# Sperm DNA Fragmentation in Normospermic and Oligospermic Men Is Associated with Blastocyst Formation and Quality in Conventional In Vitro Fertilization

Yusaku Mori *, Linji Chen, Shogo Nishii, Miwa Sakamoto, Makoto Ohara, Akihiko Sekizawa, Sho-ichi Yamagishi

Posted Date: 8 September 2025

doi: 10.20944/preprints202509.0580.v1

Keywords: Assisted reproductive therapy; blastocyst; in vitro fertilization; male infertility; sperm DNA fragmentation

 doi:10.20944/preprints202509.0580.v1



*Article*

# Sperm DNA Fragmentation in Normospermic and Oligospermic Men Is Associated with Blastocyst Formation and Quality in Conventional In Vitro Fertilization

**Yusaku Mori [1], Linji Chen [2,3], Shogo Nishii [2], Miwa Sakamoto [2], Makoto Ohara [1], Akihiko Sekizawa [2] and Sho-Ichi Yamagishi [1]**

[1] Department of Diabetes, Metabolism, and Endocrinology, Showa Medical University Graduate School of Medicine, Tokyo, Japan
[2] Department of Obstetrics and Gynecology, Showa Meical University Graduate School of Medicine, Tokyo, Japan
[3] Department of Obstetrics and Gynecology, Beijing Friendship Hospital, Capital Medical University, Beijing, China
* Correspondence: author: u-mori@med.showa-u.ac.jp; Tel.: +81-3-3784-8947; Fax: +81-3-3784-8948

**Abstract**

**Background**: Assisted reproductive therapy (ART) has been utilized as an effective therapeutic strategy for addressing infertility worldwide, and one of the key determinants of ART success is the acquisition of high-quality embryos through in vitro fertilization (IVF). We investigated here which male factors were associated with embryo formation and quality in conventional IVF (cIVF). **Methods**: This study was a sub-analysis of a trial conducted to examine the associations of clinical and lifestyle factors with sperm abnormalities in 42 men of infertile couples without identifiable male factor infertility. From the original cohort, 21 men whose partners underwent cIVF were included. Semen samples were evaluated for standard sperm parameters and DNA fragmentation index (DFI). Blood biochemical parameters and lifestyle habits were also evaluated. Blastocysts were assessed 5 days after cIVF, and implantation success was determined 10 days after embryo transfer. **Results**: Normospermia and oligospermia were observed in 67% and 33% of participants, respectively, with mild sperm DFI in 76%. Blastocysts were formed in 32% of the oocytes following cIVF. Among them, good blastocyst development and quality were observed in 71% and 39%, respectively. Eighteen women underwent blastocyst transfer, resulting in an implantation success rate of 50%. Multiple regression analysis identified sperm DFI as the only variable inversely associated with blastocyst outcomes. In contrast, only female age was associated with implantation success. **Conclusions**: The present findings suggest that sperm DNA fragmentation may negatively affect high-quality embryo formation in cIVF, even among normospermic and oligospermic men with non-severe sperm DFI.

**Keywords:** assisted reproductive therapy; blastocyst; in vitro fertilization; male infertility; sperm DNA fragmentation

## 1. Introduction

The declining birth rate has become a serious social issue in many developed countries, particularly those with a birth rate below 2.0 [1]. Epidemiological studies have indicated that the increasing number of infertility may be one contributing factor to this trend [2–4]. Assisted reproductive technologies (ART) are widely used to support individuals who are unable to conceive

 doi:10.20944/preprints202509.0580.v1



naturally, and the number of births resulting from ART has been steadily increasing worldwide [2–4], thus highlighting the growing importance of ART for infertile couples.

In ART, oocytes and sperm are collected and fertilized outside the body through in vitro fertilization (IVF), and the resulting fertilized oocytes are further cultured to develop embryos, which are subsequently transferred to the uterus [5]. A cohort study reported that overall pregnancy success rate with ATR is approximately 28% in women aged 25 to 35 years, whereas success rate increases up to approximately 45–50% when embryo transfer is performed [4]. Furthermore, embryo development and quality are also closely associated with pregnancy outcomes following embryo transfer [6]. Therefore, the acquisition of well-developed embryos through IVF is one of the critical determinants of ART success.

While female factors affecting oocyte quality are well established, male factors influencing sperm may be also critical for fertilization and subsequent embryo development. Indeed, male factors are estimated to account for up to approximately 30–50% of infertility cases among couples [7,8]. However, the underlying causes of male infertility remain unidentified in most cases [7,8]. Furthermore, the specific male factors associated with successful fertilization and high-quality embryo development in IVF are not fully understood.

Recently, we have found in a prospective observational study that blood and semen oxidative stress markers are associated with reduced sperm count and motility in men from infertile couples without identifiable causes of male infertility [9]. We investigated here which male factors were associated with embryo formation and quality in conventional IVF (cIVF).

## 2. Materials and Methods

### *2.1. Ethics Statement*

This study was a sub-analysis of a trial conducted to examine the associations of clinical and lifestyle factors with sperm parameters [9]. The study design was approved by the Ethics Committee of Showa University (Approval No: 2023-047-B, approval date: 18 July 2023). All procedures were performed in accordance with the ethical standards of Showa University policy on human experimentation and the Helsinki Declaration of 1964 and its later version. Informed consent was obtained from all participants.

