Is sperm dna damage associated with IVF embryo quality? A systematic review

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Abstract

Purpose Sperm DNA damage is common amongst infertile men and may adversely impact natural reproduction, IUI-assisted reproduction and to a lesser degree IVF pregnancy. The objective of this study was to examine the influence of sperm DNA damage on embryo quality and/or development at IVF and ICSI.

Methods We conducted a systematic review of studies that evaluated sperm DNA damage and embryo development and/or quality after IVF and/or ICSI.

Results We identified 28 studies (8 IVF, 12 ICSI and 8 mixed IVF-ICSI studies) that evaluated the relationship between sperm DNA damage and embryo quality. These 28

studies evaluated 3226 treatment cycles (1033 IVF and 873 ICSI, 1320 mixed IVF-ICSI cycles) and demonstrated highly variable characteristics. In 11 of the 28 studies (1/8 IVF, 5/12 ICSI and 5/8 mixed IVF-ICSI studies), sperm DNA damage was associated with poor embryo quality and/or development, whereas the remaining 17 studies showed no relationship between sperm DNA damage and embryo quality and/or development.

Conclusions This systematic review indicates that the evaluable studies are heterogeneous and that overall, there is no consistent relationship between sperm DNA damage and embryo quality and/or development. The data also suggest that the influence of sperm DNA damage on embryo quality/development may be more significant in ICSI compared to IVF cycles.

Capsule Current studies suggest that there is no consistent relationship between sperm DNA damage and embryo quality and/or development after IVF or ICSI.

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Introduction

Experimental studies have shown that mammalian embryo development and implantation depend in part on the integrity of the sperm DNA, with a threshold of sperm DNA damage beyond which these events are impaired [1]. Moreover, there is also evidence from animal studies that sperm DNA fragmentation increases the risk of adverse post-natal events (cancer development and reduced longevity) [20]. In contrast, human studies indicate that DNA-damaged spermatozoa can fertilize successfully at IVF and ICSI and allow for normal embryo development and subsequent pregnancy [14, 22, 56]. These observations have raised questions regarding the risks of using DNA-damaged sperm for IVF and ICSI [5, 39].

There is now good evidence to show that sperm DNA and chromatin damage is associated with male infertility and reduced natural conception rates [18, 19, 23, 46]. This is highly pertinent given that infertile men (especially those with severe male-factor infertility) will be seeking treatment with ARTs and will be at risk of contributing to the development of embryos derived from spermatozoa with DNA damage. These observations have led several investigators to examine the relationship between sperm DNA damage and assisted reproductive technologies (ARTs) results in order to assess the impact of this sperm abnormality on reproductive outcomes.

A systematic review of the literature demonstrates that sperm DNA damage is associated with lower pregnancy rates after ARTs, namely, intrauterine insemination and standard in-vitro fertilization (IVF) [10, 14, 56]. In contrast, sperm DNA damage has not been associated with reduced pregnancy rates following intracytoplasmic sperm injection (ICSI), a technique that has emerged to salvage infertile couples with severe male factor infertility or repeated IVF failures [14, 56]. Nonetheless, sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and after ICSI, and because ICSI bypasses the natural defense barriers and allows for fertilization with DNA damaged sperm, there is growing concern regarding the health of the resulting offspring [20, 54].

In keeping with animal studies, several clinical studies have suggested that sperm DNA damage is associated with poor embryo quality and/or development [1, 2, 44, 55]. However, these observations have not been consistently reproduced and, moreover, the association between DNA damage and embryo quality has not yet been subjected to a systematic review. As such, the objective of this study was to conduct a systematic review of the clinical studies on sperm DNA damage and embryo quality and/or development after IVF and ICSI in order to gain further insight on the impact of sperm DNA damage (if any) on human reproduction.

