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Exploring the Influence of Mild Sperm DNA Fragmentation on In Vitro Fertilization Outcomes in Women With Polycystic Ovary Syndrome

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Abstract

Objectives: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 4–20% of women worldwide and contributes to infertility in up to 80% of cases. Previous studies have shown that severe sperm DNA fragmentation index (DFI ≥25%) adversely affects IVF success and increases pregnancy complications. However, delaying IVF to treat DFI may lead to reduced ovarian reserve and greater psychological burden in women. The objective of this study is to assess the effect of mild sperm DNA fragmentation (15% ≤ DFI ≤ 25%) on IVF outcomes in PCOS women compared with controls.

Materials and Methods: This retrospective study included 282 infertile couples undergoing their first In vitro fertilization (IVF)/Intracytoplasmic sperm injection (ICSI) cycle. Couples were stratified according to DFI level (<15% vs. 15–25%). Sperm DFI was measured using the halo test. Outcomes assessed included fertilization, embryo quality, clinical pregnancy, and live birth.

Results: Men with DFI 15–25% had significantly lower sperm concentration (14.0±13.3 million/mL vs. 30.0±13.7 million/mL, $P<0.001$), progressive motility (4.7±5.6% vs. 10.3±6.5%, $P<0.001$), vitality (63.1±27.2% vs. 85.3±7.2%, $P<0.001$), and fertilization rate (65.3±17.8% vs. 72.0±22.3%, $P=0.01$). Clinical pregnancy was achieved in 40.1% of participants (34.4% live birth, 5.7% pregnancy loss), with no significant difference between DFI groups ($P=0.99$).

Conclusions: Mild DFI (15–25%) is associated with poorer sperm parameters but does not significantly affect pregnancy outcomes in IVF/ICSI cycles. Mild DFI elevation alone may not warrant delaying IVF treatment in PCOS patients.

Keywords: Sperm DNA fragmentation, DFI, Polycystic ovary syndrome (PCOS), In vitro fertilization (IVF), Intracytoplasmic sperm injection (ICSI), Infertility, Pregnancy outcomes

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogeneous and multifactorial endocrine disorder that affects approximately 4–20% of women worldwide (1-5). This syndrome is characterized by hormonal imbalances, insulin resistance, and ovulatory dysfunction, leading to infertility in up to 80% of cases (5-8). The exact causes of PCOS remain unclear, but it is thought to be driven by a complex interplay of environmental, genetic, and epigenetic factors (9).

Despite in vitro fertilization (IVF) being a successful treatment option for many couples, PCOS patients still face elevated risks of miscarriage, biochemical pregnancy loss, and lower fertilization rates (10-12).

Furthermore, sperm quality plays a crucial role in embryo development. Sperm DNA fragmentation index (DFI), which measures chromatin integrity, has been linked to male fertility potential and pregnancy outcomes (13). Moreover, infertile men tend to have higher DFI levels than fertile men (14).

Prior investigations have shown correlations between severe SDF and the development of embryos in the IVF center (15,16). Specifically, DFI at the fertilization step correlates with reduced fertilization rates for IVF and intracytoplasmic sperm injection (ICSI) (17).

Given the significance of sperm quality in the context of PCOS, it is essential to explore the association between SDF and IVF outcomes in this population (12). Investigating the effect of SDF in women with PCOS is particularly important because these women may have a higher risk of complications during pregnancy. For example, a study published in the *Al-Azhar International Medical Journal* reported that patients with PCOS and obesity were at a higher risk of spontaneous abortion(18). Prior study demonstrated the inverse effect of severe sperm DNA fragmentation index (DFI ≥ 25%) on the IVF outcome and pregnancy disorders; thus, it has been accepted that male treatment for 3-4 months may improve sperm DFI and IVF outcome. On the other hand, delaying IVF treatment may increase psychological pressure and even decrease ovarian reserve in women (19).

