

REVIEW

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Correlation of sperm DNA damage with blastocyst formation: systematic review and meta-analysis

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Abstract

Background: The routine semen analysis fails to detect sperm DNA damage which contributes to the majority of male factor infertility. Sperm DNA fragmentation test (DFI) measures the sperm DNA damage. Blastocyst formation is an important step in IVF ± ICSI. At present, the literature lacks any data that correlates DFI and blastocyst formation.

Main body of the abstract: We searched MEDLINE and other databases till 2020 for the studies that reported on sperm DNA damage and blastocyst formation in assisted reproductive technology (ART). The outcomes analyzed were (1) a comparison of blastulation rates in high DFI and low DFI groups. (2) Comparison of blastulation rates in high DFI and low DFI groups based on (a) different sperm DNA fragmentation assays (COMET, SCD, SCSA, TUNEL), (b) different types of ART (IVF/IVF + ICSI/ICSI). 10 studies were included in this review. A non-significant increase in the blastocyst formation was observed in high DFI group (OR = 0.70; 95% CI = 0.4 to 1.21; $P = 0.20$) and with SCD and TUNEL assays.

Short conclusion: Our study emphasizes on sperm DNA fragmentation (sperm DNA damage) as an important marker of blastocyst formation. The results of this meta-analysis suggest that the high sperm DNA fragmentation may not adversely affect the blastocyst formation.

Keywords: DFI, Blastocyst, IVF, ICSI, ART

Background

Sperm is the motile male gamete with the ability to capacitate, penetrate, and fertilize the oocyte (female gamete) to form a blastocyst (implantable embryo). Sperms are remarkably complex cells with a single important mission to deliver paternal DNA (male genetic material) and its associated factors to the oocyte to start a new life [20, 24]. This function is carried out successfully due to modified DNA architecture in the spermatid nucleus wherein protamines replace histones and adopt doughnut shape configuration. This modification is called chromatin condensation of spermatid DNA and any disruption or disturbance in the condensation

process will lead to unsuccessful conception/pregnancy in humans [12]. The male factor infertility is an important cause of infertility. Sperm DNA damage contributes to the majority of male factor infertility because of damage or defects in chromatin condensation of spermatid DNA [15, 31, 51].

Semen analysis remains the cornerstone of the evaluation of the infertile man and is widely used to evaluate and predict the fertilization capability of sperm [24]. Semen parameters routinely evaluated are sperm concentration, motility, live sperm ratio, and morphological examination [51]. However, routine semen analysis fails to identify sperm DNA damage [14, 40].

Many patients with male factor infertility need Assisted Reproductive Technology (ART) such as intracytoplasmic sperm injection (ICSI) for successful conception with limited success [1, 42]. Sperm (male gamete) with high

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DFI may result in poor fertilization and poor blastocyst formation, ultimately leading to poor pregnancy rates [29, 49]. However, the effect of sperm DNA damage (high DFI) on fertility outcomes are contentious. The meta-analysis by Simon et al. suggests that sperm DNA damage affects clinical pregnancy following IVF and/or ICSI treatment [46], whereas studies by Zhang et al., Chi et al., Yang et al., and Chen et al. have not shown that high DFI affects the outcome of ICSI [8, 9, 52, 54].

The test developed to assess the measure of sperm DNA damage is called sperm DNA fragmentation test, and its value is expressed as sperm DNA fragmentation index (DFI) [7, 20].

DFI is expressed as the percentage of DNA-damaged sperms in the total ejaculate. The value of DFI is related to the extent and type of sperm DNA damage and may also predict the reproductive outcomes [25].

Currently, there are 4 widely used techniques to calculate the level of sperm DNA damage such as comet assay, sperm chromatin structure assay (SCSA), sperm chromatin dispersion test (SCD), and TUNEL assay (terminal deoxynucleotidyl transferase dUTP nick end labeling). Each assay measures different aspects of sperm DNA damage [21, 32, 33].

- i. Comet assay is used to study single- or double-strand DNA breaks and measures the migration of the DNA fragments in the electric field. The intensity of the comet tail represents the amount of fragmented DNA.
- ii. In the SCSA, the extent of DNA damage is measured by the metachromatic shift from green fluorescence to red fluorescence following acid denaturation and acridine orange staining.
- iii. In the SCD assay, sperm with fragmented DNA fails to produce the characteristic halo following acid denaturation and removal of nuclear proteins.
- iv. The TUNEL assay quantifies the level of labeled nucleotide incorporated at single- and double-strand DNA breaks in a reaction catalyzed by the template-independent enzyme deoxynucleotidyl transferase.

When DFI is $\leq 20\%$, the fertility potential is assumed to be good for SCSA [48], while it is considered good if DFI is $\leq 30\%$ for SCD [5, 34] and TUNEL test [4].

Despite differences in the principle and methodology of DFI assays, the levels of DNA damage measured by these assays show a degree of correlation [21, 32, 33].

Sperm DFI has been hypothesized as one of the investigations to differentiate between infertile and fertile men [58] but not recommended in the initial evaluation of the

infertile couple by ASRM guidelines on the basis of grade C evidence [41].

Male infertility with relatively high fragmented DNA (represented as high sperm DNA fragmentation index-DFI) may correlate with a low number and quality blastocysts which will negatively affect the pregnancy even after ICSI. Thus, blastocyst formation assumes paramount importance in patients with sperm DNA damage [33, 43, 50].

The published data regarding the actual blastocyst formation in relation to sperm DNA damage are limited because they have focused only on the outcomes of pregnancy rates. The objective of this study was to evaluate the actual blastocyst formation in patients with increased damaged sperm DNA.

Main text

The review is reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Literature search

We searched MEDLINE, EMBASE, CINAHL, Google Scholar, Web of Science databases, and SCISEARCH from the inception of the database to January 2020 for studies that reported on sperm DNA damage and blastocyst formation in assisted reproductive technology (ART). We also manually reviewed the bibliographies of retrieved original papers, review papers, previous meta-analyses, and relevant studies for additional articles. In this way, missing data from our search criteria were identified and included.