Methods

Search strategy and selection criteria

We searched the Medline database from 1995 to August 2010 using the following search terms: “human sperm DNA”, “human sperm DNA damage”, “human sperm chromatin”, in combination with “embryo”, “pregnancy”, “assisted reproduction”, “in vitro fertilization”, “IVF” and “ICSI”. Additional studies were identified from the study reference lists. Only full articles published in English were searched. Two investigators (AZ and NA) independently reviewed the papers for eligibility and discrepancies were resolved by group discussion.

Data extraction

We selected studies that evaluated sperm DNA damage (in whole semen) and embryo quality after IVF and/or ICSI. We recorded the accrual type (i.e. consecutive), patient selection, female inclusion/exclusion criteria, treatment type, sperm DNA assay type, sperm DNA damage cut-off point, number of cycles or patients, and relationship between embryo quality and/or development and sperm DNA test results. Embryo quality was reported differently in the various studies: (1) embryo development (e.g. cleavage rate), (2) embryo grade or score, (3) embryo fragmentation or (4) multi-nucleation.

Results

Studies selected

Of the initial 285 citations retrieved, review of the titles and abstracts indicated that 255 were not relevant. Full papers were obtained for the remaining 30 citations. After reviewing the 30 papers, 7 were excluded [7, 8, 10, 12, 24, 47, 49] because embryo quality/development was not reported. The 23 remaining papers included a total of 28 studies on the association between sperm DNA damage and embryo quality/development after IVF, ICSI or both.

Study characteristics

The 23 eligible papers (with 28 studies: 8 IVF, 12 ICSI and 8 mixed IVF-ICSI studies) involved 3226 treatment cycles (1033 IVF and 873 ICSI, 1320 mixed IVF-ICSI cycles). Of the 28 studies, 16 reported on embryo quality, 11 on embryo development and 1 reported on both embryo quality and embryo development. 11 of the 28 studies reported a significant inverse relationship between sperm DNA damage and embryo quality and/or embryo development. Of the 17 studies that evaluated embryo quality, 5 reported a significant association between sperm DNA damage and embryo quality. Of the 12 studies that evaluated embryo development, 7 reported a significant association between sperm DNA damage and embryo development.

Of the 28 studies assessed, 8 evaluated day 2 embryos, 14 evaluated day 3 embryos, 3 evaluated day 5 embryos and 3 did not specify the day of evaluation (see Tables 1, 2 and 3). There was no significant relationship between the day of embryo evaluation and the impact of sperm DNA damage on embryo quality: 50% (4/8) of the day 2 embryo studies, 43% (6/14) of the day 3 embryo studies and 67% (2/3) of the day 5 embryo studies reported a significant effect of sperm DNA damage on poor embryo development/quality, respectively.

Table 1 Selected characteristics of 8 studies on sperm DNA damage and embryo quality after IVF

Study	n	DD test	Female factors Inclusions (Ctl-ed)	Day of E-Eval	EQ marker	DD & poor EQ (correlation)
Filatov,'99	176	CC	unspecified (no)	2 & 3	Grade	none
Host, '00	175	TUNEL	unspecified (yes)	3	Grade	none
Tomsu,'02	40	COMET	<40 (yes)	3	Grade	positive
Henkel,'03	208	TUNEL	unspecified (no)	NR	Fragmentation	none
Huang, '05	217	TUNEL	unspecified (no)	3	Grade	none
Lin, '08	137	SCSA	<40, FSH<15 (yes)	3	Grade	none
Benchai, '07	84	TUNEL	unspecified (yes)	5	Development	none
Frydman,'08	117	TUNEL	<38, FSH<10 (yes)	NR	Grade	none

CC chromatin compaction; Ctl-ed controlled; COMET single cell gel electrophoresis assay; DD DNA damage; E-Eval embryo evaluation; EQ embryo quality; NR not reported; SCSA sperm chromatin structure assay; TUNEL Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling.