In this study, we examined the influence of mild DFI

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Key Messages

- Mild sperm DNA fragmentation impairs semen quality but does not reduce IVF pregnancy success in PCOS women.
- IVF treatment need not be delayed for PCOS patients based solely on a mild DFI elevation.

disorder (15≤ DFI≤25) on the outcome of IVF treatment in women with PCOS and a control group. The research result can help choose the protocol to start or delay the ovarian stimulation. This study aimed to evaluate whether mild sperm DNA fragmentation (15–25% DFI) impacts IVF outcomes in PCOS patients.

Materials and Methods

Patients and Experimental Design

This study enrolled 282 infertile couples undergoing their first IVF treatment at the Kamali Hospital, Karaj, Iran, during 2022-2023. All participants had normal chromosomal karyotypes and were selected for cleavage-stage embryo or blastocyst transfer. Male partners were required to be under 50 years old and had not recently used any medications harmful to sperm function. Based on the Rotterdam criteria, participants were randomly assigned to one of two groups: a PCOS group with 122 individuals and a control group with 160 participants. Both groups were further stratified according to sperm DFI: 25 ≤ DFI ≤ 15% and 15 < DFI. Control subjects were recruited with these inclusion criteria: female age < 42 years, basal follicle-stimulating hormone (FSH) level < 10 mIU/mL, BMI 18.5-24.9, and infertility solely attributed to dysfunction of the fallopian tube. PCOS patients were defined according to the 2003 Rotterdam criteria, which require at least two of these features: clinical symptoms caused by high testosterone and/or hyperandrogenemia, oligo-anovulation, or ultrasound evidence of ≥12 follicles with diameters 2-9 mm in one or both ovaries, or an ovarian volume exceeding 10 ml. Eligibility criteria for PCOS patients included: female age < 42 years, infertility attributed to fallopian tube factor, and FSH level < 10 mIU/mL.

Sample Size

Based on prior studies, an effect size of 0.3, α=0.05, and power of 80% indicated a required sample size of 270; thus, 282 couples were included.

Semen Analysis

Semen was collected in sterile containers after 3-7 days of abstinence and stored in a controlled environment to prevent changes that may influence the quality of sperm. After 30 minutes of liquefaction, sperm viability and concentration were evaluated using a Makler chamber. Sperm morphology was assessed via hematoxylin and eosin (H&E) staining. To separate the precipitate, samples were processed by two discontinuous density gradients (80%–40%) and centrifuged after liquefaction. The precipitant was then washed twice with HTF medium plus 10% human albumin and resuspended in a suitable volume of the same medium.

Sperm DNA Fragmentation Index Detection

Sperm DFI was assessed in semen samples using the Sperm DNA Fragmentation Assay Kit (IVF Co, Tehran, Iran) based on the halo test previously described by Fernández et al. Three hundred sperm were analyzed per sample. All evaluations were performed independently by two observers with <5% variability (20). After liquefaction, the semen was diluted to a concentration of 5-10 × 10^6/mL with HTF medium. According to the manufacturer's procedure, the samples were prepared and examined under a light microscope at 100× magnification. Three hundred spermatozoa were scored for DFI, which was determined by observing the formation of halos around sperm nuclei following exposure to lysing solution. Non-fragmented sperm exhibited a characteristic halo pattern, while fragmented sperm showed either minor or no halos. The DFI was calculated as a percentage of total scored spermatozoa.

Ovarian Stimulation and Laboratory Tests

Patients underwent a GnRH-antagonist protocol. Cetrotide was administered daily at a fixed dose of 0.25 mg starting from day 6 of the simulation cycle until the trigger day to prevent premature luteinizing hormone (LH) surges. Ovarian stimulation was achieved using Cinnal-f, with initial doses ranging from 150-300 IU/day, adjusted based on follicular response monitored by transvaginal ultrasound every 2-3 days. Once ≥2 follicles reached ≥18 mm, 5000-10 000 IU of hCG was given as the ovulatory trigger, and oocyte retrieval was done after 34-36 hours via ultrasound-guided aspiration. Embryo transfer was done on day 3 at the 8-cell stage, with luteal phase support provided using progesterone from the day of transfer until 16 days post-transfer. FSH, LH, and anti-Müllerian hormone (AMH) were evaluated on the third day of the IVF cycle.