### *2.2. Study Participants*

Our previous study included 42 men of infertile couples without obvious male factor infertility who visited the Reproduction section of the Obstetrics and Gynecology Department at Showa University Hospital (Tokyo, Japan) from July 2023 to April 2024; the exclusion criteria are described elsewhere [9]. Of the 42 participants, this study included men whose partners underwent in cIVF.

### *2.3. Study Design*

After enrollment, participants underwent routine clinical and physical examinations and skin AGE measurement, completed a lifestyle habits questionnaire [10], and provided semen and blood samples under non-fasting conditions. Skin AGE levels were non-invasively measured as skin autofluorescence (SAF) with AGE-Reader™ Mu (Diagnoptics Technologies B.V., Groningen, The Netherlands) [10].

### *2.4. Lifestyle Questionnaire*

Lifestyle habits were evaluated using the following 12 multiple-choice questions as described in a previous report [10] : (1) exercise frequency, (2) smoking duration, (3) alcohol consumption frequency, (4) sleep duration, (5) perceived mental stress, (6) vegetable intake, (7) breakfast frequency, (8) tendency to overeat, (9) greasy food consumption, (10) processed food consumption, (11) sugary food consumption, and (12) starting meals with vegetables. Each item was scored on a

 doi:10.20944/preprints202509.0580.v1



scale from 1 (worst) to 5 (best). Exercise-, smoking-, and alcohol-related habits were assessed using items (1), (2), and (3), respectively. Mental stress–related lifestyle habits were evaluated using the average score of items (4) and (5). Diet-related lifestyle habits were assessed by averaging the scores of items (6) through (12).

### *2.5. Blood Sample Measurements*

Serum levels of oxidative stress, AGE, glucose, lipids, zinc (Zn), and free testosterone levels were measured using the following methods, respectively: the d-ROMs test (Wismerll Company Limited, Bunkyo, Tokyo, Japan), an enzyme-linked immunosorbent assay, an enzyme electrode method, colorimetric assay, atomic absorption spectrophotometry, and radioimmunoassay [11,12]. Normal values are described previously [9].

### *2.6. Sperm Sample Measurement*

Fresh semen samples were collected by masturbation after 2–7 days of sexual abstinence and used for the assessment of sperm parameters as previously described [9,13]. Semen oxidative stress levels were evaluated by measuring the oxidation-reduction potential (ORP) of semen samples with the MiOXSYS™ analyzer (Aytu BioScience, Englewood, CO, USA) [14,15]. Sperm DNA fragmentation index (DFI) was measured in semen smears using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method and was expressed as the percentage of TUNEL-positive sperm to the total sperm count [9]. Sperm DFI was classified as mild (< 15%), moderate (15–30%), and severe (> 30%) [16,17]. Normal reference values for sperm parameters were defined as follows [13–15]: semen volume of > 1.4 mL; sperm concentration of > 160 × $10^6$/mL; sperm number of > 390 × $10^6$/ejaculate; total motility of ≧ 42%; progressive motility of ≧ 30%; and ORP of < 1.34 mV/$10^6$ sperm/mL.

### *2.7. Stimulation Protocols in cIVF*

Controlled ovarian stimulation was performed using either a gonadotropin-releasing hormone (GnRH) antagonist or a progestin-based protocol. Recombinant follicle-stimulating hormone (FSH) was initiated on menstrual cycle days 2–4, with doses adjusted individually. In the GnRH antagonist protocol, a GnRH antagonist was added once the leading follicle exceeded 14 mm. In the progestin-based protocol, dydrogesterone was administered from the start of FSH stimulation. Final oocyte maturation was induced when at least two follicles reached ≥18 mm using a buserelin nasal spray combined with recombinant human chorionic gonadotropin (hCG), followed by standard oocyte retrieval.

### *2.8. cIVF Procedure*

IVF was performed approximately 3 hours after oocyte retrieval. Fresh semen samples were prepared by standard density gradient centrifugation, and motile sperm were resuspended in fertilization medium. Cumulus–oocyte complexes were cultured with sperm (100,000–150,000/mL) in HTF medium supplemented with serum substitute (Irvine Scientific) at 37 °C under 5% $O_2$, 6% $CO_2$, and balance $N_2$. Fertilization was assessed 16–18 hours later by the presence of two pronuclei.

### *2.9. Blastocyst Assessment*

Fertilized oocytes were further cultured to the blastocyst stage for cryopreservation, and evaluated on day 5 using the Gardner and Schoolcraft grading system [18]. Blastocyst development stage was defined as: 0, pre-blastocyst; 1, blastocoel cavity occupying < 50%; 2, blastocoel cavity occupying approximately 50% of embryo; 3, blastocoel cavity occupying > 50%; 4, blastocoel cavity fully expanded with thinning zona pellucida, 5, partially hatching blastocyst; 6, completely hatched blastocyst. Blastocyst formation rate was calculated as the percentage of embryos reaching stage ≥ 1 among total cultured oocytes. The developmental stage was averaged across all cultured oocytes as

 doi:10.20944/preprints202509.0580.v1



an individual value. Blastocysts at stage ≥ 3 were further assessed for inner cell mass (ICM) and trophectoderm (TE) quality. ICM and TE were graded as follows: ICM: A = tightly packed distinct cells, B = loosely grouped cells, C = few/disorganized cells; TE: A: cohesive epithelium, B = fewer/looser cells, C = sparse/disorganized cells. Each blastocyst was classified as good (ICM and TE: AA, AB, BA), moderate (BB), low (BC, CB), or very low (CC) grade [6], and scored 3, 2, 1, or 0, respectively. The average score was calculated as an individual value.