The 8 IVF study characteristics are depicted in Table 1. Of these 8 studies, 6 were reportedly prospective [6, 21, 26, 27, 30, 50] but sampling was consecutive in only 1 paper [27]. Three studies excluded couples with poor ovarian reserve or advanced maternal age [21, 30, 50]. Sperm DNA damage was not associated with embryo quality in 7 of the 8 IVF studies. Only the report of Tomsu et al., (the smallest of the IVF studies) reported an inverse relationship between sperm DNA damage and embryo quality [50].

Of the 12 ICSI studies, 10 were reportedly prospective [2, 6, 22, 25–27, 30, 34, 37, 55] but sampling was consecutive in only 2 papers [27, 55] (see Table 2). Three studies excluded couples with poor ovarian reserve or advanced maternal age [30, 34, 55]. In nearly half of the studies (5/12 ICSI studies accounting for 443 of the 873 cycles), sperm DNA damage was associated with poor

embryo quality (2 studies) and/or impaired development (3 studies).

Of the 8 mixed IVF-ICSI studies, 4 were reportedly prospective [35, 38, 44, 52] but sampling was consecutive in only 2 papers [38, 52] (see Table 3). One study excluded couples with advanced maternal age [35] and one study involved an egg-donor program [32]. In over half of the studies (5/8 studies accounting for 1018 of the 1320 cycles), sperm DNA damage was associated with poor embryo quality (1 study) and/or impaired development (4 studies).

Discussion

We conducted a systematic review of studies on sperm DNA damage and embryo quality/development after IVF

Table 2 Selected characteristics of 12 studies on sperm DNA damage and embryo quality after ICSI

Study	n	DD test	Female factors Inclusions (ctl-ed)	Day of E-Eval	EQ marker	DD & poor EQ (correlation)
Hammadeh,'96	60	ABBlue	unspecified (no)	2	Development	none
Host, '00	61	TUNEL	unspecified (yes)	3	Grade	none
Henkel,'03	54	TUNEL	unspecified (no)	NR	Fragmentation	none
Gandini, '04	22	SCSA	unspecified (yes)	2	Grade	none
Huang, '05	86	TUNEL	unspecified (no)	3	Grade	none
Zini, '05	60	SCSA	<40 (yes)	3	Multinucleation	positive
Nasr-Esfahani,'05	28	COMET	unspecified (no)	2	Development	positive
Muriel,'06	85	SCD	unspecified (no)	3 & 5	Development	positive
Benchai, '07	218	TUNEL	unspecified (yes)	5	Development	positive
Lin, '08	86	SCSA	<40, FSH<15 (yes)	3	Grade	none
Micinski,'09	50	SCSA	<38 (no)	2	Development	none
Avendano,'10	36	TUNEL	unspecified (no)	3	Grade	positive

ABBlue aniline blue; Ctl-ed controlled; COMET single cell gel electrophoresis assay; DD DNA damage; E-Eval embryo evaluation; EQ embryo quality; NR not reported; SCD sperm chromatin dispersion assay; SCSA sperm chromatin structure assay; TUNEL Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling.

Table 3 Selected characteristics of 8 studies on sperm DNA damage and embryo quality after IVF and/or ICSI (mixed studies)

Study	n	DD test	Female factors Inclusions (Ctl-ed)	Day of E-Eval	EQ marker	DD & poor EQ (correlation)
Morris, '02	60	COMET	<40 (no)	2	Development	positive
Larson-Cooke, '03	89	SCSA	unspecified (yes)	3	Development	none
Seli, '04	49	TUNEL	unspecified (no)	2	Development	positive
Virro, '04	249	SCSA	unspecified (no)	2-3	Development	positive
Payne, '05	100	SCSA	unspecified (yes)	2	Development	none
Bakos, '08	113	TUNEL	unspecified (yes)	2	Grade	none
Meseguer, '09	38	DNA-Ox	Egg-donor (yes)	3	Development	positive
Velez de Calle, '08	622	SCD	unspecified (no)	2	Grade	positive