A computerized search was performed using the Medical Subjects Headings (MeSH) search strategy to generate two subsets of citations. One subset included the search terms and words related to "sperm DNA damage," "sperm DNA fragmentation," "sperm DNA integrity," or "sperm DNA" along with "Comet," "TUNEL," "SCSA," "Acridine orange," "Halo," or "SCD."

The other subset included the search terms and words related to "ART," "IVF," "ICSI," "Late embryo development," "blastocyst," "embryo," or "day 5 culture."

Both subsets were combined and searched again to capture all the relevant articles or citations for our study. The search was restricted to clinical studies in human subjects.

Two authors (SV and PK) conducted the search and reviewed them independently. The discrepancies for inclusion and exclusion of the studies were resolved by group consensus with other reviewers.

Authors were contacted whenever possible if full manuscripts or translations were not available. We also considered the inclusion of studies that collected relevant

data but were excluded from the previous meta-analysis due to the inability to extract two-by-two tables.

Inclusion criteria and study selection based on outcomes

Sperm DNA damage involves DNA fragmentation, breakage of DNA cross-links (single or double strands), abnormal protamination, and chromatin condensation. For the purpose of our review and meta-analysis, we included studies that looked into sperm DNA fragmentation or breakage of DNA cross-links (single or double strands) and reported DNA damage as either low DFI (sperm DNA fragmentation index) or high DFI in the couples who underwent In vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) as treatment of their infertility. In the studies included, the female demographics responsible for infertility were comparable and hence the inconsistencies with regard to female factors were also comparable. The male factors like varicocele, infections, and smoking also contribute to sperm DNA damage (Smith [47]). In this study, we tried to find out the impact of DFI on blastocyst formation.

Outcomes

- Our main outcome of interest was to compare blastulation rates in two groups viz., high DFI (high sperm DNA fragmentation) and low DFI (low sperm DNA fragmentation), based on the percentage of sperm DNA damage expressed as DFI.

(Blastulation rate is defined as the rate of blastocyst formation resulting from in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) of metaphase II (M II) oocytes with sperm DNA damage expressed as high or low DFI).

Our other outcomes of interest were to compare of blastulation rate in high DFI and low DFI sperm DNA fragmentation groups based on the following:

- i. Different sperm DNA fragmentation assays (COMET, SCD, SCSA, TUNEL).
- ii. Different types of ART treatments (IVF/IVF + ICSI/ICSI).

Inclusion criteria

Studies were included if they analyzed the relationship between the following:

- i. Sperm DNA fragmentation detected by the Comet, SCSA, SCD, or TUNEL assays

- ii. Blastocyst formation in couples who underwent ICSI, IVF, or mixed (IVF + ICSI)
- iii. Blastulation rate (blastocyst formation rate): sufficient data available to calculate the blastocyst formation rate from the studies (number of blastocysts after fertilization of M II oocytes either by ICSI, IVF, or mixed (IVF + ICSI) and sperm damage was reported by DFI).

Exclusion criteria

The following studies were excluded from our analysis that did report on the following:

- i. Studies measuring sperm DNA fragmentation using slide-based acridine orange staining method or any other methods other than COMET, SCSA, SCD, and TUNEL assays.
- ii. Studies that did not define sperm DNA fragmentation as low and high DFI.
- iii. Studies which did not report on blastocyst formation.
- iv. Studies with insufficient data on blastocysts formation to construct 2×2 table.
- v. Studies were excluded if they had no original data available for retrieval or overlapping or duplication of the data.

Data collection and extraction

Data extraction was done by selecting the titles and abstracts, from the electronic search using the search strategy mentioned. They were scrutinized independently by reviewers (SV, SR, and PK). Full manuscripts of studies that fulfilled our selection criteria were retrieved. The inclusion and exclusion of studies were decided after careful evaluation of the full manuscripts.

The information extracted from the included articles to perform the meta-analysis is as follows: name of the author, year of publication, study design, type of ART treatment (ICSI, IVF, IVF + ICSI), and method of sperm DNA fragmentation assay, after excluding the female factors. The two-by-two table was constructed for the blastulation rate (number blastocysts formed in low and high DFI groups).

Statistical analysis

We retrieved the data for each individual study with respect to the number of blastocysts formed from the total number of metaphase 2 (M II) oocytes from IVF/ICSI cycle.

The outcomes were summarized as the comparison of pooled odds ratio (OR) with 95% CI for blastocyst

formation in the high DFI (high sperm DNA fragmentation) group and low DFI (low sperm DNA fragmentation) group.

We also performed separate subgroup meta-analyses in different sperm DNA fragmentation methods and also in different types of ART treatments (ICSI/IVF, IVF + ICSI) for the same pooled odds ratio (OR) with 95% CI for blastocyst formation in high and low groups of DFI.

We used the random effect models for meta-analysis to calculate an overall OR and its 95% confidence intervals (CI) with the forest plots.

We used the Review Manager [RevMan] Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, [38] to perform these meta-analyses. The presence of heterogeneity was assessed by the I^2 statistic. An $I^2 > 50\%$ was taken to indicate substantial heterogeneity. Heterogeneity of the exposure effects was evaluated graphically using forest plots and statistically using the I^2 statistic to quantify heterogeneity across studies.

The random effects model was used to calculate the odds risks if the I^2 statistic was greater than 50%. Exploration of the causes of heterogeneity was planned using variation in features of the population, exposure, and study quality.

Sensitivity analysis

A sensitivity analysis was conducted by repeating meta-analyses in following ways: Excluding and including retrospective studies, applying fixed as well as random effect models to note any effect on the outcomes. We also performed subgroup analysis of the studies comparing different DFI cutoff values to note any effect on the outcomes.

