weakness) of the sperm DNA studies warrant further research on the predictive value of sperm DNA fragmentation on pregnancy outcomes after ART.

References

1. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update. 2002;8(4):333–43.
2. Swain N, et al. Proteomics and male infertility, in proteomics in human reproduction: biomarkers for millennials. Cham: Springer International Publishing; 2016. p. 21–43.
3. Swain N, Cirenza C, Samanta L. Role of proteomics in female infertility, in proteomics in human reproduction: biomarkers for millennials. Cham: Springer International Publishing; 2016. p. 45–63.
4. Marteil G, Richard-Parpaillon L, Kubiak JZ. Role of oocyte quality in meiotic maturation and embryonic development. Reprod Biol. 2009;9(3):203–24.
5. Coughlan C, et al. Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. Asian J Androl. 2015;17(4):681–5.
6. Bach PV, Schlegel PN. Sperm DNA damage and its role in IVF and ICSI. Basic Clin Androl. 2016;26:15.
7. Zini A, Libman J. Sperm DNA damage: clinical significance in the era of assisted reproduction. CMAJ. 2006;175(5):495–500.
8. Zini A, et al. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2008;23(12):2663–8.
9. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online. 2006;12(4):466–72.
10. Muriel L, et al. 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. 2006;85(2):371–83.
11. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod. 2004;19(3):611–5.
12. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis. 2004;19(3):169–85.
13. Zini A, Sigman M. Are tests of sperm DNA damage clinically useful? Pros and cons. J Androl. 2009;30(3):219–29.
14. Irvine DS, et al. DNA integrity in human spermatozoa: relationships with semen quality. J Androl. 2000;21(1):33–44.
15. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010;93(4):1027–36.
16. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod. 2010;16(1):3–13.
17. Evenson D. Reply to: 'The predictive value of the sperm chromatin structure assay (SCSA)' – a response from the SCSA inventor. Hum Reprod. 2006;21(2):570–2.
18. Samanta L, Mohanty G, Agarwal A. Male factors in recurrent pregnancy loss, in recurrent pregnancy loss. New York: Springer; 2016. p. 109–29.
19. Gonzalez-Marin C, Gosalvez J, Roy R. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci. 2012;13(11):14026–52.
20. Esterhuizen AD, et al. Defective sperm decondensation: a cause for fertilization failure. Andrologia. 2002;34(1):1–7.
21. Sakkas D, et al. Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. Hum Reprod. 1996;11(4):837–43.

22. Tavalaee M, Razavi S, Nasr-Esfahani MH. Influence of sperm chromatin anomalies on assisted reproductive technology outcome. Fertil Steril. 2009;91(4):1119–26.
23. Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool. 1999;284(6):696–704.
24. Ahmadi A, Ng SC. Developmental capacity of damaged spermatozoa. Hum Reprod. 1999;14(9):2279–85.
25. Wdowiak A, Bakalczuk S, Bakalczuk G. The effect of sperm DNA fragmentation on the dynamics of the embryonic development in intracytoplasmatic sperm injection. Reprod Biol. 2015;15(2):94–100.
26. Tesarik J, Mendoza C, Greco E. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum Reprod. 2002;17(1):184–9.
27. Speyer BE, et al. Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Hum Reprod. 2010;25(7):1609–18.
28. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four-and eight-cell stages of preimplantation development. Nature. 1988;332(6163):459–61.
29. Banerjee S, et al. Does blastocyst culture eliminate paternal chromosomal defects and select good embryos?: inheritance of an abnormal paternal genome following ICSI. Hum Reprod. 2000;15(12):2455–9.
30. Virro MR, Larson-Cook KL, Evenson DP. 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. 2004;81(5):1289–95.
31. Seli E, et al. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril. 2004;82(2):378–83.
32. Benchaib M, et al. Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique. Hum Reprod. 2003;18(5):1023–8.
33. Avendano C, et al. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril. 2010;94(2):549–57.
34. Marchetti F, et al. Disruption of maternal DNA repair increases sperm-derived chromosomal aberrations. Proc Natl Acad Sci U S A. 2007;104(45):17725–9.
35. Menezo Y Jr, et al. Expression profile of genes coding for DNA repair in human oocytes using pangenomic microarrays, with a special focus on ROS linked decays. J Assist Reprod Genet. 2007;24(11):513–20.
36. Jaroudi S, et al. Expression profiling of DNA repair genes in human oocytes and blastocysts using microarrays. Hum Reprod. 2009;24(10):2649–55.
37. Gosalvez J, et al. Can DNA fragmentation of neat or swim-up spermatozoa be used to predict pregnancy following ICSI of fertile oocyte donors? Asian J Androl. 2013;15(6):812–8.
38. Zhao J, et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril. 2014;102(4):998–1005. e8
39. Esbert M, et al. Impact of sperm DNA fragmentation on the outcome of IVF with own or donated oocytes. Reprod Biomed Online. 2011;23(6):704–10.
40. Meseguer M, et al. Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertil Steril. 2011;95(1):124–8.
41. Nunez-Calonge R, et al. An improved experimental model for understanding the impact of sperm DNA fragmentation on human pregnancy following ICSI. Reprod Sci. 2012;19(11):1163–8.
42. Alvarez JG. The predictive value of sperm chromatin structure assay. Hum Reprod. 2005;20(8):2365–7.
43. Payne JF, et al. Redefining the relationship between sperm deoxyribonucleic acid fragmentation as measured by the sperm chromatin structure assay and outcomes of assisted reproductive techniques. Fertil Steril. 2005;84(2):356–64.
44. Mohanty G, et al. Histone retention, protein carbonylation, and lipid peroxidation in spermatozoa: possible role in recurrent pregnancy loss. Syst Biol Reprod Med. 2016;62(3):201–12.