In Vitro Fertilization and Embryo Transfer

Sperm preparation involved a two-step process: sperm swim-up technique after density gradient centrifugation using 40% and 80% solutions, resulting in a final concentration of 1 × 10^6/mL. ICSI was performed on the retrieved eggs one day post-fertilization, with verification of fertilization confirmed by the presence of 2pn (male pronucleus). Embryos obtained on day three of the cycle were evaluated and cryopreserved via vitrification, with subsequent transfer occurring during the freeze cycle. The assessed embryos on day three were characterized as having 6-8 cells and grades A and B.

Clinical Follow-Up

After embryo transfer at 14 days, patients underwent

serum β-human chorionic gonadotropin (β-hCG) analysis to detect biochemical pregnancy. A serum level exceeding 50 IU/L was indicative of a biochemical pregnancy. At 35 days post-transfer, patients underwent transvaginal ultrasound examination to confirm the presence of a viable pregnancy. A normal fetal heart rate detected in the uterus was considered a reliable indicator of clinical pregnancy. Pregnancy loss was described as the failure of the embryo or fetus to progress to viability, resulting in abortion.

Statistical Analysis

Statistical analyses were performed using SPSS version 24, with continuous variables presented as mean ± SD and categorical variables as percentages; comparisons were made using the t-test or chi-square test, and multivariable logistic regression was adjusted for female age, body mass index (BMI), anti-Müllerian hormone (AMH) levels, and embryo quality. Exact *P* values are reported, with statistical significance defined as $P < 0.05$.

Results

Characteristics of the study participants

The study enrolled 282 women, comprising 122 cases with PCOS and 160 controls. Demographic analysis revealed that the mean age of female participants was 33±5 years (range: 19-42), while the mean age of male partners was 37±5 years (range: 23-50). Of the male partners, 21.3% (60 cases) had ≥15 DFI, and 78.7% (222 cases) had <15 DFI (Table 1).

Fertility and Pregnancy Outcomes

Among the total participants, 40.1% (113 cases) achieved a clinical pregnancy through IVF, with 34.4% (97 cases) resulting in a live birth and 5.7% (16 cases) in a negative outcome. Conversely, 59.9% (169 cases) did not respond to assisted reproductive methods (Figure 1).

Comparison of Fertility Parameters Between PCOS Patients and Control Subjects

Table 2 presents the results of comparing fertility parameters between PCOS patients and control subjects. Our data showed that PCOS patients had significantly increased levels of AMH (5.91 ± 3.66 ng/mL) compared to control subjects (2.57 ± 1.59 ng/mL, $P=0.0001$), indicating a potential impact of PCOS on ovarian reserve. Additionally, PCOS patients had higher numbers of metaphase II oocytes (11.89 ± 7.45 vs. 9.33 ± 6.56, $P=0.003$) and embryos at day 3 (8.41 ± 5.88 vs. 6.68 ± 5.22, $P=0.01$). However, the normal fertilization rate due to the ICSI was lower in PCOS patients (70.41 ± 21.41%) compared to control subjects (81.42 ± 17.67%, $P=0.0001$), indicating a potential impact on fertilization capacity.

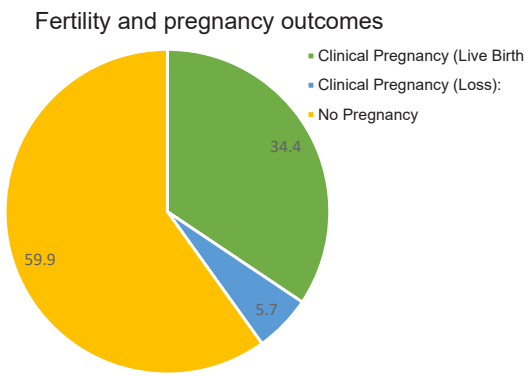

Figure 1. Fertility and Pregnancy Outcomes Among IVF Participants. Data are expressed as percent.