### *2.10. Embryo Transfer Procedure*

Embryo transfer was performed under transvaginal ultrasound guidance using a soft embryo transfer catheter. All transfers were conducted in hormone replacement cycles. Endometrial preparation was initiated on day 2 of the menstrual cycle with estradiol and continued until endometrial thickness reached ≥8 mm on ultrasound. Once the target endometrial thickness was achieved, oral dydrogesterone (30 mg/day) and intravaginal progesterone (300 mg/day) were administered for 5 consecutive days before embryo transfer. A single embryo was selected for transfer based on morphological quality and developmental stage, typically on day 5. The selected embryo was loaded into the catheter in a minimal volume of culture medium and gently deposited approximately 1–2 cm from the uterine fundus. Implantation success was assessed 7 days after transfer by detecting serum hCG. Implantation success rate was expressed as the value per embryo transfer.

### *2.11. Statistical Analyses*

Statistical analyses were performed with JMP Pro statistical software version 17.0.0 (SAS Institution Inc., Cary, NC, USA). The normality of data distribution was tested with the Shapiro–Wilk test. Data with normal and non-normal distributions and category were expressed as mean ± standard deviation (SD), median with 25 and 75 percentiles, and percentage, respectively. Correlations were analyzed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed or categorical data. Multiple stepwise regression analyses were conducted with dependent variables including blastocyst formation rate, development stage, and quality grade, and independent variables including male and female ages and male factors such as body mass index (BMI), SAF, serum laboratory parameters, lifestyle habits, sperm parameters. Two groups were compared with unpaired t-test, Wilcoxon signed-rank test, or Fisher's exact test, as appropriate. Statistical significance was set at p-values of $<0.05$.

## 3. Results

### *3.1. Background Characteristics of the Study Participants*

As shown in Table 1, 67% of the participants and 71% of their partners were ≤ 40 years of age. Most participants had no comorbidities, whereas 57% of the partners had any comorbidity that could potentially affect fertilization. Table 2 summarizes the participants' metabolic parameters. Most of them were not obese, and SAF values were within the age-adjusted normal range. Serum d-ROMs levels were normal in 48% of the participants, slightly high in 24%, and high in 28%. Serum glucose levels were normal in all participants, while serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), Zn, and free testosterone were normal in 81%, 76%, 62%, 48%, and 76% of the participants; respectively. Lifestyle habits are presented in Table 3. Approximately 50% of the participants exercised regularly, 65% were non-smokers, and 48% did not drink regularly. More than half reported not experiencing mental stress and having healthy dietary habits.

 doi:10.20944/preprints202509.0580.v1



**Table 1.** Background characteristics of the study participants.

| Participants (men) | | Partners (women) | |
|---|---|---|---|
| Number | 21 | Number | 21 |
| Age (years old) | 38.9 ± 6.0 | Age (years old) | 36.3 ± 5.1 |
| Comorbidities | | Comorbidities | |
| Hyperuricemia (*n*) | 1 [4.8%] | Polycystic ovary syndrome (*n*) | 6 [28.6%] |
| Hypercholesterolemia (*n*) | 1 [4.8%] | Uterine fibroids (*n*) | 3 [14.3%] |
| Hypertension (*n*) | 1 [4.8%] | Endometriosis (*n*) | 2 [9.5%] |
| | | Sexual dysfunction (*n*) | 1 [4.8%] |

Square brackets indicate percentages. *n* represents the number of subjects.

**Table 2.** Clinical parameters of the study participants.

| | |
|---|---|
| Height (cm) | 171.5 ± 5.7 |
| Body weight (kg) | 69.0 ± 11.5 |
| BMI (kg/m$^2$) | 23.4 ± 3.2 |
| Wasit circumstance (cm) | 83 [78–91] |
| SAF (AU) | 1.7 [1.6–1.9] |
| Serum AGEs (μg/mL) | 0.24 [0.20–0.34] |
| Serum d-ROMs (U.CARR) | 309 ± 55 |
| Serum glucose (mg/dL) | 103 [99–117] |
| Serum LDL-C (mg/dL) | 113 ± 30 |
| Serum HDL-C (mg/dL) | 53 ± 16 |
| Serum TG (mg/dL) | 168 ± 89 |
| Serum Zn (ug/dL) | 77 [69–89] |
| Serum free testosterone (pg/mL) | 11.8 ± 3.4 |

Data with normal and non-normal distributions and categorical data were expressed as mean ± standard deviation (SD), median with 25th and 75th percentiles, and percentage, respectively. BMI: body mass index, SAF: skin autofluorescence intensity, AGEs: advanced glycation end products, d-ROMs: diacron-reactive oxygen metabolites, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglycerides, Zn: zinc.