Results of the search

A total of 1272 citations were initially identified after electronic search and 1249 citations were excluded after screening the titles and /or abstracts. A total of 23 publications were identified and scrutinized in full text in Fig. 1—PRISMA.

Thirteen studies were excluded with reasons, and a total of 10 studies were included for the meta-analysis.

Study characteristics are summarized in Tables 1 and 2.

Included studies are presented in Table 1.

A total of ten studies were included in the meta-analysis. Of those, five were prospective [4, 5, 34, 44, 48] and the remaining five were retrospective studies [16, 17, 43, 56]. There were no randomized controlled studies based on these outcomes.

Excluded studies are presented in Table 2.

Thirteen studies were excluded from the meta-analysis. Of those, eight studies [2, 3, 22, 25, 37, 45, 50, 53] had insufficient data on blastulation rates.

Two studies (Muriel et al. [30]; Loutradi et al. [28]) did not define the low and high DFI threshold for sperm DNA fragmentation, one study [19] had inappropriate inclusion of only high DFI, one study [11] reported on sperm DNA methylation defects rather than sperm DNA fragmentation, and one study (Piccolomini et al. [36]) reported on analyzed.

Outcomes: Comparison of blastulation rates in low DFI and high DFI groups are presented in Figs. 2a, b.

A total of 9083 blastocysts were formed from 22,369 metaphase II oocytes from the 10 studies included. Overall, non-significant increase in the blastulation rates was observed in the high DFI group in comparison to the low DFI group. The combined pooled odds ratio (OR) with confidence intervals (CI) are as follows: random OR=0.75; 95% CI=0.4 to 1.21; $P=0.20$; I^2 (inconsistency)=98%. Fixed OR=0.85; 95% CI=0.79 to 0.92; $P \leq 0.00001$.

A sensitivity analysis was conducted by repeating meta-analyses by excluding and including retrospective studies did not show any difference in the outcomes. Similarly, subgroup analysis comparing different DFI cutoff values also did not show any difference in the outcomes as shown in Fig. 2a, b.

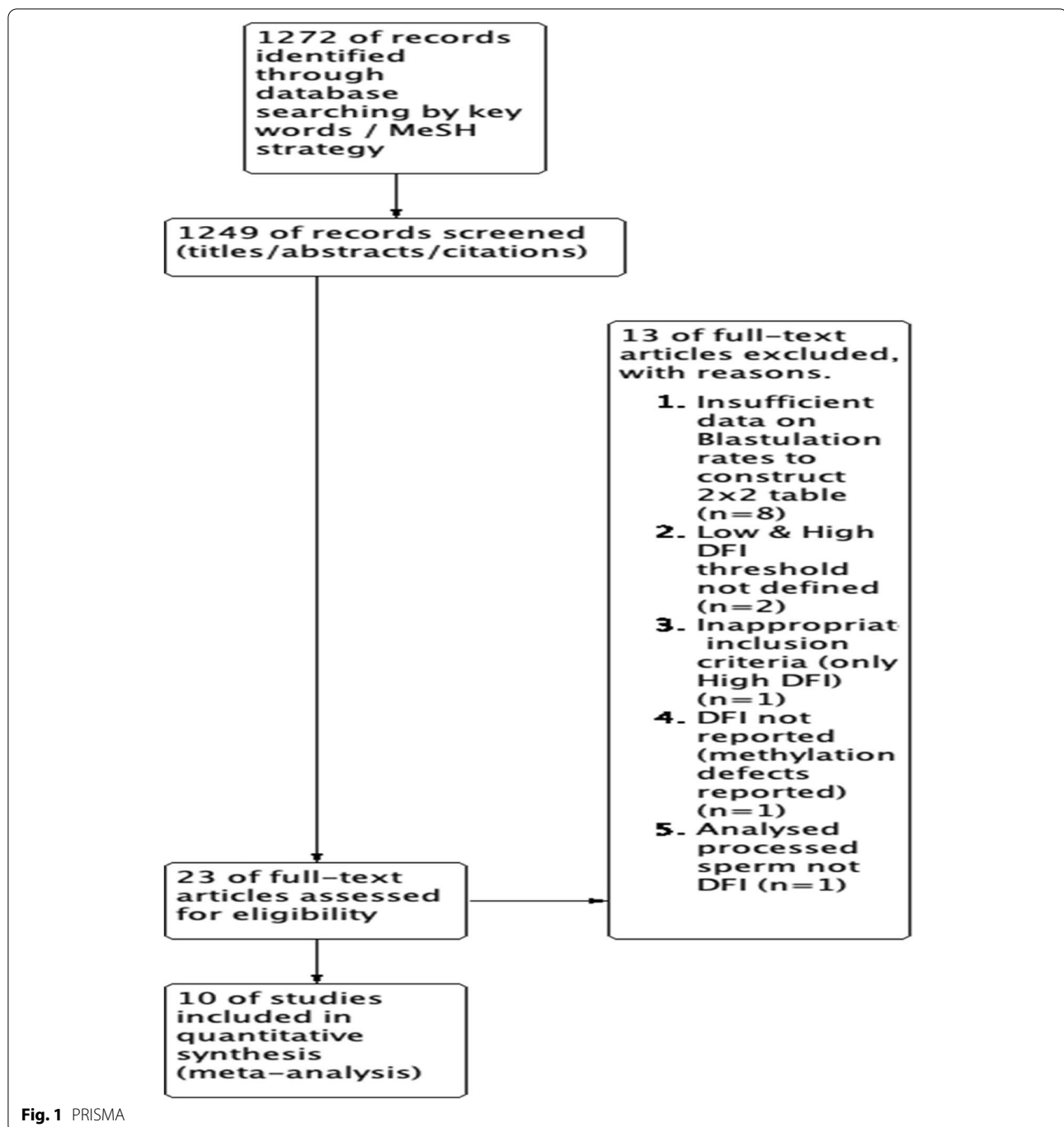
Our other outcomes of interest were to compare of blastulation rate in high DFI and low DFI sperm DNA fragmentation groups based on the following:

- Different sperm DNA fragmentation assays (COMET, SCD, SCSA, TUNEL).
- Different types of ART treatments (IVF/IVF + ICSI/ICSI).