Ctl-ed controlled; *COMET* single cell gel electrophoresis assay; *DD* DNA damage; *DNA-Ox* DNA oxidation; *E-Eval* embryo evaluation; *EQ* embryo quality; *SCD* sperm chromatin dispersion assay; *SCSA* sperm chromatin structure assay; *TUNEL* Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling.

and/or ICSI. We identified 28 evaluable studies (from 23 papers) involving 3226 cycles of treatment (1033 IVF, 873 ICSI and 1320 mixed IVF-ICSI cycles) and observed mixed results in terms of the relationship between sperm DNA damage and embryo quality and/or development. Overall, sperm DNA damage was associated with poor embryo quality/development in fewer than half of the 28 evaluable studies (11/28), with the remaining studies reporting no significant relationship. In 7 of the 11 positive studies, poor embryo development was associated with sperm DNA damage and in 5 of the 11 positive studies poor embryo quality was associated with sperm DNA damage. An evaluation of the 8 IVF studies revealed that only 1 of the studies (accounting for 40 of the 1033 IVF treatment cycles) reported that sperm DNA damage was associated with poor embryo quality. A similar evaluation of ICSI studies demonstrated that close to half of these studies (5/12 ICSI studies with 873 treatment cycles in total) reported that sperm DNA damage was associated with poor embryo quality and/or delayed development.

Although a direct and/or quantitative comparison of the IVF vs. ICSI studies is not possible, the data suggest that the influence of sperm DNA damage on embryo quality/development (if any) is less important in the context of IVF compared to ICSI. One might speculate that the lack of influence of sperm DNA damage on IVF embryo quality/development may be a result of (1) sperm DNA repair in the oocyte and, more importantly, on (2) the natural selection that occurs during IVF. Indeed, the integrity of the sperm DNA is closely related to sperm motility and sperm membrane characteristics (with the latter characteristic being important for sperm-cumulus and sperm-zona binding) and, as such, natural selection processes should in theory reduce the probability of fertilization with DNA-damaged sperm at IVF [17, 28, 29, 31, 43]. Although experimental IVF studies have shown that sperm DNA

damage is associated with poor embryo development, these studies are not entirely translatable to human studies. Unlike DNA-damaged human spermatozoa where multiple other sperm defects are observed (e.g. membrane and motility defects), artificially DNA-damaged animal spermatozoa (e.g. radiation-induced) do not generally possess other sperm defects [1].

With ICSI, on the other hand, natural selection barriers are bypassed entirely and fertilization with highly DNA-fragmented sperm is possible. Although this damage may also be repaired in the oocyte, excessive damage may potentially result in early reproductive failures (e.g. poor embryo quality or development). Avendano et al., have reported that in semen samples with teratozoospermia, even the morphologically normal spermatozoa (as would be hand-selected for ICSI) may possess high levels of DNA damage [3]. Experimental ICSI studies have shown that sperm DNA damage will result in poorer embryo development [13, 20]. The differences in the IVF and ICSI studies (i.e. the more notable relationship between DNA damage and embryo quality/development with ICSI compared to IVF) may also reflect the fact that the selection criteria for IVF and/or ICSI are such that the sperm quality (including DNA integrity) of ICSI samples is generally poorer than that of IVF samples.

A formal meta-analysis (with estimation of combined odds ratio) would provide a more quantitative estimation of the relationship between sperm DNA damage and embryo quality/development. However, such an analysis was not possible because in several of the studies a cutoff or threshold value of sperm DNA damage was not reported and/or the data on embryo quality/development were not dichotomized according to DNA test result (positive/negative DNA test). Moreover, the definition of embryo quality/development was highly variable amongst the studies. Arguably, these shortcomings may weaken the

strength of our systematic review but this may not be relevant in light of the largely negative results in this study (with fewer than half of the studies showing an association between sperm DNA damage and embryo quality/development). Nonetheless, these shortcomings clearly highlight the importance of ensuring that future studies on embryo quality should report on highly objective findings (based on validated embryo scoring systems) and with sufficient detail (e.g. # embryos transferred, day of transfer of embryos, score of transferred embryos, embryo culture media, IVF protocol and female factors such as age, presence of polycystic ovaries, obesity and/or endometriosis) to allow for valid between study comparisons and formal systematic analyses.