 doi:10.20944/preprints202509.0580.v1



**Table 3.** Lifestyle habits of the study participants.

| | | | | |
|---|---|---|---|---|
| A. How often do you engage in physical activity such as a 30-minute walk or equivalent exercise | | | | |
| 1. Not at all | 2. No exercise, but walk around at home or office | 3. Once a week | 4. 2–3 times a week | 5. More than 4 times a week |
| 3 [14.3%] | 8 [38.1%] | 3 [14.3%] | 4 [19.0%] | 3 [14.3%] |
| B. How long have you been smoking? | | | | |
| 1. Daily smoking for ≥10 years | 2. Daily smoking for <10 years | 3. Quit smoking within 1 year | 4. Quit smoking more than 1 year ago | 5. Never smoked |
| 6 [28.6%] | 1 [4.8%] | 2 [9.5%] | 1 [4.8%] | 11 [52.4%] |
| C. How frequently do you consume alcoholic beverages? | | | | |
| 1. More than 4 times a week | 2. 2–3 times a week | 3. Once a week | 4. Sometimes | 5. Never |
| 6 [28.6%] | 2 [9.5%] | 3 [14.3%] | 6 [28.6%] | 4 [19.0%] |
| D. How many hours do you sleep each day? | | | | |
| 1. Less than 4 hours | 2. 4–5 hours | 3. 5–7 hours | 4. 7–8 hours | 5. More than 8 hours |
| 3 [14.3%] | 8 [38.1%] | 0 [0%] | 9 [42.9%] | 1 [4.8%] |
| E. Do you feel mentally stressed? | | | | |
| 1. Strongly agree | 2. Agree | 3. Neutral | 4. Disagree | 5. Strongly disagree |
| 1 [4.8%] | 7 [33.3%] | 0 [0%] | 11 [52.4%] | 2 [9.5%] |
| F. Do you eat plenty of vegetables? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 2 [9.5%] | 3 [14.3%] | 0 [0%] | 6 [28.6%] | 10 [47.6%] |
| G. Do you eat breakfast daily? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 6 [28.6%] | 3 [14.3%] | 1 [4.8%] | 4 [19.0%] | 7 [33.3%] |
| H. Do you try to avoid overeating beyond about 80% fullness? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 3 [14.3%] | 4 [19.0%] | 2 [9.5%] | 10 [47.6%] | 2 [9.5%] |
| I. Do you try to avoid consuming greasy foods? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 6 [28.6%] | 6 [28.6%] | 6 [28.6%] | 2 [9.5%] | 1 [4.8%] |
| J. Do you try to avoid consuming processed foods? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |

 doi:10.20944/preprints202509.0580.v1



| | | | | |
|---|---|---|---|---|
| 5 [23.8%] | 5 [23.8%] | 4 [19.0%] | 5 [23.8%] | 2 [9.5%] |
| K. Do you try to avoid consuming sugary foods, such as cakes and candies? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 6 [28.6%] | 7 [33.3%] | 0 [0%] | 2 [9.5%] | 6 [28.6%] |
| L. Do you eat vegetables first during your meals? | | | | |
| 1. Strongly disagree | 2. Disagree | 3. Neutral | 4. Agree | 5. Strongly agree |
| 2 [9.5%] | 3 [14.3%] | 4 [19.0%] | 7 [33.3%] | 5 [23.8%] |

Values show the number of the participants with the percentage in square brackets.

*3.2. Background Sperm Parameters and cIVF Outcomes*

Sperm parameters are presented in Table 4. Total sperm count was normospermia in 67% and oligospermia in 33% of the participants. Total motility and progressive motility of sperm were within the normal ranges in 90% of cases. Sperm DFI was classified as mild in 76%, moderate in 19%, and severe in 5% of the participants. Semen ORP levels were within the normal range in all participants. Blastocyst formation was observed in 32% of the oocytes used for cIVF. Among the formed blastocysts, good blastocyst development stage (stage ≥ 4) and quality grade (score = 3) were observed in 71% and 39%, respectively. Of the 21 couples, 18 women underwent blastocyst transfer, with an implantation success rate of 50%.

**Table 4.** Background sperm parameters and in vitro fertilization outcomes.

| | |
|---|---|
| Sperm volume (mL) | 3.3 ± 1.1 |
| Sperm concentration ($10^6$/mL) | 130 [101–201] |
| Total sperm count (×$10^6$ per ejaculate) | 432 [261–666] |
| Sperm total motility (%) | 64 ± 15 |
| Sperm progressive motility (%) | 55 ± 16 |
| Sperm ORP (mV/$10^6$ sperm/mL) | 0.22 [0.17–0.41] |
| Sperm DFI (%) | 6.5 [3.0–15.4] |
| Blastocyst formation rate (%) | 29.6 [16.7–39.2] |
| Blastocyst development stage | 2.6 ± 1.4 |
| Blastocyst quality grade | 1.3 ± 0.9 |
| Implantation success after transfer (%) | 52 [0–100] |

Data with normal and non-normal distributions were expressed as mean ± SD and median with 25th and 75th percentiles, respectively. Data on blastocyst growth stage, blastocyst quality grade, and pregnancy success after blastocyst transfer were available for 18 participants due to blastocyst formation failure in 3 cases. ORP, oxidation-reduction potential; DFI, DNA fragmentation index.

 doi:10.20944/preprints202509.0580.v1



*3.3. Correlation of Metabolic, Lifestyle, and Sperm Factors with cIVF Outcomes*

As shown in Table 5, sperm DFI was negatively correlated with blastocyst formation rate, development stage, and quality grade. Furthermore, male age was also negatively associated with blastocyte quality grade. However, multiple regression analysis showed that sperm DFI was the only variable inversely associated with blastocyst formation rate, developmental stage, and quality. Implantation success was significantly associated with female age ($r$ = –0.50, $p$ = 0.04), but not with any male parameters.