Various methods of sperm DNA fragmentation assays with different cutoff values of high and low DFI are the confounding factors that can affect the blastulation rates. Therefore, subgroup analysis was performed to compare blastulation rates in different sperm DNA fragmentation assays as shown in Fig. 3a, b.

The blastulation rates in the four SCD studies were pooled. Non-significant increase in blastulation rates were observed in high DFI group in comparison to low DFI group. The combined pooled OR with CI are as follows: random pooled OR=0.52; 95% CI=0.35 to 0.78; $P=0.001$; I^2 (inconsistency)=90% and fixed pooled fixed OR=0.6; 95% CI=0.54 to 0.67; $P < 0.00001$.

The blastulation rates in the three TUNEL studies were pooled, and increased blastulation rates were observed in high DFI group in comparison to low DFI group. The combined pooled OR with CI are as follows: pooled



random OR=0.44; 95% CI=0.10 to 1.91; $P=0.27$; I^2 (inconsistency)=98%. Pooled fixed OR=0.73; 95% CI=0.61 to 0.87; $P=0.0005$.

The blastulation rates in the three SCSA studies were pooled, and higher blastulation rates were observed in low DFI group in comparison to high DFI group. The combined pooled OR with CI are as follows: pooled

random OR=1.56; 95% CI=0.43 to 5.6; $P=0.5$; I^2 (inconsistency)=99% and fixed pooled fixed OR=1.67; 95% CI=1.45 to 1.93; $P<0.00001$.

Different types of ART treatments (IVF/IVF + ICSI/ICSI)

Various methods of ART treatments (IVF/IVF + ICSI/ICSI) can have different blastulation rates and that can

Table 1 Study characteristics (included studies)

S. No	Author year	Type of study	ART treatment	No. of cycles	DNA fragmentation assay	Low and high DFI threshold cutoff
1	Borges et al., [5]	Prospective	ICSI	475 cycles Low DFI $n=42$ High DFI $n=433$	SCD	DFI < 30% & DFI > 30%
2	Ni et al. [34]	Prospective	IVF/ICSI-FET	1082 cycles Low DFI $n=910$ High DFI: $n=172$	SCD	DFI < 30% & DFI > 30%
3	Zheng et al., [56]	Retrospective	IVF/ICSI	161 cycles Low DFI $n=68$ High DFI $n=93$	SCD	DFI < 10% & DFI > 10%
4	Garcia-Ferreya et al., [16]	Retrospective	IVF/ICSI/OD	32 cycles Low DFI = 05 High DFI = 27	SCD	DFI < 17% & DFI > 17%
5	Speyer et al., [48]	Prospective	IVF/ICSI	347 cycles Low DFI $n=297$ High DFI $n=50$	SCSA	DFI < 20% & DFI > 20%
6	Gat et al., [17]	Retrospective	ICSI-FET	177 cycles Low DFI $n=141$ High DFI $n=36$	SCSA	DFI > 30%- high DFI < 15%- low
7	Gat et al. [17]	Retrospective	ICSI-OD-FET	45 cycles Low DFI $n=18$ High DFI $n=27$	SCSA	DFI < 15% & DFI > 15%
8	Benchaib et al., [4]	Prospective	IVF/ICSI-OD	322 cycles of ICSI + IVF Low DFI $n=87$ High DFI = 235	TUNEL	DFI < 30% & DFI > 30%
9	Seli et al., [44]	Prospective	IVF/ICSI	49 cycles Low DFI $n=28$ High DFI = 21	TUNEL	DFI < 20% & DFI > 20%
10	Sedo et al., [43]	Retrospective	IVF/ICSI/OD	82 cycles Low DFI $n=54$ High DFI = 28	TUNEL	DFI < 15% & DFI > 15%

affect the outcomes. Therefore, a subgroup analysis was conducted in different ART treatment methods to compare the blastulation rates as shown in Fig. 4a, b.

The blastulation rates in the seven studies were pooled, and a non-significant increase in blastulation rates was observed in the high DFI group in comparison to the low DFI group.

The combined pooled odds ratio with confidence intervals are as follows: pooled random OR=0.68; 95% CI=0.30 to 1.54; $P=0.35$; I^2 (inconsistency)=98% and pooled fixed OR=0.95; 95% CI=0.85 to 1.05; $P<0.00001$.

The blastulation rates in the four studies were pooled, and no significant difference in blastulation rates was observed in low and high groups of the DFI group.

Table 2 Study characteristics (excluded studies)

