Correlation of sperm DNA damage with IVF and ICSI outcomes: A systematic review and meta-analysis

Zhongxiang Li · Liquan Wang · Jie Cai · Hefeng Huang

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Abstract *Purpose:* To assess the effects of sperm DNA damage, as determined by the TUNEL assay and the SCSA respectively, on the outcomes of IVF/ICSI treatment.

Methods: A Medline search (from Jan 1978 to Apr 2006) was performed, together with a manual search of the bibliographies of retrieved original papers and review articles. 8 articles met all inclusion/exclusion criteria, of which, 5 used the TUNEL assay and the other 3 used the SCSA. All these articles were included in separate meta-analysis. The meta-analysis was conducted using the RevMan software with fixed-effect model or random-effects model.

Results: As for articles using the TUNEL assay, the pooled results of IVF outcomes indicated that the clinical pregnancy rate (RR 0.68, 95% CI 0.54 to 0.85, $P = 0.006$), but not the fertilization rate (RR 0.79, 95% CI 0.54 to 1.16, $P = 0.23$) decreased significantly for patients with high degree of sperm DNA damage compared with those with low degree of sperm DNA damage. RRs of the ICSI outcomes indicated that there was no significant difference in either fertilization rate (RR 1.03, 95% CI 0.89 to 1.18, $P = 0.70$) or clinical pregnancy rate (RR 0.76, 95% CI 0.55 to 1.04, $P = 0.09$) between these two groups. As for the SCSA papers, the pooled results showed no significant effects of sperm DNA damage on the clinical pregnancy rate after IVF (RR 0.58, 95% CI 0.25 to 1.31, $P = 0.19$) or ICSI (RR 1.18, 95% CI 0.81 to 1.74, $P = 0.38$).

Conclusion(s): Our meta-analysis indicates that sperm DNA damage, as assessed by the TUNEL assay, signifi-

cantly decreases only the chance of IVF clinical pregnancy, but not that of either IVF fertilization or ICSI fertilization or ICSI clinical pregnancy. Besides, our results also reveal that sperm DNA damage, when assessed by the SCSA, has no significant effect on the chance of clinical pregnancy after IVF or ICSI treatment.

Keywords Spermatozoa · DNA damage · In vitro fertilization · Intracytoplasmic sperm injection · Fertilization · Clinical pregnancy · Meta-analysis

Introduction

Sperm DNA integrity has been recognized as one of the vital determinants of normal fertilization and embryo growth in both natural and assisted conception [1, 2]. Moreover, DNA-damaged sperm still has a chance to form pronuclei at fertilization and even probably allow for a subsequent embryo development in the context of assistant reproduction technology (ART) [3, 4], which raises the concern that transmission of damaged DNA to the offspring, particularly at levels that exceed DNA repair capacity of the oocyte, could have serious consequences [5, 6]. On account of these factors, several techniques have been developed to detect sperm DNA damage, such as the terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL), sperm chromatin structure assay (SCSA) and comet assay [7]. Using these methods, researchers have performed numerous studies to evaluate the adverse influence of sperm DNA damage on the reproductive outcomes.

In natural conception, animal studies have shown that normal fertilization and subsequent embryo development depend in part on the integrity of sperm DNA [8–11]. Besides, clinical evidences in human have also indicated that

Zhongxiang Li is Postdoctoral fellow supervised by Hefeng Huang

Z. Li · L. Wang · J. Cai · H. Huang (✉)
Center for Reproductive Medicine, Women's Hospital, School of
Medicine, Zhejiang University,
Hangzhou, Zhejiang 310006, P.R. China
e-mail: huanghefg@hotmail.com

sperm DNA damage (DNA fragmentation and/or abnormal chromatin packaging) adversely affects the reproductive outcomes, and that infertile men possess substantially more sperm DNA damage than do fertile men [12–15]. More importantly, Sergerie et al reported recently a cut-off value of 20% sperm DNA damage between fertile controls and infertile men, giving 96.5% sensitivity and 89.4% specificity. The results by Sergerie et al indicates that sperm DNA integrity can be taken as an effective indicator of male fertility potential in natural conditions [16].