A weakness of systematic reviews and meta-analyses is the heterogeneity of the study characteristics. In this particular review, the study characteristics were highly variable: data collection (prospective or retrospective), day of embryo evaluation, definition of embryo quality (development, fragmentation, grade, multinucleation), population characteristics (unselected, egg-donor), female inclusion/exclusion criteria, IVF protocol and sperm DNA test type. Another important limitation of this systematic review is that most of the studies failed to control for female factors and the IVF protocol when assessing the relationship between sperm DNA damage and embryo quality/development. For example, obesity may adversely affect the quality of embryos in younger women [33]. The IVF ovarian stimulation protocol could also introduce a source of bias as it may influence embryo quality. Conventional GnRH (Gonadotrophin releasing hormone) agonist long suppression followed by ovarian stimulation protocol has been commonly used as the default IVF protocol in most of the IVF programs worldwide (it is the protocol used in most of the analyzed studies in this report). This conventional protocol aims to produce multiple mature oocytes and hence multiple embryos. However, it has been shown that the conventional ovarian stimulation protocol may produce a higher proportion of chromosomally abnormal and mosaic embryos compared to a mild ovarian stimulation [4, 51]. As such, it may be reasonable for future studies in this area to evaluate the relationship between sperm DNA damage and embryo quality/development in the context of milder ovarian stimulation protocols.

Another significant limitation of this review lies in the definition and evaluation of embryo quality/development. There is a growing body of literature suggesting that the morphology-based embryo scoring systems (as reported in the analyzed studies) are imperfect. Indeed, one of the main drawbacks of these scoring systems is that they are poor predictors of the genetic integrity and chromosomal composition of the embryo [36]. Emerging techniques for evaluating the embryos might provide more accurate information on overall the quality and chromosomal

integrity of the cultured embryo (e.g. cleavage time, extended culture to blastocyst and metabolic assessment of culture media) [42].

The mechanisms by which sperm DNA damage may influence embryo quality/development are not known. It has been proposed that paternal genomic abnormalities (e.g. sperm DNA damage) can influence embryonic development but that such defects may only be detected at later stages of embryo development and post embryonic genome activation. One of the potential weaknesses of this review is the fact that a number of the included studies (8/28) evaluated day 2 embryos and one might expect that sperm DNA damage may not have a notable influence on embryo morphology at day 2. Nonetheless, 50% (4/8) of the studies that evaluated day 2 embryos reported a relationship between sperm DNA damage and embryo development/quality. Embryonic genome activation may occur progressively through preimplantation development (i.e. between the 4–8 cell stage) [40, 41, 44, 49] with paternal genome deficiencies potentially having a negative effect on continued development to the blastocyst stage [9, 16, 45, 48]. Moreover, studies in bovine fertilization suggest that the onset of the first S phase and embryo cleavage depend on paternal factors (the source of the bull sperm) [15, 53]. It has been suggested that defects in embryo quality/development may be due to sperm epigenetic alterations [11].

In summary, this systematic review demonstrates that there is no consistent relationship between sperm DNA damage and embryo quality/development after IVF and IVF/ICSI. The data also suggest that the influence of sperm DNA damage on embryo quality may be less significant in IVF compared to ICSI cycles. Moreover, the influence of sperm DNA damage on the embryo appears to be related to embryo development more so than embryo quality. However, the conclusions of this study are very much weakened by the limitations of the current embryo scoring systems and the heterogeneity of the analyzed studies (e.g. data collection, day of embryo evaluation, definition of embryo quality, population characteristics, female inclusion/exclusion criteria, IVF protocol and sperm DNA test type). As such, additional, well-designed prospective studies are needed to further evaluate the relationship between sperm DNA damage with both embryo development and quality.

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