45. Donnelly ET, et al. Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. Hum Reprod. 2000;15(7):1552–61.
46. Morrell JM, et al. Reduced senescence and retained nuclear DNA integrity in human spermatozoa prepared by density gradient centrifugation. J Assist Reprod Genet. 2004;21(6):217–22.
47. Caglar GS, et al. Semen DNA fragmentation index, evaluated with both TUNEL and Comet assay, and the ICSI outcome. In Vivo. 2007;21(6):1075–80.
48. Simon L, et al. Sperm DNA damage measured by the alkaline Comet assay as an independent predictor of male infertility and in vitro fertilization success. Fertil Steril. 2011;95(2):652–7.
49. Sakkas D, et al. The use of two density gradient centrifugation techniques and the swim-up method to separate spermatozoa with chromatin and nuclear DNA anomalies. Hum Reprod. 2000;15(5):1112–6.
50. Piomboni P, et al. Ultrastructural and DNA fragmentation analyses in swim-up selected human sperm. Arch Androl. 2006;52(1):51–9.
51. Jayaraman V, et al. Sperm processing by swim-up and density gradient is effective in elimination of sperm with DNA damage. J Assist Reprod Genet. 2012;29(6):557–63.
52. Zini A, et al. Influence of semen processing technique on human sperm DNA integrity. Urology. 2000;56(6):1081–4.
53. Hammadeh ME, et al. Comparison of sperm preparation methods: effect on chromatin and morphology recovery rates and their consequences on the clinical outcome after in vitro fertilization embryo transfer. Int J Androl. 2001;24(6):360–8.
54. Bungum M, et al. Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive for the outcome of ART. Hum Reprod. 2008;23(1):4–10.
55. Tomlinson MJ, et al. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod. 2001;16(10):2160–5.
56. Host E, Lindenberg S, Smidt-Jensen S. The role of DNA strand breaks in human spermatozoa used for IVF and ICSI. Acta Obstet Gynecol Scand. 2000;79(7):559–63.
57. Oliveira JB, et al. Correlation between semen analysis by motile sperm organelle morphology examination and sperm DNA damage. Fertil Steril. 2010;94(5):1937–40.
58. Aitken RJ, Hanson AR, Kuczera L. Electrophoretic sperm isolation: optimization of electrophoresis conditions and impact on oxidative stress. Hum Reprod. 2011;26(8):1955–64.
59. Wang W, et al. Effects of a microfluidic sperm sorter on sperm routine parameters and DNA integrity. Zhonghua Nan Ke Xue. 2011;17(4):301–4.
60. Kheirollahi-Kouhestani M, et al. Selection of sperm based on combined density gradient and Zeta method may improve ICSI outcome. Hum Reprod. 2009;24(10):2409–16.
61. Zahedi A, et al. Zeta potential vs apoptotic marker: which is more suitable for ICSI sperm selection? J Assist Reprod Genet. 2013;30(9):1181–6.
62. Petersen CG, et al. Relationship between DNA damage and sperm head birefringence. Reprod Biomed Online. 2011;22(6):583–9.
63. Rappa KL, et al. Sperm processing for advanced reproductive technologies: where are we today? Biotechnol Adv. 2016;34(5):578–87.
64. Tandara M, et al. Sperm DNA integrity testing: big halo is a good predictor of embryo quality and pregnancy after conventional IVF. Andrology. 2014;2(5):678–86.
65. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod. 1997;56(3):602–7.
66. Bakos HW, et al. Sperm DNA damage is associated with assisted reproductive technology pregnancy. Int J Androl. 2008;31(5):518–26.
67. Marchetti C, et al. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum Reprod. 2002;17(5):1257–65.
68. Zhang X, et al. Localization of single-stranded DNA in human sperm nuclei. Fertil Steril. 2007;88(5):1334–8.
69. Pregl Breznik B, Kovacic B, Vlaisavljevic V. Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil Steril. 2013;99(5):1233–41.