Serial no	Author and year	Type of assisted treatment	Type of study, no. of cycles	Sperm DNA fragmentation assay	Reason for exclusion
1	Benchabib et al. [3]	IVF/ICSI	Prospective study <i>n</i> = 104 cycles They have studied the formation of day 3 and not blastocysts	TUNEL	Insufficient data on blastulation rates and blastocyst formation rates cannot be calculated
2	Larson-Cook et al. [25]	IVF/ICSI	Retrospective <i>n</i> = 89 cycles Blastulation rates with respect to high and low DFI are not mentioned	SCSA	
3	Virro et al. [50]	IVF and or ICSI	Retrospective study <i>n</i> = 249 cycles Low DFI = 178 High DFI = 71	SCSA	
4	Huang et al. [22]	IVF/ICSI	Retrospective study <i>n</i> = 303 cycles	TUNEL	
5	Breznik et al. [37]	IVF/ICSI	Prospective study 883 cycles for IVF 878 cycles for ICSI	Sperm function tests	
6	Anifandis et al. [2]	IVF/ICSI	Prospective study <i>n</i> = 156 cycles Low DFI <i>n</i> = 71 High DFI <i>n</i> = 85	SCD	
7	Simon et al. [45]	IVF/ICSI	Prospective study <i>n</i> = 238 cycles only sperm DNA damage was studied using the 3 assays COMET assay <i>n</i> = 238 TUNEL assay <i>n</i> = 235 FCCE <i>n</i> = 102	COMET TUNEL SCSA (FCCE)	
8	Muriel et al. [30]	IVF/ICSI	Prospective study <i>n</i> = 85 cycles	SCD	
9	Loutradi et al. [28]	ICSI	Prospective study <i>n</i> = 219 cycles Since semen analysis was used, we could not categorize as low DFI or high DFI	Semen analysis	Threshold for low and high DFI was not defined inappropriate inclusion of only high DFI Only patients with high DFI were considered and subjected to intervention
10	Greco et al. [19]	ICSI	prospective study <i>n</i> = 38 cycles	TUNEL	
11	Denomme et al. [11]	ICSI	Prospective study <i>n</i> = 246 cycles	Methylome and transcriptome analysis	DNA fragmentation index was not studied. Sperm DNA methylation defects were studied Since methylome and transcriptome analysis was done, there were no DFI categorization
12	Piccolomini et al. [36]	IVF/ICSI	Retrospective study <i>n</i> = 4205 cycles Since semen analysis was used, we could not categorize as low DFI or high DFI	Semen analysis as per WHO recommendations	DNA fragmentation index was not studied. Processed sperms were analyzed
13	Yang et al. [53]	ICSI	Prospective study <i>n</i> = 90 cycles High DFI = 60 Low DFI = 30	DFI assay was not mentioned	Assay for sperm DNA fragmentation was not mentioned

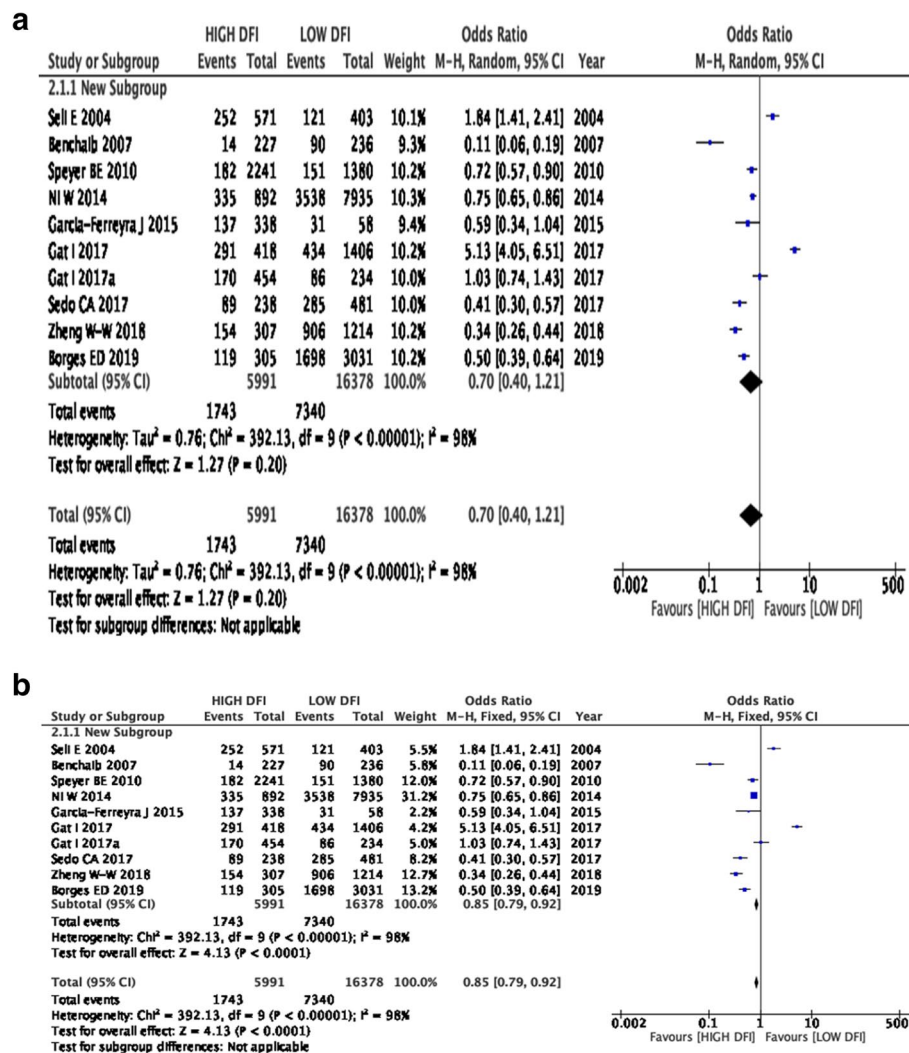


Fig. 2 Comparison of blastulation rate in high DFI and low DFI (random). **b** Comparison of blastulation rate in high DFI and low DFI (fixed)

The combined pooled odds ratio with confidence intervals are as follows pooled random OR=1.07; 95% CI=0.35 to 3.25; $P=0.92$; I^2 (inconsistency)=92% and pooled fixed OR=1.49; 95% CI=1.17 to 1.89; $P=0.001$.

The blastulation rates in the two studies were pooled, and a significant increase in blastulation rates was observed in the high DFI group.

The combined pooled odds ratio with confidence intervals are as follows: pooled random OR=0.44; 95% CI=0.23 to 0.83; $P=0.01$; I^2 (inconsistency)=94% and pooled fixed OR=0.63; 95% CI=0.55 to 7.2; $P<0.0001$.