**Table 5.** Correlation of metabolic, lifestyle, and sperm factors associated with blastocyst outcomes.

| | Blastocyst formation rate | | Blastocyst development stage | | Blastocyst quality grade | |
|---|---|---|---|---|---|---|
| Variables | *r* | *p* | *r* | *p* | *r* | *p* |
| Male age (years old) | | 0.98 | | 0.26 | −0.44 | 0.04 |
| Female age (years old) | | 0.37 | | 0.20 | | 0.07 |
| Body weight (kg) | | 0.55 | | 0.96 | | 0.94 |
| BMI (kg/m$^2$) | | 0.78 | | 0.66 | | 0.62 |
| Wasit circumstance (cm) | | 0.90 | | 0.80 | | 0.90 |
| SAF (AU) | | 0.76 | | 0.19 | | 0.08 |
| Serum AGEs (μg/mL) | | 0.96 | | 0.90 | | 0.86 |
| Serum d-ROMs (U.CARR) | | 0.80 | | 0.52 | | 0.40 |
| Serum glucose (mg/dL) | | 0.80 | | 0.61 | | 0.49 |
| Serum LDL-C (mg/dL) | | 0.66 | | 0.55 | | 0.48 |
| Serum HDL-C (mg/dL) | | 0.23 | | 0.17 | | 0.35 |
| Serum TG (mg/dL) | | 0.35 | | 0.83 | | 0.70 |
| Serum Zn (ug/dL) | | 0.51 | | 0.29 | | 0.30 |
| Serum free testosterone (pg/mL) | | 0.70 | | 0.93 | | 0.88 |
| **Exercise-related lifestyle habits** | | 0.11 | | 0.61 | | 0.39 |
| **Smoking-related lifestyle habits** | | 0.14 | | 0.09 | | 0.19 |
| **Alcohol-related lifestyle habits** | | 0.66 | | 0.60 | | 0.86 |
| **Mental stress–related lifestyle habits** | | 0.81 | | 0.80 | | 0.57 |
| **Diet-related lifestyle habits** | | 0.46 | | 0.87 | | 0.75 |
| Sperm volume (mL) | | 0.59 | | 0.46 | | 0.44 |
| Sperm concentration (10$^6$/mL) | | 0.30 | | 0.32 | | 0.65 |
| Total sperm count (×10$^6$ per ejaculate) | | 0.56 | | 0.55 | | 0.99 |
| Sperm total motility (%) | | 0.89 | | 0.21 | | 0.47 |
| Sperm progressive motility (%) | | 1.00 | | 0.14 | | 0.44 |
| Sperm ORP (mV/10$^6$ sperm/mL) | | 0.50 | | 0.61 | | 0.81 |
| Sperm DFI (%) | −0.50 | 0.03 | −0.45 | 0.04 | −0.45 | 0.04 |

All data presented refer to male parameters, except for female age. *r* values correspond to Pearson's correlation coefficient for variables with normal distribution and to Spearman's correlation coefficient for variables without normal distribution.

 doi:10.20944/preprints202509.0580.v1



*3.4. Correlation of Metabolic, Lifestyle, and Sperm Factors with Sperm DFI*

As shown in Supplemental Table 1, serum HDL-C level was the sole male factor associated with sperm DFI ($r$ = 0.52, $p$ = 0.02). Subgroup analysis according to DFI severity revealed no significant differences in metabolic, lifestyle, and sperm factors between the mild and moderate/severe groups (Supplemental Table 2).

## 4. Discussion

ART is a widely utilized therapeutic strategy for assisting infertility in many countries [2–4]. While the acquisition of well-developed embryos through IVF is one of the key determinants of ART success [6], the contribution of specific male factors remains incompletely understood. This study aimed to identify male factors associated with embryo formation and quality in cIVF. In the present study, most participants exhibited generally normal metabolic parameters, except for high ratio of hypertriglyceridemia, and maintained healthy lifestyle habits. Regarding sperm parameters, 33% met the criteria for oligospermia, while other sperm parameters, including DFI, were mostly within normal ranges. In this population, multiple regression analyses revealed that, among evaluated metabolic, lifestyle, and sperm parameters, sperm DFI was the only factor that correlated with impaired embryo formation and quality in cIVF. Recent studies have reported that, in men with infertility, sperm DNA fragmentation index (DFI) is associated with fertilization ability independently of conventional sperm parameters, such as concentration, motility, and morphology [19, 20]. Furthermore, in men diagnosed with male infertility, high sperm DFI (>30%) has been reported to be associated with lower high-quality embryo rates, blastocyst development rates, and implantation success rates in IVF compared with mild (<15%) or moderate (15–30%) DFI [16, 17]. However, it remains unclear whether sperm DFI is associated with IVF outcomes in men with non-severe DFI, particularly those with relatively preserved spermatogenesis. The present findings suggest that sperm DFI may be a negative predictor of blastocyst formation and quality in cIVF, even among normospermic and oligospermic men with non-severe DFI, and that its assessment should be considered in cases of poor embryo development or quality in this population.