70. Gu LJ, et al. Sperm chromatin anomalies have an adverse effect on the outcome of conventional in vitro fertilization: a study with strictly controlled external factors. Fertil Steril. 2009;92(4):1344–6.
71. Tarozzi N, et al. Anomalies in sperm chromatin packaging: implications for assisted reproduction techniques. Reprod Biomed Online. 2009;18(4):486–95.
72. Filatov MV, et al. Relationship between abnormal sperm chromatin packing and IVF results. Mol Hum Reprod. 1999;5(9):825–30.
73. Haidl G, Schill WB. Assessment of sperm chromatin condensation: an important test for prediction of IVF outcome. Arch Androl. 1994;32(3):263–6.
74. Claassens OE, et al. The Acridine Orange test: determining the relationship between sperm morphology and fertilization in vitro. Hum Reprod. 1992;7(2):242–7.
75. Liu DY, Baker HW. Sperm nuclear chromatin normality: relationship with sperm morphology, sperm-zona pellucida binding, and fertilization rates in vitro. Fertil Steril. 1992;58(6):1178–84.
76. Janny L, Menezo YJ. Evidence for a strong paternal effect on human preimplantation embryo development and blastocyst formation. Mol Reprod Dev. 1994;38(1):36–42.
77. Henkel R, et al. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril. 2004;81(4):965–72.
78. Lopez G, et al. Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment. Asian J Androl. 2013;15(6):790–4.
79. Simon L, et al. Sperm DNA damage has a negative association with live-birth rates after IVF. Reprod Biomed Online. 2013;26(1):68–78.
80. Tomsu M, Sharma V, Miller D. Embryo quality and IVF treatment outcomes may correlate with different sperm comet assay parameters. Hum Reprod. 2002;17(7):1856–62.
81. Osman A, et al. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online. 2015;30(2):120–7.
82. Lopes S, et al. Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertil Steril. 1998;69(3):528–32.
83. Cebesoy FB, Aydos K, Unlu C. Effect of sperm chromatin damage on fertilization ratio and embryo quality post-ICSI. Arch Androl. 2006;52(5):397–402.
84. Borini A, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21(11):2876–81.
85. Micinski P, et al. The sperm chromatin structure assay (SCSA) as prognostic factor in IVF/ICSI program. Reprod Biol. 2009;9(1):65–70.
86. Dar S, et al. In vitro fertilization-intracytoplasmic sperm injection outcome in patients with a markedly high DNA fragmentation index (>50%). Fertil Steril. 2013;100(1):75–80.
87. Rubio C, et al. Incidence of sperm chromosomal abnormalities in a risk population: relationship with sperm quality and ICSI outcome. Hum Reprod. 2001;16(10):2084–92.
88. Calogero AE, et al. High sperm aneuploidy rate in unselected infertile patients and its relationship with intracytoplasmic sperm injection outcome. Hum Reprod. 2001;16(7):1433–9.
89. Burrello N, et al. Lower sperm aneuploidy frequency is associated with high pregnancy rates in ICSI programmes. Hum Reprod. 2003;18(7):1371–6.
90. Pang MG, et al. Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridization in spermatozoa from nine patients with oligoasthenoteratozoospermia undergoing intracytoplasmic sperm injection. Hum Reprod. 1999;14(5):1266–73.
91. Nicopoullos JD, et al. Sperm DNA fragmentation in subfertile men: the effect on the outcome of intracytoplasmic sperm injection and correlation with sperm variables. BJU Int. 2008;101(12):1553–60.
92. Sergerie M, et al. Sperm DNA fragmentation: threshold value in male fertility. Hum Reprod. 2005;20(12):3446–51.
93. Benchaib M, et al. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertil Steril. 2007;87(1):93–100.

94. Bungum M, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod. 2007;22(1):174–9.
95. Jiang H, et al. The relationship of sperm DNA fragmentation index with the outcomes of in-vitro fertilisation-embryo transfer and intracytoplasmic sperm injection. J Obstet Gynaecol. 2011;31(7):636–9.
96. Bungum M, et al. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination IVF and ICSI. Hum Reprod. 2004;19(6):1401–8.
97. Huang CC, et al. Sperm DNA fragmentation negatively correlates with velocity and fertilization rates but might not affect pregnancy rates. Fertil Steril. 2005;84(1):130–40.
98. Velez de la Calle JF, et al. Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: results of a large prospective multicenter study. Fertil Steril. 2008;90(5):1792–9.
99. Larson-Cook KL, et al. Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril. 2003;80(4):895–902.
100. Henkel R, et al. DNA fragmentation of spermatozoa and assisted reproduction technology. Reprod Biomed Online. 2003;7(4):477–84.
101. Kennedy C, et al. Sperm chromatin structure correlates with spontaneous abortion and multiple pregnancy rates in assisted reproduction. Reprod Biomed Online. 2011;22(3):272–6.
102. Li Z, et al. Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. J Assist Reprod Genet. 2006;23(9–10):367–76.
103. Robinson L, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012;27(10):2908–17.
104. Simon L, et al. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl. 2017;19(1):80–90.
105. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril. 2008;89(4):823–31.
106. Zhang Z, et al. Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: a meta-analysis. J Assist Reprod Genet. 2015;32(1):17–26.
107. Pabuccu EG, et al. Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high sperm DNA fragmentation and previous ART failures. Andrologia. 2017;49(2):e12609.