Table 1. Characteristics of the Study Participants

Characteristic	Value
Total number of participants	282
Number of PCOS cases	122
Number of controls	160
Mean age of female participants	33 ± 5 years (range: 19–42)
Mean age of male partners	37 ± 5 years (range: 23–50)
Male partners with DFI ≥15%	60 cases (21.3%)
Male partners with DFI <15%	222 cases (78.7%)

Baseline demographic and clinical characteristics of the study population, stratified by PCOS diagnosis and sperm DNA Fragmentation Index (DFI) status in male partners.

Comparison of Pregnancy Outcomes

Table 3 presents the comparison of pregnancy outcomes between PCOS patients and control subjects. Our data revealed no significant difference between the two groups regarding pregnancy outcome, as indicated by a *P* value of 0.42.

Comparison of Sperm Parameters in Male Partners With ≥15 DFI Versus <15 DFI

The comparison of study variables in male partners with sperm samples exhibiting ≥15 DFI and <15 DFI revealed significant differences in several key sperm parameters (Table 4). As shown in Figure 1, men with abnormal DFI had significantly lower ICSI normal fertilization rates (65.35% ± 17.83% vs. 71.99% ± 22.26%, $P=0.01$) compared to those with normal DFI. Additionally, they exhibited lower sperm concentration (14,037,931 ± 13 328 498.3 vs. 30 043 010.75 ± 13, 722 335 per ml, $P=0.0001$), lower progressive sperm motility (4.65% ± 5.63% vs. 10.3% ± 6.54%, $P=0.0001$), and higher rates of necrozoospermia (85.62% ± 12.42% vs. 69.23% ± 14.96%, $P=0.0001$). Besides, men with abnormal DFI had lower rates of normal morphology (0.65% ± 0.85% vs. 1.23% ± 0.91%, $P=0.003$) and significantly lower sperm vitality (63.13% ± 27.25% vs. 85.35% ± 7.24%, $P=0.0001$).

Association Between DFI level and Pregnancy Outcome

The association between DFI level and pregnancy outcome was investigated in PCOS patients and control

Table 2. Comparison of Fertility Parameters Vetween PCOS Patients and Control Subjects

Parameter	PCOS Group (n=122)	Control Group (n=160)	*P* value
AMH (ng/mL)	5.91 ± 3.66	2.57 ± 1.59	0.0001
Metaphase II oocytes	11.89 ± 7.45	9.33 ± 6.56	0.003
Embryos at day-3	8.41 ± 5.88	6.68 ± 5.22	0.01
Normal fertilization rate (ICSI)	70.41 ± 21.41%	81.42 ± 17.67%	0.0001

AMH: Anti-Müllerian hormone; ICSI: Intracytoplasmic sperm injection; PCOS: Polycystic ovary syndrome.
Values are expressed as mean ± SD. Comparisons between groups were performed using the independent samples t-test. A *P* value < 0.05 was considered statistically significant.

Table 3. Pregnancy Outcomes in PCOS Patients and Control Subjects

Outcome Category	PCOS Group (n=122)	Control Group (n=160)	*P* value
Clinical pregnancy	Not specified	Not specified	0.42

PCOS: Polycystic ovary syndrome.
Values are expressed as counts and percentages. Statistical pregnancy outcome rate comparisons were conducted using the chi-square test.

Table 4. Comparison of Sperm Parameters in Male Partners With ≥15% DFI vs <15% DFI

Parameter	DFI ≥ 15% (n=60)	DFI < 15% (n=222)	*P* value
Normal fertilization rate	65.35% ± 17.83	71.99% ± 22.26	0.01
Sperm concentration (per ml)	14 037 931 ± 13 328 498.3	30 043 010.75 ± 13 722 335	0.0001
Progressive motility (%)	4.65% ± 5.63	10.3% ± 6.54	0.0001
Necrozoospermia (%)	85.62% ± 12.42	69.23% ± 14.96	0.0001
Normal morphology (%)	0.65% ± 0.85	1.23% ± 0.91	0.003
Sperm vitality (%)	63.13% ± 27.25	85.35% ± 7.24	0.0001