Several studies have reported male factors associated with s[19,20perm DFI, such as age, BMI, and smoking habits [16,21]; however, conflicting results have been reported [22,23]. In the present study, only serum HDL-C levels were associated with sperm DFI, consistent with the findings of our original study [9]. HDL is a lipoprotein that transports cholesterol from peripheral tissues to the liver [24]. In a previous study evaluating the association between serum lipids and sperm parameters, abnormal sperm morphology was more frequent in men with HDL-C levels >57 mg/dL or <42 mg/dL [25]. Another study assessing both blood and seminal lipids has reported that seminal plasma contains HDL-C at approximately 25% of serum levels, and that seminal HDL-C levels are higher in men with sperm abnormalities than in those without them [26]. These previous findings support the observed association between serum HDL-C and sperm DFI in this study. However, the molecular mechanisms linking serum HDL-C and sperm DFI remain unclear.

Sperm is known to be vulnerable to oxidative stress, exposure to which can cause DNA fragmentation [27,28]. In this analysis, systemic and seminal oxidative stress was assessed as serum d-ROMs and semen ORP, respectively. Additionally, AGEs, which form and accumulate with aging and lifestyle factors and generate oxidative stress through interactions with their cell-surface receptor, were measured in serum and skin [29–31]. However, none of these parameters were associated with sperm DFI. Consistently, a previous study also reported no correlation between systemic oxidative stress levels and sperm DFI [32]. In our previous animal study using a mouse model of diabetes and obesity, glyceraldehyde-derived AGEs, one of the most toxic AGE subtypes, accumulated in the interstitium of the testis, accompanied by increased oxidative stress levels, but these changes were not observed in the seminiferous tubules [33]. The seminiferous tubules are protected from circulating factors by the blood–testis barrier; therefore, systemic oxidative stress and AGEs may not be directly associated with sperm DFI. Regarding seminal oxidative stress, semen ORP

 doi:10.20944/preprints202509.0580.v1



levels have been reported to be positively associated with sperm DFI in men with infertility [34]. However, in the present study, sperm parameters were normal in most participants, except for oligospermia in 33%, and semen ORP levels were not elevated in any participants. This may account for the absence of an association between sperm DFI and semen ORP.

Many clinical studies have investigated whether sperm DFI is associated with implantation and pregnancy success after embryo transfer; however, findings have been conflicting. A recent meta-analysis of 92 studies reported a negative association between sperm DFI and pregnancy success in 35 studies, whereas no association was found in the remaining 57 studies [35]. In the present study, no male factors, including sperm DFI, were identified as being associated with pregnancy success after embryo transfer. In cIVF, blastocysts were formed in 32% of the oocytes, and good blastocyst development and quality were achieved in 71% and 39% of formed blastocysts, respectively. This allowed the selection of high-quality blastocysts with a greater likelihood of implantation success, which may explain why sperm DFI was associated with blastocyst formation and quality but not directly with implantation success after embryo transfer.

As a limitation of this study, IVF was performed with the conventional method [5]. Therefore, it remains unclear whether a similar association would be observed with other IVF methods, such as intracytoplasmic sperm injection. Second, sperm DFI was assessed using sperm smears with TUNEL method. Other methods for sperm DFI assessment, such as sperm chromatin structure assay and sperm chromatin dispersion assay, are widely used in clinical settings due to their simpler procedures [35]. TUNEL method is considered more sensitive than the other methods, although its measurement procedure is somewhat complex [36]. Thus, it should be noted that similar association may not be observed when sperm DFI is assessed by other methods. Third, this study had a small sample size, and therefore the present findings should be further confirmed in a large-scale study to provide more robust evidence.

## 5. Conclusions

The present findings indicate that sperm DNA fragmentation may negatively affect high-quality embryo formation in cIVF, even among normospermic and oligospermic men with non-severe sperm DFI. Sperm DFI could serve as a valuable negative predictive marker for ART success in this population.

**Supplementary Materials:** Supplemental Table S1. Correlation of metabolic, lifestyle, and sperm factors associated with sperm DFI. Supplemental Table S2. Comparison of metabolic, lifestyle, and sperm factors between groups with mild and moderate/severe sperm DFI. The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

**Author Contributions:** Conceptualization: Y.M., A.S., and S.Y.; Methodology: Y.M., L.C., S.N., M.S., and M.O.; Software: Y.M. and L.C.; Validation: Y.M. and L.C.; Formal Analysis: Y.M.; Investigation: Y.M., L.C., S.N., and M.S.; Resources: A.S. and S.Y.; Data Curation: Y.M. and L.C.; Writing—Original Draft Preparation: Y.M.; Writing—Review and Editing: L.C., S.N., M.S., M.O., A.S., and S.Y.; Visualization: Y.M.; Supervision: M.O., A.S., and S.Y.; Project Administration: Y.M., A.S., and S.Y.; Funding Acquisition: Y.S. All authors approved the final version of the manuscript. Y.M. is the guarantor of this work and is responsible for its integrity.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethic Committee of Showa University approved this study protocol (Approval No: 2023-047-B, approval date: July 18, 2023).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

 doi:10.20944/preprints202509.0580.v1



**Conflicts of Interest:** Y.M. received financial support from Boehringer Ingelheim GmbH (Binger Strasse 173, 55216 Ingelheim Am Rhein, German) and Ono Pharmaceutical CO., LTD (Osaka, Japan). The other authors declare no other competing interests.