As for IVF and/or ICSI, although many clinical studies have been performed to assess the adverse impacts of human sperm DNA damage on reproductive outcomes, the conclusions from these studies remain controversial. Some investigators do not identify any adverse effects of sperm DNA damage on fertilization rate [17–21], while others assert a negative correlation between DNA fragmentation and fertilization rate [22–26]. In addition, there also exists a debate in terms of pregnancy rate. Some authors find no influence of DNA damage on pregnancy rate [26–28], but others propose a significant reduction of pregnancy rate for patients with high loads of DNA-damaged sperms [19, 21, 29–31].

Recently, several excellent reviews give a descriptive summary of literature regarding the relationship between sperm DNA damage and male fertile ability [7, 32–35]. Almost all of these reviews conclude that DNA damage may impair male fertility potential, but that in terms of the predictive value of each assay for ART outcomes, there still exist disagreements among a variety of studies used the same or different method. In addition, it is proposed that several factors might be responsible for these controversies remained to be clarified. First, the types and mechanisms of sperm DNA damage might be varied among different studies. As is well known, defects in the genomic material in sperm may take the form of condensation or nuclear maturity defects, DNA breaks, or sperm chromosomal abnormalities [5, 7]. The causes of these defects have been attributed to diversified conditions such as disease, drug use, elevated testicular temperature, air pollution and cigarette smoking. Second, multiple techniques have been used to measure DNA defects in human spermatozoa [5, 7]. The ability of these techniques to accurately estimate both the value and the nature of sperm DNA damage depends on the technical and biological aspects of each test. For example, the TUNEL assay, a most commonly used technique, can directly identify DNA breaks, while another commonly used test SCSA can only indirectly reflect DNA integrity through assessing the susceptibility of chromatin to acid denaturation. So, each test identifies a specific type of DNA damage that has biological influence on the some aspects of fertilization and embryo development. Finally, the sample sizes in some original studies are too small to arrive at a significant result. Together with these published reviews, a meta-analysis may help to reach a more definite

conclusion and to explain further why the discrepancy exists among different studies.

Among those that have been employed to detect sperm DNA damage, two methods are extensively studied and thought to have a higher predicative value than others. The first one is TUNEL assay, usually described as the method that can detect ‘real’ DNA damage and provide a direct measure of DNA breaks in sperm. Therefore, it should have a higher predictive value than other tests that measure “potential” DNA damage. This is further supported by recent studies which use the TUNEL assay to determine sperm DNA fragmentation and demonstrate a highly predicative value for male fertility in natural conception with 96.5% sensitivity and 89.4% specificity and good stability [16, 36]. The second one is the SCSA, often referred as being able to reflect only “potential” DNA damage. This method was first provided by Evenson et al and extensively used later in plenty of studies [37]. Furthermore, it has been testified by studies that the SCSA parameters are stable within individuals over time and correlated well with DNA strand breaks as indicated by the TUNEL assay [38].

So, it is proposed that both the TUNEL assay and the SCSA should have the same value in evaluating sperm DNA damage. However, even for these two methods, there is still a lack of consensus on their clinical values in predicting IVF/ICSI outcomes in terms of fertilization rate and clinical pregnancy rate.

In view of these confusing factors, the aim of our study was to evaluate further the relationship between sperm DNA damage and reproductive outcomes (including fertilization rate and pregnancy rate) after IVF/ICSI by conducting separate meta-analysis of the published data according to different methods used. Besides, we only included the related studies that employed the TUNEL assay and the SCSA to detect sperm DNA damage, because these two methods are most widely used and thought to have a higher value than others.

Methods

Search strategy

We searched the MEDLINE database for relevant articles published in English from Jan 1978 to Apr 2006 by using the following Medical Subject Heading terms and/or text words: spermatozoa, sperm, DNA damage, DNA fragmentation, in vitro fertilization, intracytoplasmic sperm injection and assisted reproductive technique. The search was restricted to clinical trials in human. All the titles and abstracts obtained from MEDLINE search were reviewed first for relevance, and then full text of each paper initially selected was evaluated further to determine its eligibility for the meta-analysis.

To identify additional studies that may have been missed during the MEDLINE search, we also manually reviewed the bibliographies of retrieved original papers and review articles. Two investigators (ZX Li, LQ Wang) independently reviewed each retrieved paper for eligibility and discrepancies were resolved by group discussion.