DFI: DNA fragmentation index; ICSI: Intracytoplasmic sperm injection.
Values are expressed as mean ± SD. Sperm parameters assessed include concentration (sperm/mL), progressive motility (%), necrozoospermia (%), morphology (% normal forms), and vitality (% live sperm). Group comparisons were analyzed using the independent samples t-test, and a p-value of less than 0.05 was considered statistically significant.

subjects separately and collectively. The findings revealed no significant difference in pregnancy outcome between individuals with normal and abnormal DFI levels in any of the three study groups.

As shown in Table 5, among all participants, the pregnancy outcome did not differ significantly between those with DFI levels less than 15 and those with DFI levels 15 or greater ($P = 0.99$). Similarly, among PCOS patients, the pregnancy outcome did not differ significantly between those with DFI levels less than 15 and those with DFI levels 15 or greater ($P = 0.57$). Among control subjects, the pregnancy outcome did not differ significantly between those with DFI levels less than 15 and those with DFI levels 15 or greater ($P = 0.38$). The results presented in Table 5 indicated no significant association between DFI level and pregnancy outcome.

Discussion

This study showed mild sperm DNA fragmentation (15–25%) negatively impacted sperm quality and fertilization but did not significantly affect clinical pregnancy outcomes.

These findings suggest that high levels of DFI may negatively impact sperm quality, in line with existing scientific evidence (21-23). Despite the differences in sperm parameters, our study did not provide evidence for a significant association between DFI level and pregnancy outcome in PCOS patients or control subjects. This may be because other factors, such as oocyte quality, embryo quality, and uterine factors, may have a more significant

Table 5. Association Between DFI Level and Pregnancy Outcome

Group	Pregnancy outcome	DFI <15% (n)	DFI ≥15% (n)	*P* value
All participants	Positive pregnancy	—	—	0.99
PCOS patients		—	—	0.57
Control subjects		—	—	0.38

DFI: DNA fragmentation index; ICSI: Intracytoplasmic sperm injection.
Values are expressed as mean ± SD. Pregnancy outcomes were categorized as positive or negative based on clinical confirmation.
Group comparisons for each subgroup (total, PCOS, control) were analyzed using the Chi-square test. No statistically significant associations were found ($P > 0.05$ in all groups).

impact on pregnancy outcomes (24). Furthermore, our study revealed that PCOS patients had increased levels of AMH and higher numbers of metaphase II oocytes and embryos at day 3 compared to control subjects. This suggests that PCOS may have an impact on ovarian reserve, which could potentially mask any effects of DFI on pregnancy outcome.

Notably, our research did not reveal any significant difference in pregnancy outcome between individuals with normal and abnormal DFI levels. This is in line with previous investigations suggesting that DFI may not be a reliable predictor of IVF success (25,26). However, it is essential to note that our study only involved a small number of men with DFI levels ≥15%, which may not represent the general population.

Li et al reported a statistically significant link between SDF and miscarriage rates as well as birth weight in assisted reproductive technology (ART) cycles, highlighting the potential importance of SDF as a predictor of ART outcomes (27). However, this study did not specifically focus on couples with PCOS. Our study highlights the significance of considering other variables that may influence IVF success in addition to sperm DNA fragmentation.

Our findings corroborate the findings of Fendo et al (28), who also reported no statistically significant association between sperm DFI and pregnancy outcomes in PCOS patients undergoing IVF. Nevertheless, our investigation did not investigate the impact of high DFI on high-quality blastocyst formation rates, which was a notable difference from their investigation. Fendo et al found that PCOS patients had lower high-quality blastocyst formation rates when using sperm with high DFI (>15%). This suggests that while the level of DFI may not impact pregnancy outcomes, it may still influence the quality of resulting blastocysts in PCOS patients. Future studies could benefit from exploring this relationship further, potentially using more detailed evaluations of blastocyst morphology or functional assessments to understand better the mechanisms underlying these findings.