## References


1. Fauser, B.C.J.M.; Adamson, G.D.; Boivin, J.; Chambers, G.M.; de Geyter, C.; Dyer, S.; Inhorn, M.C.; Schmidt, L.; Serour, G.I.; Tarlatzis, B.; et al. Declining global fertility rates and the implications for family planning and family building: an IFFS consensus document based on a narrative review of the literature. *Hum Reprod Update*. **2024**, 30, 153–173.
2. Smeenk, J.; Wyns, C.; De Geyter, C.; Kupka, M.; Bergh, C.; Cuevas Saiz, I.; De Neubourg, D.; Rezabek, K.; Tandler-Schneider, A.; Rugescu, I.; et al. ART in Europe, 2019: results generated from European registries by ESHRE. *Hum Reprod.* **2023**, 38, 2321–2338.
3. Wang, S.F.; Seifer, D.B. Assessment of a Decade of Change in U.S. Assisted Reproductive Technology Cumulative Live-Birth Rates: 2004-2009 Compared With 2014-2020. *Obstet Gynecol*. **2024**, 143, 839–848.
4. Katagiri, Y.; Jwa, S.C.; Kuwahara, A.; Iwasa, T.; Ono, M.; Kato, K.; Kishi, H.; Kuwabara, Y.; Harada, M.; Hamatani, T.; et al. Assisted reproductive technology in Japan: A summary report for 2020 by the Ethics Committee of the Japan Society of obstetrics and gynecology. *Reprod Med Biol.* **2023**, 22, e12494.
5. Jain, M.; Singh, M. Assisted Reproductive Technology (ART) Techniques. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Updated 2023 Jun 7; cited 2025 Aug 19. Available from: https://www.ncbi.nlm.nih.gov/books/NBK576409/
6. Zou, H.; Kemper, J.M.; Hammond, E.R.; Xu, F.; Liu, G.; Xue, L.; Bai, X.; Liao, H.; Xue, S.; Zhao, S.; et al. Blastocyst quality and reproductive and perinatal outcomes: a multinational multicentre observational study. *Hum Reprod*. **2023**, 38, 2391–2399.
7. Agarwal, A.; Mulgund, A.; Hamada, A.; Chyatte, M.R. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. **2015**, 13, 37.
8. Huang, B.; Wang, Z.; Kong, Y.; Jin, M.; Ma, L. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of global burden of disease study. *BMC Public Health*. **2023**, 23, 2195.
9. Chen, L.; Mori, Y.; Nishii, S.; Sakamoto, M.; Ohara, M.; Yamagishi, S.I.; Sekizawa, A. Impact of Oxidative Stress on Sperm Quality in Oligozoospermia and Normozoospermia Males Without Obvious Causes of Infertility. *J. Clin. Med*. **2024**, 13, 7158.
10. Isami, F.; West, B.J.; Nakajima, S.; Yamagishi, S.I. Association of advanced glycation end products, evaluated by skin autofluorescence, with lifestyle habits in a general Japanese population. *J Int Med Res*. **2018**, 46, 1043–1051.
11. Ohara, M.; Nagaike, H.; Fujikawa, T.; Kohata, Y.; Ogawa, M.; Omachi, T.; Sasajima, R.; Chiba, H.; Ara, T.; Sugawara A.; et al. Effects of omarigliptin on glucose variability and oxidative stress in type 2 diabetes patients: A prospective study. *Diabetes Res Clin Pract*. **2021**, 179, 108999.
12. Takeuchi, M.; Takino, J.; Furuno, S.; Shirai, H.; Kawakami, M.; Muramatsu, M.; Kobayashi, Y.; Yamagishi, S. Assessment of the concentrations of various advanced glycation end-products in beverages and foods that are commonly consumed in Japan. *PLoS One*. **2015**, 10, e0118652.
13. World Health Organization (WHO). WHO laboratory manual for the examination and processing of human semen, 6th ed; World Health Organization: Geneva, Switzerland, 2021; pp. 9–64.
14. Agarwal, A.; Panner, Selvam. M.K.; Arafa, M.; Okada, H.; Homa, S.; Killeen, A.; Balaban, B.; Saleh, R.; Armagan, A.; Roychoudhury, S.; et al. Multi-center evaluation of oxidation-reduction potential by the MiOXSYS in males with abnormal semen. *Asian J Androl*. **2019**, 21, 565–569.
15. Cicek, O.S.Y.; Kaya, G.; Alyuruk, B.; Doger, E.; Girisen, T.; Filiz, S. The association of seminal oxidation reduction potential with sperm parameters in patients with unexplained and male factor infertility. *Int Braz J Urol*. **2021**, 47, 112–119.
16. Al Omrani, B.; Al Eisa, N.; Javed, M.; Al Ghedan, M.; Al Matrafi, H.; Al Sufyan, H. Associations of sperm DNA fragmentation with lifestyle factors and semen parameters of Saudi men and its impact on ICSI outcome. *Reprod Biol Endocrinol*. **2018**, 16, 49.