Inclusion criteria

All the published studies analyzing the relationship between sperm DNA damage and IVF/ICSI outcomes were considered for inclusion in the meta-analysis if they satisfied the following criteria: 1) clinical study in human; 2) sperm DNA damage detected by the TUNEL assay or the SCSA; and 3) fertilization rate and/or clinical pregnancy rate calculated as the study endpoints. Studies were excluded if they had no original data available for retrieval and duplicate publications were also excluded.

Data extraction

Following the MOOSE (Meta-analysis Of Observational Studies in Epidemiology) statement, we used a standardized reporting form to abstract data from each included study [39]. Some number data, if not available directly, were calculated or transformed for the meta-analysis according to original data in the papers. Two reviewers (ZX Li, LQ Wang) independently extracted data from each study using a predefined review spreadsheet. Any differences were resolved by group discussion to reach consensus. We also assessed methodological quality of each retrieved paper according to the criteria for observational studies [40].

The primary outcome measures were the fertilization rate respectively for IVF (per 100 oocytes co-cultured in vitro) and ICSI (per 100 oocytes injected) and the clinical pregnancy rate respectively for IVF and ICSI (per 100 oocyte retrieval cycles). Fertilization was defined as formation of the pronuclei and clinical pregnancy was defined as the presence of a positive pregnancy test (HCG positive) together with the ultrasound visualization of intrauterine gestation sac.

Statistical analysis

We performed separate meta-analyses for the different study outcomes from each method used to determine DNA damage. For each method, the relative likelihood of DNA damage effect on IVF/ICSI outcomes was expressed as a risk ratio (RR). We obtained pooled estimates of the RR by combining the separate estimates of inverse variance-weighted log RR estimates from each study [41, 42]. Thus, the larger studies offering more precise estimates of the relative likelihood were given greater weight than other studies in the

analysis [43]. Random-effects model or fixed-effects model was used for pooling according to homogeneity test result [41, 42]. Confidence intervals and heterogeneity of estimates were evaluated in standard fashion [44, 45]. Publication bias was also assessed using the funnel plots when appropriate.

The meta-analysis was conducted by using the Rev-Man software. The significance level was set at $P = 0.05$.

Results

Study selection and characteristics

98 potentially relevant studies were searched from the MEDLINE database. 1 more article was identified by manual search of the references cited sections. Among these studies, a total of 8 studies met the predefined selection criteria, of which 5 used the TUNEL assay and the other 3 used the SCSA, to evaluate sperm DNA damage [4, 19, 21, 25, 26, 28, 46, 47]. A typical flow schematic for the selection process is shown in Fig. 1. These selected studies were included in separate meta-analysis according to which method (i.e. the TUNEL assay or the SCSA) was used to evaluate sperm DNA damage. The patients involved in each study were divided into two groups, namely the group with high degree of sperm DNA damage and the group with low degree damage, according to their DNA damage levels above or below a cut-off value.

The 5 studies that used the TUNEL assay included a total of 816 subjects [19, 21, 25, 26, 28]. The percentage of TUNEL-positive sperms was calculated to indicate the levels of sperm DNA damage. In addition, the cut-off values for defining high or low degree of DNA damage were decided according to the ROC curves in some studies [19, 21], but not stated clearly in others [25, 26, 28]. There were some inconsistencies among the TUNEL assay studies regarding sperm preparation, methods used to perform the TUNEL assay, as well as in the cut-off levels selected for groups division. The other 3 that used the SCSA collected a total of 299 subjects [4, 46, 47]. Among these studies, there were also differences in definition of thresholds for DNA damage evaluation and study design was not stated clearly. Main characteristics of the included studies are presented in Table 1.

Quality of the included studies

On the whole, the reporting quality of the included studies was moderate: the methods of recruitment, total case numbers, inclusion criteria and study design were generally clearly stated. However, in some studies, data suitable for meta-analysis were not presented directly and needed to be calculated according to available data in these papers. In

Table 1 Characteristics of included studies ($n = 8$) addressing effects of sperm DNA damage on IVF/ICSI outcomes