Heterogeneity (inconsistency) of the studies included for the meta-analysis

The overall heterogeneity of the effects among the evaluated studies was high ($I^2=98\%$). Heterogeneity

remained high even after the subgroup analysis based on types of DNA assay (SCD, TUNEL, and SCSA) and types of ART treatments (ICSI, IVF + ICSI, and IVF).

Among these studies, there were differences in study design, selection of subjects, and definition of low and high DFI cutoff threshold values for DNA damage (for a given assay).

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis to evaluate the effect of sperm DNA fragmentation on the rate of blastocyst formation (blastulation).

According to our meta-analysis, there is an overall non-significant increase in the blastocyst formation rate in the high sperm DNA fragmentation/damage (high

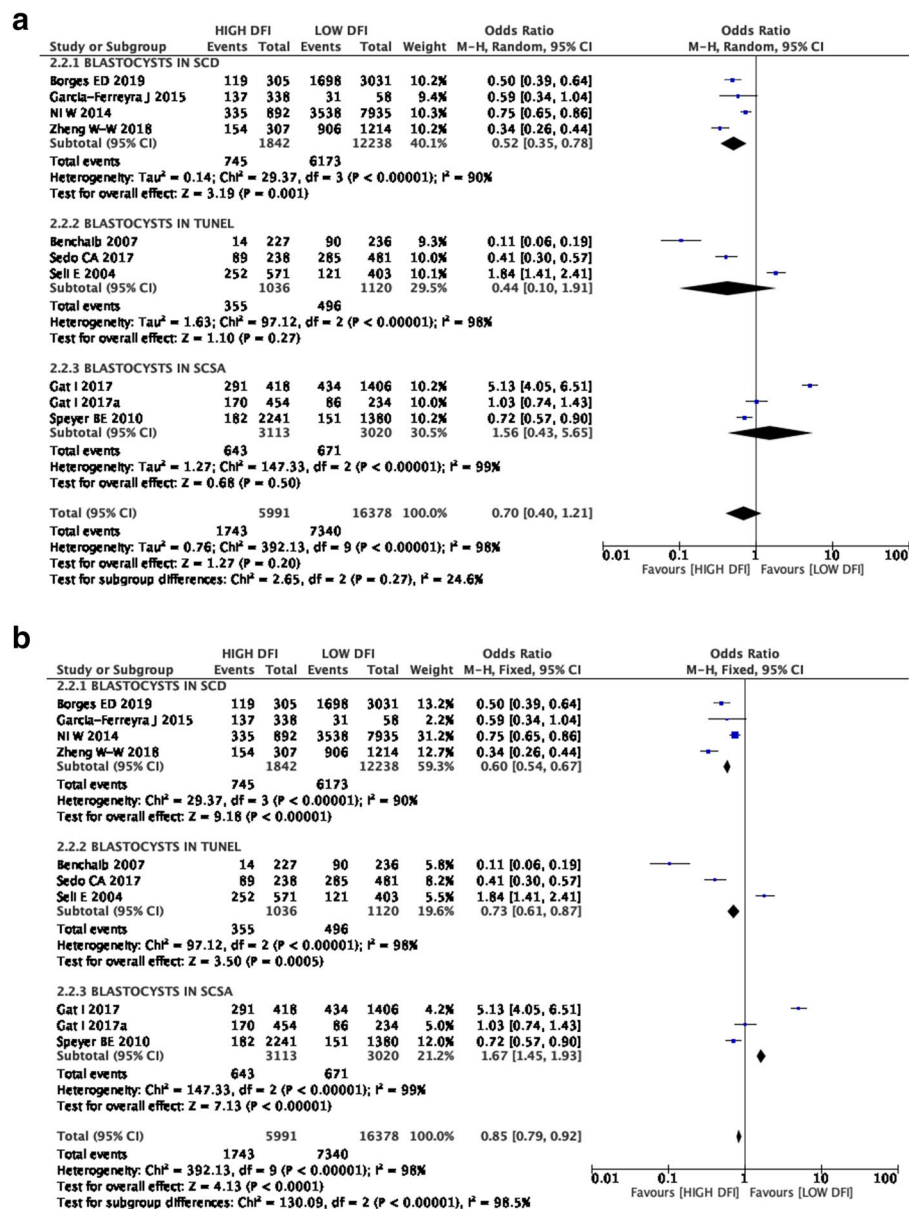


Fig. 3 a Comparison of blastulation rates in different sperm DNA assays (random). b Comparison of blastulation rates in different sperm DNA assays (fixed)

DFI) group in comparison to the low DFI group. The random pooled OR was 0.70 with a CI of 0.40–1.21 and a P value of 0.20.

When we did subgroup analysis in different types of sperm DNA assays used to evaluate sperm DNA damage and in different types of ART procedures, we found that the similar positive trend (higher blastulation rates) in the high DFI group with SCD and TUNEL assays but blastulation rates were better in low DFI group with SCSA.

We noticed a slight positive effect on blastulation rates (higher blastulation rates) in the high DFI group when IVF was used as a method of ART, and there was no difference in blastulation rates between high and low DFI groups when ICSI or when mixed IVF+ICSI is used as methods of ART.