 doi:10.20944/preprints202509.0580.v1




17. Du, C.; Tuo, Y. Correlation of DNA fragments with routine semen parameters and lifestyle and their impact on assisted reproductive outcomes. *Rev Int Androl*. **2023**, 21, 100337.
18. Gardner, D.K.; Schoolcraft, W.B. Culture and transfer of human blastocysts. *Curr Opin Obstet Gynecol*. **1999**, 11, 307–311.
19. Alahmar, A.T.; Singh, R.; Palani, A. Sperm DNA Fragmentation in Reproductive Medicine: A Review. *J Hum Reprod Sci*. **2022**, 15, 206–218.
20. Zhao, J.; Zhang, Q.; Wang, Y.; Li, Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril*. **2014**, 102, 998–1005.e8.
21. Kort, H.I.; Massey, J.B.; Elsner, C.W.; Mitchell-Leef, D.; Shapiro, D.B.; Witt, M.A.; Roudebush, W.E. Impact of body mass index values on sperm quantity and quality. *J Androl*. **2006**, 27, 450–452.
22. Rybar, R.; Kopecka, V.; Prinosilova, P.; Markova, P.; Rubes, J. Male obesity and age in relationship to semen parameters and sperm chromatin integrity. *Andrologia*. **2011**, 43, 286–291.
23. Nicopoullos, J.D.; Gilling-Smith, C.; Almeida, P.A.; Homa, S.; Norman-Taylor, J.Q.; Ramsay J.W. Sperm DNA fragmentation in subfertile men: the effect on the outcome of intracytoplasmic sperm injection and correlation with sperm variables. *BJU Int*. **2008**, 101, 1553–1560.
24. Albitar, O.; D'Souza, C.M.; Adeghate E.A. Effects of Lipoproteins on Metabolic Health. *Nutrients*. **2024**, 16, 2156.
25. Liu, C.Y.; Chou, Y.C.; Lin, S.H.; Wu, S.T.; Cha, T.L.; Chen, H.I.; Tsao, C.W. Serum lipid profiles are associated with semen quality. *Asian J Androl*. **2017**, 19, 633–638.
26. Lu, J.C.; Jing, J.; Yao, Q.; Fan, K.; Wang, G.H.; Feng, R.X.; Liang, Y.J.; Chen, L.; Ge, Y.F.; Yao, B. Relationship between Lipids Levels of Serum and Seminal Plasma and Semen Parameters in 631 Chinese Subfertile Men. *PLoS One*. **2016**, 11, e0146304.
27. Agarwal, A.; Virk, G.; Ong, C.; du Plessis, S.S. Effect of oxidative stress on male reproduction. *World J Mens Health*. **2014**, 32, 1–17.
28. Nowicka-Bauer, K.; Nixon, B. Molecular Changes Induced by Oxidative Stress that Impair Human Sperm Motility. *Antioxidants (Basel)*. **2020**, 9, 134.
29. Yamagishi, S.; Matsui, T. Pathologic role of dietary advanced glycation end products in cardiometabolic disorders, and therapeutic intervention. *Nutrition*. **2016**, 32, 157–165.
30. Yamagishi, S.I.; Matsui, T. Therapeutic Potential of DNA-aptamers Raised Against AGE-RAGE Axis in Diabetes-related Complications. *Curr Pharm Des*. **2018**, 24, 2802–2809.
31. Yamagishi, S.; Nakamura, N.; Suematsu, M.; Kaseda, K.; Matsui, T. Advanced Glycation End Products: A Molecular Target for Vascular Complications in Diabetes. *Mol Med*. **2015**, 21, S32–40.
32. Guz, J.; Gackowski, D.; Foksinski, M.; Rozalski, R.; Zarakowska, E.; Siomek, A.; Szpila, A.; Kotzbach, M.; Kotzbach, R.; Olinski, R. Comparison of oxidative stress/DNA damage in semen and blood of fertile and infertile men. *PLoS One*. **2013**, 8, e68490.
33. Mori, Y.; Terasaki, M.; Osaka, N.; Fujikawa, T.; Yashima, H.; Saito, T.; Kataoka, Y.; Ohara, M.; Higashimoto, Y.; Matsui, T.; et al. DNA Aptamer Raised against Advanced Glycation End Products Improves Sperm Concentration, Motility, and Viability by Suppressing Receptors for Advanced Glycation End Product-Induced Oxidative Stress and Inflammation in the Testes of Diabetic Mice. *Int J Mol Sci*. **2024**, 25, 5947.
34. Furtado, T.P.; Osadchiy, V.; Furtado, M.H. Semen static oxidation-reduction potential is not helpful in evaluating male fertility. *Andrology*. **2024** Oct 10. doi: 10.1111/andr.13759. Epub ahead of print.
35. Simon, L.; Emery, B.R.; Carrell, D.T. Review: Diagnosis and impact of sperm DNA alterations in assisted reproduction. *Best Pract Res Clin Obstet Gynaecol*. **2017**, 44, 38–56.
36. Sharma, R.K.; Sabanegh, E.; Mahfouz, R.; Gupta, S.; Thiyagarajan, A.; Agarwal A. TUNEL as a test for sperm DNA damage in the evaluation of male infertility. *Urology*. **2010**, 76, 1380–1386.