Study profiles	Study design	Subjects	Sperm preparation	Cut-off value (%)	IVF outcome (<i>n^a/N^b</i>)				ICSI outcome (<i>n^a/N^b</i>)			
					Fertilization		Pregnancy	LD ^d	Fertilization		Pregnancy	LD ^d
					HD ^c	LD ^d	HD ^c		LD ^d	HD ^c	LD ^d	
TUNEL assay												
Henkel, et al., 2004, (14)	Prospective	Randomly selected	Sperm suspensions centrifuged	36.5	40/63	60/104	12/63	37/104	–	–	–	–
Henkel, et al., 2003, (12)	Not clear	Randomly selected	Sperm suspensions centrifuged	IVF 36.5	41/64	84/144	12/64	50/144	–	–	–	–
Host, et al., 2000, (18) Huang, et al., 2005, (19)	Prospective	Consecutive selection	Liquefied raw sperms	ICSI 24.3	–	–	–	–	25/29	21/25	6/29	12/25
	Retrospective	Not clear	Prepared with PureSperm gradients	4	17/55	87/123	12/52	47/123	24/36	17/25	15/36	9/25
			Density gradient centrifugation	4	26/42	161/175	21/42	99/175	42/49	30/37	22/49	22/37
Seli, et al., 2004, (21)	Prospective	Not clear	Density gradient centrifugation	20	–	–	10/22	14/27	–	–	–	–
SCSA												
Bungum et al., 2004, (4)	Not clear	Consecutive selection	Raw semen sample	27	–	–	4/18	30/82	–	–	9/17	17/41
Zini et al., 2005, (46)	Not clear	Consecutive selection	Raw semen sample	30	–	–	–	–	–	–	6/11	25/49
Boe-Hansen et al., 2006, (47)	Not clear	Not clear	Raw semen sample	27	–	–	1/7	38/132	–	–	6/18	8/29

^a N = number of IVF or ICSI cycles performed.^b n = number of fertilized or pregnancy-positive IVF or ICSI cycles.^cHD High degree of sperm DNA damage group.^dLD Low degree of sperm DNA damage group.

Fig. 1 Article selection process for meta-analysis

addition, some of the inconsistencies among included studies may introduce biases into the meta-analysis and decrease power to detect the actual effects.

Meta-analysis of the studies using the TUNEL assay

The pooled IVF fertilization rate was 55.36% (124/224) for patients with high degree of sperm DNA damage and 71.80% (392/546) for patients with low degree of sperm DNA damage, giving a summary RR of 0.79 (95% CI 0.54 to 1.16, $P=0.23$) (Fig. 2A). This summary RR indicated a 21% reduction in the odds of having fertilization for IVF patients with high degree of sperm DNA damage compared with those with low degree of sperm DNA damage. But the difference between these two groups was not statistically significant ($P=0.23$). Also between these two groups, the pooled IVF pregnancy rate was 27.57% (67/243) and 43.11% (247/573) respectively, giving a summary RR of 0.68 (95% CI 0.54 to 0.85, $P=0.0006$) (Fig. 2B). This summary RR indicated a significant reduction of 32% in the odds of having

pregnancy for IVF patients with high degree of sperm DNA damage compared with those with low degree of sperm DNA damage.

Among the pooled studies, the test for heterogeneity was statistically significant for IVF fertilization analysis (Fig. 2A, $P=0.001$, $I^2=87.3\%$), but not for IVF pregnancy analysis (Fig. 2B, $P=0.32$, $I^2=14.8\%$). No evidence of publication bias was found graphically from the funnel plot (Fig. 3).

The pooled ICSI fertilization rate was 79.83% (91/114) for patients with high degree of sperm DNA damage and 78.16% (68/87) for patients with low degree of sperm DNA damage, giving a summary RR of 1.03 (95% CI 0.89 to 1.18, $P=0.70$) (Fig. 2C). There was no significant difference in ICSI fertilization rate between these two groups ($P=0.70$). Also for these two groups, the pooled ICSI pregnancy rate was 37.72% (43/114) and 49.43% (43/87) respectively, giving a summary RR of 0.76 (95% CI 0.55 to 1.04, $P=0.09$) (Fig. 2D). This result indicated a 24% reduction in the odds of having pregnancy for ICSI patients with high degree of sperm DNA damage compared with those with low degree of