So far, all the published meta-analyses [10, 27, 35, 39, 46, 55] studied the effect of high DNA sperm fragmentation on pregnancy rates and miscarriage rates but did not address the causal relationship between sperm DNA

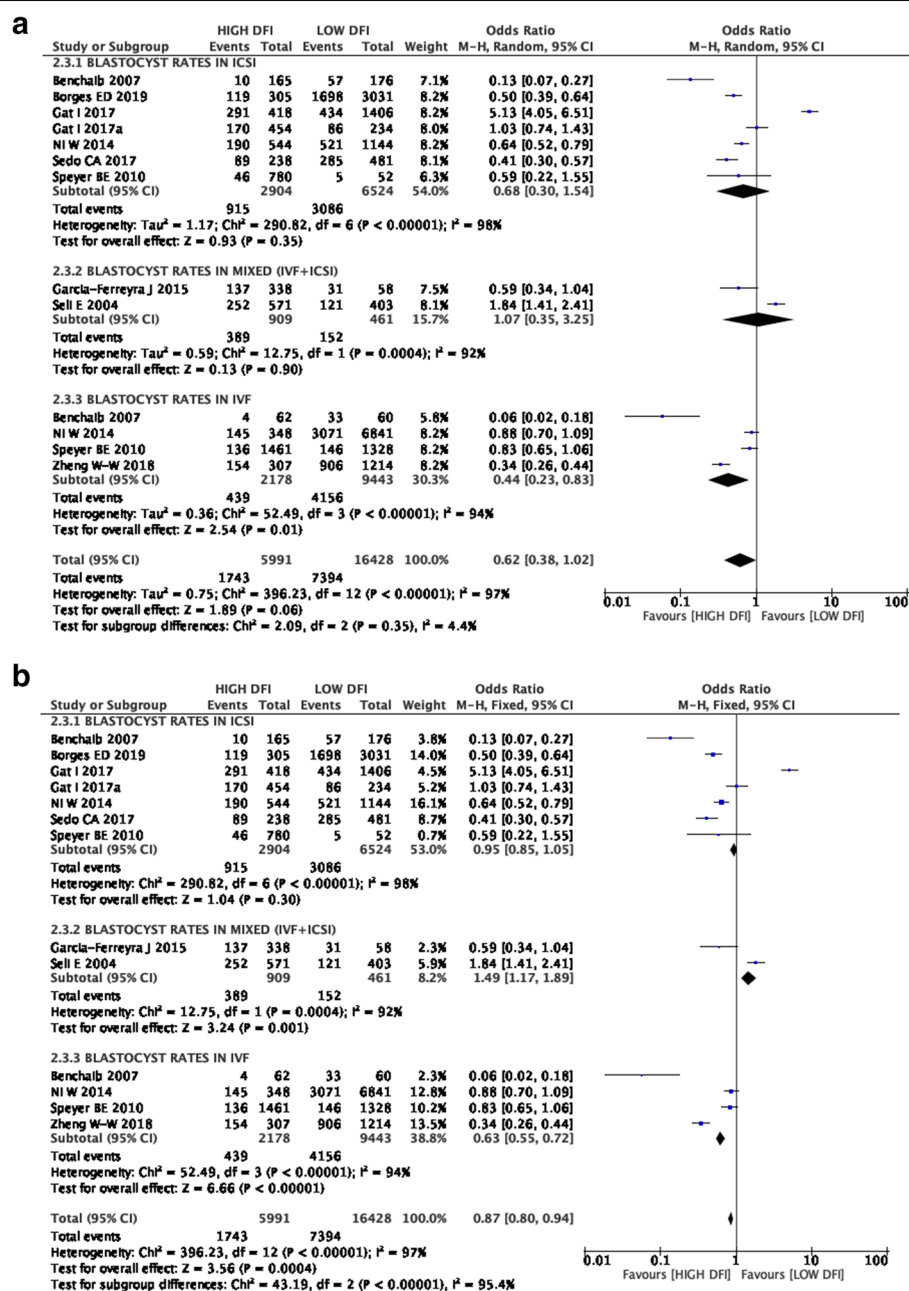


Fig. 4 **a** Comparison of blastulation rates in different ART methods (random). **b** Comparison of blastulation rates in different ART methods (fixed)

fragmentation and the blastulation rates, the crucial step for any pregnancy and its related outcomes. Pregnancy rates and miscarriage rates in ART are dependent and confounded by other female factors like elevated levels of progesterone on the day of hCG trigger, fresh and frozen embryo transfers not just limited to high sperm DNA fragmentation. Hence, pregnancy rates and miscarriage

rates are not true reflections of blastulation rates in men with high DNA sperm fragmentation.

It is assumed that poor rate of blastocyst formation (blastulation rates) with high sperm DNA fragmentation is the plausible cause for the low pregnancy rates and this may be due to the embryonic arrest (inactivation of paternal embryonic genome, i.e., failure of 4–8 cell stage embryo to form blastocyst) [6].

The overall slight improvement of blastulation rates with high sperm DNA fragmentation (high DFI) in our study is not entirely supportive of the above hypothesis. Seven out of ten studies had a combined odds ratio of just less than unity favoring a high DFI group with a small increase in blastulation rates. This increase may be due to the inherent ability of the oocyte to repair the effect of fertilizing sperm DNA damage. However, the oocyte loses its ability to repair with increasing maternal age, poor ovarian reserve, and poor quality of oocytes retrieved [18]. The inclusion of subjects with lower maternal age may be the reason for better blastulation rates in our analysis with high DFI. The largest study included in our analysis [34] which represents 31% in our analysis had subjects with maternal age of less than 32 years concluded that no significant association between sperm DNA fragmentation and pregnancy outcomes. The younger age of the women might have compensated for the male factor. Studies by Gat et al., [17] are also in agreement with the same, where the donor oocytes were used for ICSI.

Another factor which might have influenced our results is the variable threshold cutoff levels for high DNA fragmentation in the studies included in our analysis. Seven out ten studies had threshold cutoff levels for high DNA fragmentation when $DFI > 15\%$ and three studies had when $DFI > 30\%$. It is possible that the average DFI in the high DFI group might be closer to the lower threshold cutoff values which might have resulted in better blastulation rates in the high DFI group especially if the high DFI threshold cutoff is $> 15\%$.