The existing literature suggests that oocytes of PCOS women or those with advanced maternal age may exhibit impaired capacity to repair sperm DNA damage, which can lead to reduced high-quality blastocyst rates (28,29). However, our findings do not support this notion, potentially due to the careful choosing of embryos for transfer. In our study, we deliberately selected embryos with 6-8 cells and grades A and B, which may have minimized the impact of DNA damage on blastocyst quality. This approach may have contributed to the discrepancy between our results and previous studies.

The GnRH-antagonist procedure used in this investigation is effective in reducing the risk of ovarian hyperstimulation syndrome (OHSS) and improving the number of oocytes retrieved in PCOS women who underwent IVF (30,31). Our data are consistent with previous research that has reported similar outcomes with this protocol, which is often preferred for its reduced risk of OHSS and improved patient safety. However, it is essential to note that the optimal stimulation protocol for women with PCOS remains a topic of ongoing debate, and future investigations are necessary to identify the most effective strategies for managing the patient population. Additionally, individual variables such as BMI, age, and response to ovarian stimulation may influence the choice of stimulation protocol, highlighting the importance of personalized treatment approaches in assisted reproductive technology. El-Sayed et al reported that women with PCOS have a significantly higher risk of spontaneous abortion compared to women without the condition. They proposed that this higher risk is likely attributed to the high prevalence of obesity among PCOS women and suggest that women with PCOS may require more vigilant monitoring during pregnancy to identify potential complications earlier and improve outcomes (18). In the present work, no significant difference was found in the IVF outcome between the PCOS group and controls. Although we did not evaluate obesity as a cofounding factor, based on the objective of our research, DFI as a potential risk factor did not influence the outcome. In this regard, Dou et al investigated the abortion rates in PCOS patients undergoing IVF or ICSI treatments. They revealed that PCOS did not affect early or overall late abortion rates after IVF/ICSI treatments. Still, the rate of late abortion was significantly higher in PCOS patients who became pregnant with twins. On the other hand, obese patients were more likely to experience late abortion in twin pregnancies (32). Our study revealed no significant association between SDF levels and pregnancy outcome in both PCOS patients and control subjects. This suggests that SDF may not significantly predict IVF outcomes in either group. Our data are in contrast to some prior investigations that reported a significant association between high SDF levels and poor IVF outcomes (33,34). However, several factors could explain these discrepancies, such as differences in study populations, sample sizes, methods used to measure SDF, and the cut-off designated for high SDF. It's also important to note that SDF is just one of many factors that can influence IVF outcomes. Other factors, such as female age, embryo quality, and treatment protocols, can also significantly affect success rates.

Limitations

This study has several limitations, including its retrospective design, a limited sample size for the mild DFI subgroup, and the potential for confounding despite adjustments made in the analysis. Additionally, we did not evaluate obesity, which is an important modifier in the outcomes of PCOS.

Conclusions

Mild sperm DNA fragmentation (15–25% DFI) was

associated with impaired sperm parameters but did not significantly affect pregnancy outcomes in PCOS patients or controls. Therefore, mild DFI elevation alone should not be considered a reason to postpone IVF/ICSI treatment.

Authors' Contribution

Conceptualization: Ramesh Baradaran Bagheri, Mitra Rahimi Fathkouhi.
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Methodology: Ramesh Baradaran Bagheri.
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Software: Mitra Rahimi Fathkouhi.
Supervision: Ramesh Baradaran Bagheri.
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Visualization: Ramesh Baradaran Bagheri, Samaneh Sadat Hosseini Quchani, Mitra Rahimi Fathkouhi.
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Conflict of Interests

None.

Ethical Issues

This study was approved by the Ethics Committee of Alborz University of Medical Sciences (IR.ABZUMS.REC.1399.292). Written informed consent was obtained from all participants.

Declaration of AI-Assisted Tools in the Writing Process

The authors used DeepSeek (V3) AI tool for editing in order to enhance the quality of this manuscript. All content was checked by the authors, who accept full responsibility for its accuracy.

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