Fig. 2 Meta-analysis of the TUNEL data on correlation of IVF/ICSI outcome with sperm DNA damage. Black squares indicate the risk ratio (RR) in each study and horizontal lines represent 95% credible interval (CI) of the RR. Black diamonds show the pooled estimates of RR with 95% CI. Fixed and random models, which assume homogenous and heterogenous studies respectively, were used to calculate the pooled RR. (A) IVF fertilization rate is not influenced by DNA damage (RR 0.79; 95% CI 0.54–1.16; $P = 0.23$). (B) IVF pregnancy rate is decreased by DNA damage (RR 0.68; 95% CI 0.54–0.85; $P = 0.0006$). (C) ICSI fertilization rate is not influenced by DNA damage (RR 1.03; 95% CI 0.89–1.18; $P = 0.70$). (D) ICSI pregnancy rate not is influenced by DNA damage (RR 0.76; 95% CI 0.55–1.04; $P = 0.09$)

sperm DNA damage. However, the difference between these two groups was not significant ($P = 0.09$).

No significant heterogeneity among included studies was found (Fig. 2C, $P = 0.93$, $I^2 = 0\%$ and Fig. 2D, $P = 0.18$, $I^2 = 42.0\%$ respectively) in either ICSI fertilization or ICSI pregnancy analysis.

Meta-analysis of the studies using SCSA

For meta-analysis of the studies using SCSA, the pooled clinical pregnancy rate for IVF was 20.00% (5/25) in high degree damage group and 31.78% (68/214) in low degree damage group respectively, giving a summary RR of

Fig. 3 Funnel plot of studies of IVF pregnancy with sperm DNA damage

0.58 (95% CI 0.25 to 1.31, $P=0.19$) (Fig. 4A); in the main while the pooled clinical pregnancy rate for ICSI was 45.65% (21/46) and 40.021% (50/119) in these two groups respectively, showing a summary RR of 1.18 (95% CI 0.81 to 1.74, $P=0.38$) (Fig. 4B). These results revealed that sperm DNA damage had no significant effects on clinical pregnancy after either IVF or ICSI treatment. Moreover, no significant statistical heterogeneity was detected between studies (Fig. 4).

Discussion

Principal findings from meta-analysis of the TUNEL assay articles

In the separate meta-analysis of 5 articles using the TUNEL assay, we found a significant decreased likelihood for IVF pregnancy in patients with high level of sperm DNA damage (Fig. 2B). However, we did not demonstrate any significant adverse effect of sperm DNA damage on the chance of IVF fertilization (Fig. 2A). These results indicate that during IVF treatment sperm DNA damage has only an adverse effect on the pregnancy outcome, but no influence on the fertilization. In accordance with our results, several other original studies, which used the SCSA and the Comet assay to evaluate sperm DNA integrity, have also demonstrated that only clinical pregnancy but not fertilization rate is adversely affected by sperm DNA damage during IVF treatment [31, 48, 49]. Furthermore, our conclusion is supported by another study. This study has verified that the embryonic genome is not expressed until after the second cleavage division, i.e. at the four to eight-cell stage in human embryo [50], suggesting that an adverse effect posed by the paternal genome abnormality

comes out only at later development stage. Therefore, we deduce that sperms with high degree of DNA damage may complete fertilization as normal during in vitro co-culture with oocyte, but embryo development in uterus will be impaired at later stage, thus leading to pregnancy failure.

Just as alike in IVF, we have not found any relationship between sperm DNA damage and fertilization rates in ICSI. In the main while, we also have not observed any adverse effect of sperm DNA damage on clinical pregnancy rates, which is just contrary to what has been found in IVF. So, as for the relationship between sperm DNA damage and pregnancy outcome, the discrepancy is presented between IVF and ICSI. Possible causes have yet to be determined. One explanation is that sperm selection during ICSI process may arbitrarily bypass some abnormal sperms, so increasing the chance of fertilization and pregnancy with “normal sperm.” Another is that only three original studies included in our meta-analysis of ICSI outcome have no power to give a significant result. The later explanation seems to be supported by our meta-analysis result which demonstrated a 24% reduction tendency of ICSI pregnancy rate in patients with a high level of sperm DNA damage. Moreover, several other original studies using different DNA damage detection assays also suggested a definite adverse effect of DNA damage on ICSI pregnancy [27, 30, 51]. Therefore, the conclusion about correlation of sperm DNA damage with ICSI pregnancy outcome in our meta-analysis should be taken cautiously and further clinical studies on this issue deserve to be conducted.

