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Original Article

Sperm DNA fragmentation index, as measured by sperm chromatin dispersion, might not predict assisted reproductive outcome

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ABSTRACT

Objective: Routine semen parameters have limited clinical diagnostic value for predicting male infertility. The aim of this study was to investigate the association between sperm DNA fragmentation index (DFI) and semen quality, and between DFI and clinical pregnancy rate of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Methods and materials: A total of 390 couples undergoing sperm fragmentation prior to receiving conventional IVF (n = 238) or ICSI (n = 152) were evaluated.

Results: We found that there were no significant differences in fertilization rate, good embryo rate, or pregnancy rate between high (≥30%) and low (<30%) DFI groups after IVF or ICSI. However, statistically different decreasing motility trends under higher DFI values in the IVF and ICSI groups were detected. Comparison of ROC curve of motility and DFI scores for achieved pregnancy revealed that the best DFI cut-off value was 20%. Also, no significant change was found when 20% DFI level was taken in IVF and ICSI outcomes.

Conclusion: DFI scores did not provide independent information regarding fertilization, embryo quality, or pregnancy for infertile patients who received IVF or ICSI, but were consistent with semen analysis for infertile couples, regardless of IVF or ICSI outcome.

Introduction

Routine semen parameters have limited clinical diagnostic and prognostic value for predicting male infertility. It has been estimated that more than 48.5 million couples are infertile worldwide, which has had a widespread global impact [1]. Male-related infertility is solely contribute to approximately 20% of all infertility cases; when combined with female factors, they contribute to 30–40% of cases [2]. To date, assessment of male infertility is still based on semen quality analysis according to World Health Organization (WHO) standards, including total sperm number, concentration, motility, and morphology [3]. In fact, many cases of male infertility are caused by sperm DNA defects, which routine semen quality analysis still fails to detect [4]. Therefore, routine semen have limited clinical diagnostic and prognostic value for predicting male infertility.

Recently, many studies have shown that sperm DNA fragmentation index (DFI) is used for prediction of male infertility, and it has better diagnostic and prognostic value than routine semen parameters [5–8]. It was reported that DNA integrity is essential to fertilize oocytes and is highly indicative of male infertility [9]. Recently, several studies have shown the damage rate of sperm DNA is higher in males with suspected infertility compared with fertile men [5,7,8,10–13]. Many factors can result in sperm DNA damage, including infection [14], drug use [15] and advanced age [16].

To date, various methods have been developed and introduced to measure sperm DNA fragmentation or damage, including terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay (TUNEL) [17], Comet assay

Abbreviations: DFI, Sperm DNA fragmentation; SCD, Sperm Chromatin Dispersion; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

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[18], DNA breakage detection-fluorescent in situ hybridization assay [19,20], sperm chromatin dispersion (SCD) [21], and sperm chromatin structure assay [6,22]. Recently, some investigators created a novel synthetic oligopeptide that showed significant value for detecting DNA damage in human spermatozoa [23].

More recently, contradictory findings have been published regarding the association between sperm DNA damage and outcome of assisted reproduction technology (ART). A systematic review showed that sperm DNA damage is associated with lower pregnancy rate in natural, intrauterine insemination [24], and in vitro fertilization (IVF) [25,26], and is associated with increased risk of pregnancy loss in couples who underwent IVF or intracytoplasmic sperm injection (ICSI) [25–28]. Alternatively, some studies suggested that DFI is not associated with ART outcome [29,30]. Therefore, evidence regarding association between sperm DNA damage and ART outcome is inconclusive.

In the present study, we investigate the associations among sperm DNA damage or fragmentation and semen parameters, fertilization rate, good embryo rate, and pregnancy rate after IVF or ICSI.

Materials and methods

Patients

This was a retrospective study at Peking University People's Hospital the data was collected during the month of September 2014 to June 2016. All experimental procedures and sample collection were approved by the Medical Ethics Committee of Peking University People's Hospital, and a written informed consent was obtained from each participant. A total of 390 infertile couples undergoing IVF or ICSI were included in this study. The following data were collected: sperm concentration, sperm motility, sperm morphology, DFI, fertilization rate, good embryo rate, and clinical pregnancy rate.

All female participants without poor ovary response had day 3 serum FSH levels <15 IU/L. Only freshly ejaculated sperm and sperm samples with a concentration of at least 1 million/mL were included for this study.