Blastulation rates in different sperm DNA fragmentation assays

The blastulation rates favor high sperm DNA fragmentation (high DFI) in SCD and TUNEL assays, which is similar to overall blastulation rates and favors low DFI in SCSA. The SCD assay was used for sperm DNA fragmentation in four studies and accounts for 60% weightage in the meta-analysis. TUNEL and SCSA were used in three studies each, respectively. No studies with COMET analysis were included in the meta-analysis. The SCSA assay demonstrates more negative effects of sperm with high DFI on blastulation rate than with other two assays (SCD and TUNEL). This may be related to the lower threshold cutoff value for sperm DNA fragmentation used in all three studies ($< 15\%$), whereas in SCD and TUNEL assays lower threshold cutoff value for sperm DNA fragmentation varied from 15 to 30%.

Blastulation rates with TUNEL and SCD assays from our review may be comparable to the pregnancy rates of the previous metanalysis done by Simon et al. [46] but not with

meta-analysis done by Li et al. [27] in the high DFI group. In contrast, blastulation rates of our review with SCSA assay are not comparable to pregnancy rates reported by Simon et al. [46] and Li et al. [27] in their meta-analysis.

The SCD and SCSA assays are indirect tests and measure possible single-strand DNA damage after denaturation where as the TUNEL assay is the direct test and measures actual double-stranded DNA damage. TUNEL assay is more predictive of the outcomes with the sperm DNA damage, but the high interoperable variation and lack of standardization makes it unreliable. On the other hand, SCD and SCSA are easily reproducible and less interoperable variability makes it user-friendly.

Despite differences in the principle and methodology of these assays, the levels of DNA damage measured by these assays show some degree of correlation. This is in agreement with studies done by Nasr-Esfahani et al., [32, 33] and Henkel et al., [21].

Blastulation rates in different methods of ART (IVF/IVF + ICSI/ICSI)

Our meta-analysis did not demonstrate any significant difference in the blastulation rates between high DFI and low DFI groups when ICSI was employed as a method of ART but demonstrated an increase in the blastulation rates when IVF is employed as a method of ART, and favors low DFI group when mixed IVF + ICSI ART method is employed.

Our blastulation results with ICSI or IVF do not correlate with previous meta-analysis done by Osman et al. [35], Zhao et al. [55], and Li et al. [27], where the rates of fertilization improved when ICSI was employed as a method of ART in comparison to IVF. Instead, our blastulation rates were better with IVF in the high DFI group, and this may be due to the fact that in IVF process only the sperm with less DNA damage will be naturally selected which has better capacity and ability to fertilize and divide to form blastocysts.

Our results of no difference in blastulation rates in the high and low DFI group with ICSI agrees with pregnancy rates reported by Simon et al. [46] and Cissen et al. [10], but not with Zhao et al. [55]; Osman et al. [35]; Zini et al., [57]; Li et al. [27]; Evenson and Wixon, [13], based on the hypothesis that morphologically normal-looking sperm selected for the ICSI in high DFI group (high damaged sperm DNA) may bypass or will have reduced impact of robust rigors of the normal fertilization and will lead to better fertilization rates [26].

The results of this study emphasize the importance of sperm DNA fragmentation (sperm DNA damage) as a marker for blastocyst formation rather than pregnancy rates.

Strategies like lifestyle modifications, antioxidant therapies, infection control, varicocele repair, ICSI, intracytoplasmic morphologically selected sperm (IMSI), and physiologic ICSI are available to improve the sperm damage prior to ART procedure [23] and subsequently better blastocyst formation rates and pregnancies. However, the above findings need to be interpreted with caution because of high heterogeneity in the study population, mixture of prospective and retrospective studies without randomization, different threshold levels applied to define low- and high-DNA sperm fragmentation among the studies, lack of standardization (due to intra- and intervariability among the different assays), and the inability of these assays to predict the actual DNA damage of the sperm which will fertilize the oocyte irrespective of the ART methods.

Limitations

First, the extreme heterogeneity in the study populations makes it difficult to draw concrete conclusions on the effects of sperm DNA damage (high DFI) on the blastocyst formation. Second, meta-analysis suggests sperm DNA damage may not affect blastocyst formation. However, studies summarizing the relationship between sperm DNA damage and blastocyst formation were of low quality (lack of RCTs on this subject). Nevertheless, the effect of high-sperm DNA fragmentation on blastulation rates warrants further adequately powered and well-designed randomized studies with standardized threshold cutoff levels of DFI for individual assays.

Conclusion

In conclusion, this study emphasizes on sperm DNA fragmentation (sperm DNA damage) as an important marker of blastocyst formation. The results of this meta-analysis suggest that the high-sperm DNA fragmentation may not significantly affects the fertilization or blastocyst formation.

Nevertheless, the effect of high-sperm DNA fragmentation on blastulation rates warrants further adequately powered and well-designed randomized studies with agreed threshold cutoff levels with the individual assay. This is not only to understand the implications of sperm DNA fragmentation tests on fertilization/blastocyst formation, pregnancy rates, and miscarriage rates but also to assess the role of therapeutic interventions such as lifestyle modifications, antioxidant therapies, infection control, varicocele repair, ICSI, intracytoplasmic morphologically selected sperm (IMSI), physiologic

ICSI to improve the sperm damage prior to ART procedure, and subsequently better blastocyst formation rates and pregnancies.

Abbreviations

ART: Assisted reproductive technology; CI: Confidence interval; DNA: Deoxyribonucleic acid; DFI: DNA fragmentation Index; ICSI: Intracytoplasmic sperm injection; IMSI: Intracytoplasmic morphologically selected sperm; IVF: In vitro fertilization; M II: Metaphase II; MeSH: Medical Subjects Headings; OR: Odds ratio; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RevMan: Review manager; SCD: Sperm chromatin dispersion; SCSA: Sperm chromatin structure assay; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labelling.

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Authors' contributions

SV and PK wrote the protocol and manuscript. SV, PK, and SR managed the literature search and SV and SR performed calculations, estimations, and meta-analysis in RevMan. All authors critically revised the manuscript and approved the final version.

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Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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