Principal findings from meta-analysis of the SCSA articles

As for the meta-analysis of SCSA articles, results suggested that there is not any significant adverse effect of sperm DNA

Fig. 4 Meta-analysis of the SCSA data on correlation of IVF/ICSI outcome with sperm DNA damage. (A) IVF pregnancy rate and (B) ICSI pregnancy rate are not influenced by DNA damage

damage on clinical pregnancy after either IVF or ICSI treatment. This is in agreement with result from meta-analysis of articles using the TUNEL assay regarding ICSI clinical pregnancy, but contrary to that regarding IVF clinical pregnancy. Recently, several reviews also confessed that SCSA has no definite value in predicting IVF/ICSI outcomes, but that its predictivity for in vivo and IUI fertilizations are clear for reduced pregnancy odds [32–35]. Possible reasons for the lack of agreement with the TUNEL assay in part may be due to the facts that there exists only small number of patients involved and defects of study design in the SCSA studies, and the differences in natures of both methodologies used to detecting DNA.

The TUNEL assay vs SCSA in sperm DNA evaluation

Both the TUNEL assay and SCSA have been thought to provide the same value in evaluating sperm DNA integrity and proposed for clinical use in ART setting [32, 33]. However, the results from our meta-analysis demonstrate that both the TUNEL assay and the SCSA do not

agree with each other completely. This disagreement may be attributed to some inconsistency among original studies and/or the different nature of each method itself. Therefore, further large and well-designed clinical studies and cross-sectional comparison of both methods are necessary to reach a more consistent conclusion, and more importantly it may be possible that not any single test can reveal the whole nature of sperm DNA integrity and multi-methods in combination should be better used to detect DNA damage.

In fact, many aspects of sperm quality, which can be represented by a number of parameters such as mobility, morphology and DNA integrity etc, have long been accepted as vital factors contributing to successful fertilization and ART pregnancy. However, the routine parameters in present clinic do not include assessment of sperm DNA integrity, and more importantly these parameters can not testify whether sperm DNA occurs or not [23, 52, 53]. Thus, based on these facts and meta-analysis results, we propose that sperm DNA integrity, assessed by the TUNEL assay and SCSA, should be taken into consideration when applying useful parameters to predict IVF/ICSI success in clinic

practice. Unfortunately, our meta-analysis lacks a summery evaluation on the value and effectiveness of both methods in predicting IVF/ICSI success, because some limitations in the original studies make it unsuitable to do a meta-analysis of ROC curve. Therefore, future works should be done further to determine their usefulness in clinical ART setting.

Strengths and weaknesses of the review

Our study has several strengths. First, we conducted a comprehensive search of MEDLINE database and two reviewers (ZX Li, LQ Wang) completed the study selection, quality assessment and data extraction independently. Second, we performed separate meta-analysis of published data employing only the TUNNL assay or SCSA to detect sperm DNA integrity, but to other methods such as the Comet assay and acid staining, thus increasing homogeneity among studies. Finally, we also explored heterogeneity among studies and potential publication bias in accordance with widely accepted guidelines [44, 45].

Several limitations also exist in our meta-analysis. Similar to most other systematic reviews of observational studies, our meta-analysis is vulnerable to biases and confounding factors inherent in the original data. In addition, heterogeneity has been observed across the included studies about the effect of sperm DNA damage on IVF fertilization, but we could not conduct a statistical test to thoroughly explore possible sources of this variability because of the severe constraints of the available study number and sample size. However, our systematic review suggested that this heterogeneity might be due to uncontrolled confounding factors and selection bias inherent in the studies. For example, different cut-off values of DNA damage presented among included studies and patient selection was also hospital based, sometimes non-systemic and arbitrary in some studies. These confounding factors and bias might lead to difficulties in estimating a true effect.

Implications

On the whole, our meta-analysis indicates that sperm DNA damage, when assessed by the TUNEL assay, significantly decreases only the chance of IVF clinical pregnancy, but not that of either IVF fertilization or ICSI fertilization or ICSI clinical pregnancy. Besides, our results also reveal that sperm DNA damage, when assessed by the SCSA, has no significant effect on the chance of clinical pregnancy after IVF or ICSI treatment. More works should be done further to determine the usefulness of these two methods before their widely applying in IVF/ICSI clinic.

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