Semen analysis

Semen samples were collected from 390 men after 2–7 days of sexual abstinence and on the day of their partners' oocyte retrieval for IVF or ICSI. Semen analysis was performed according to WHO guidelines on a Makler R chamber (Sefi Laboratories, Tel Aviv, Israel) [3]. Sperm morphology was analyzed using strict criteria for all men [31]. Normal sperm samples were defined as those with concentrations $\geq 15 \times 10^6$/mL, progressive motility ≥32%, total motility ≥40%, and normal strict morphology ≥4%. Only normal sperm samples with concentrations $\geq 15 \times 10^6$/mL, motility ≥40%, and normal strict morphology ≥4% were used for IVF; and only sperm samples with at least one of the following criteria: concentration $<4 \times 10^6$/mL, and normal strict morphology <4% were used for ICSI.

ART procedures

All patients received ovarian stimulation using a standard luteal down-regulation regimen (long protocol) or flare-up short regimen (short protocol) [32–35]. Standard IVF or ICSI techniques were assessed as follows: the oocytes were assessed to determine whether fertilization had occurred at 16–18 h after insemination or microinjection. After 18 h, fertilization was determined to be normal if two pronuclei and two polar bodies were identified, and pronuclei size and position, as well as nucleoli size, distribution, and number were evaluated [36].

The day 3 embryo scoring system were observed according to their cell number, symmetry, blastomeres, type, and percentage of fragmentation [37]. Fresh embryo transfer was performed on day 3 after oocyte retrieval using the best quality embryos among a cohort of resultant embryos. The grading criteria were as follows: grade I: no fragmentation with equal-sized cells; grade II: <20% fragmentation with equal-sized cells; grade III: a lot of fragmentation with unequal-sized cells; grade IV: ≥20% fragmentation with unequal-sized cells; and grade V: ≥50% fragmentation. Embryos classified as grade I or II were denoted as good embryos. The day 5–6 blastocyst that were cryopreserved had at least grade 3BB [38]. Freezing and thawing were performed using a Kitazato Vitrification Freeze kit and Kitazato Thaw kit according to the manufacturer's protocols. No more than three surviving embryos were transferred into the uterine cavity. The luteal phase was routinely supported with progesterone 40–60 mg IM per day for 14 days and continued for another 4 weeks if pregnancy was established. Serum hCG levels were measured 14 days after embryo transfer. Clinical pregnancy was confirmed by ultrasound 4 weeks after embryo transfer.

SCD test

After liquefaction, an aliquot of 100 μL of the raw semen sample was used for SCD test [39]. Using the Halosperm® kit (INDAS Laboratories, Madrid, Spain), the SCD test was performed according to the manufacturer's protocol [40]. The procedure of measuring sperm DNA fragmentation by SCD test was performed as follows. A minimum of 500 spermatozoa per sample were scored under the × 100 microscope objective. The SCD test is based on the principle that sperm with non-fragmented DNA produce a big halo of dispersed DNA loops. Otherwise, sperm with fragmented DNA which size of halo smaller than 1/2 of minor diameter of the core [41]. It is widely accepted that a DFI value of 30% can be used as the cut-off to distinguish between potentially fertile and infertile men [5].

Semen preparation

IVF samples were prepared by swim up: raw semen were diluted 1: 1 (v: v) with Sperm Medium (SAGE, Cooper Surgical-Origio, Denmark). Then they were pelleted at 500 g for 5 min and the supernatants were discarded. Another process was to add 0.5–1 mL fresh medium and the incubation for 45 min of the tubes with 45° inclination. Finally, the upper 0.1–0.5 mL was taken for IVF procedures. ICSI samples were pelleted at 500 g for 5 min and the supernatants were discarded. Then, careful addition of 0.1 mL fresh medium was taken for ICSI procedures.

Statistical analysis

Statistical analysis was performed using SPSS (version 18.0, Inc., Chicago, USA). The Student's t-test for independent samples was used for comparison between groups. The correlations between parameters were examined using linear regression techniques with Pearson's correlation coefficient. ROC curves for variables were performed according to ROC analyses. The positive predictive value, negative predictive value, and their 95% CI were also calculated for significant variables. p values less than 0.05 were considered statistically significant.

Results

DFI and semen parameters

This study included 390 infertile couples undergoing IVF (n = 238) and ICSI (n = 152). The IVF and ICSI groups were further