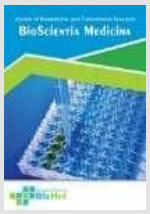
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Sperm DNA Fragmentation Index: A Comparison Study of Success Rates among Natural, Intrauterine Insemination (IUI) and In Vitro Fertilization (IVF)-Intra Cytoplasmic Sperm Injection (ICSI) Pregnancy Programs

Silvia Werdhy Lestari[1*], Eva Zakiyah[2], Gita Pratama[1,2]

[1]Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[2]Department of Obstetrics and Gynecology, Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

ARTICLE INFO

Keywords:
Male infertility
DNA fragmentation
Insemination
In vitro fertilization
Pregnancy

***Corresponding author:**
Silvia Werdhy Lestari

E-mail address:
finallysilvia@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i9.559

A B S T R A C T

Background: Some studies have reported a relationship between the sperm DNA fragmentation index (DFI) and the rate of fertilization and pregnancy. This study was designed to assess the mean sperm DNA fragmentation in pregnancies that occur in infertile couples, whether in natural pregnancy, intrauterine insemination (IUI), and in vitro fertilization (IVF) – intra cytoplasmic injection (ICSI). **Methods:** This research is an observational analytic study with a cross-sectional design. The sample taken in this study were infertile patients that underwent natural pregnancy or IUI or IVF-ICSI at Yasmin Infertility Clinic of Dr. Ciptomangunkusumo Hospital period 2018-2020 with a consecutive sampling technique. The research data was processed and analyzed by the Mann-Whitney test using the SPSS application. **Results:** The mean DFI of sperm in men with infertile couples who successfully conceived naturally was 10.7% (mild), while IUI was 20.4% (moderate), and IVF-ICSI was 30.5% (poor). The mean DFI in semen samples of men from infertile couples who underwent a natural program was significantly lower in those who successfully conceived compared to those who did not. Similar results were also shown in the IUI and IVF-ICSI programs, which showed a significantly lower DFI compared to non-pregnant women. **Conclusion:** DFI can be applied as a marker for selecting the type of pregnancy program in infertility management.

1. Introduction

Infertility affects 1 in 6 or about 15% of the number of couples of childbearing age. Male factors contribute to about half of the total infertility cases.[1,2] Routinely, male factors are identified through semen analysis which provides data in the form of sperm concentration, motility, and morphology. However, in some cases of male infertility, semen analysis results in the form of normozoospermic, or other words, sperm concentration, motility, and morphology are within normal limits. In this case, of course, a semen analysis cannot explain other abnormalities that may occur. Standard and routine examinations in the form of sperm concentration, motility, and morphology are no longer the best predictors of male fertility, so it is necessary to consider other predictors, one of which is sperm DNA fragmentation which reveals abnormalities in the integrity of the male genome.[3]

Increased DFI affects fertility status due to disruption of the male genome, and causes a decrease in pregnancy rates, both naturally and in assisted reproduction. Several studies have shown that DFI leads to lower fertilization, impaired embryonic development, lower pregnancy and live birth rates, and high rates of miscarriage in assisted reproductive techniques (ART) such as intrauterine insemination

(IUI) and in vitro fertilization (IVF) - intracytoplasmic sperm injection (ICSI).[4-6]

As a relatively new test for examining sperm abnormalities in male infertility, the sensitivity, specificity, and predictive value of DFI have been investigated and have shown better results than semen analysis parameters.[7] Nevertheless, the mean or cut-off of DFI in the success of natural pregnancy, IUI, and IVF-ICSI is still unknown. Therefore, this study was conducted to determine the value of DFI on the success of pregnancy in various types of pregnancy programs so that it can be a guide for clinicians in selecting sperm quality, especially sperm DNA fragmentation, before starting a pregnancy program.

2. Methods

This research is an observational analytic study with a cross-sectional design. The data used in this study is secondary data from the medical record of Dr. Ciptomangunkusumo Hospital Jakarta. The population of this study was infertile patients that underwent natural pregnancy or IUI or IVF-ICSI at Yasmin Infertility Clinic of Dr. Ciptomangunkusumo Hospital period 2018-2020. The sample size taken in this study was 62 respondents with a consecutive sampling technique. This research was conducted from January to May 2022. This study has also been approved by the ethics committee of the Faculty of Medicine Universitas Indonesia (FMUI) – Ciptomangunkusumo Hospital No. KET-439/UN2.F1/ETIK/PPM.00.02/2022.

Semen analysis, according to WHO standards, sperm preparation, and DNA fragmentation, was performed on samples of male sperm from infertile couples who underwent pregnancy programs.[8,9] Exclusion criteria were men who did not agree to undergo semen analysis, severe oligozoospermia (sperm concentration < 1 million/ml), and azoospermia (no sperm in seminal fluid). The independent variable in this study was. Pregnancy, while the dependent variables were sperm analysis parameters and DFI. Statistical analysis of the comparison between sperm parameters and DFI from the pregnant and non-pregnant groups was performed using the Mann-Whitney test, with the level of significance at $p<0.05$ by using SPSS (version 20). The data is presented in the form of tables and narratives.

3. Results

Comparison of semen analysis and sperm DNA fragmentation index (DFI) in infertile couples, in the pregnant and non-pregnant groups.

In this study, the characteristics of research subjects based on the results of semen analysis and sperm DNA fragmentation index (DFI) in infertile couples who underwent a pregnancy program were divided into two groups who were pregnant and those who were not, which were shown in table 1. In semen analysis, sperm concentration, and motility parameters, there were significant differences between the pregnant and non-pregnant groups ($p<0.05$), while there was no significant difference in morphological parameters ($p>0.05$). Similar to the parameters of sperm concentration and motility, there were also significant differences in DFI parameters in the pregnant and non-pregnant groups ($p>0.05$).

Table 1. Comparison of semen analysis and sperm DNA fragmentation index (DFI) in infertile couples who underwent a program of pregnancy in the pregnant and non-pregnant groups.

	Pregnant	Non-Pregnant	p
Sperm concentration	22.0	25.5	0.24
Motility			
A. Progressive	54	53	0.68
B. Non-progressive	12	10	0.67
C. Immotile	34	37	0.75
Morphology	3.7	3.5	0.65
DFI	19.8	26.8	0.01*

* = significant difference

Comparison of semen analysis and sperm DNA fragmentation index (DFI) in infertile couples who successfully conceived at natural, intra-uterine insemination (IUI) and in vitro fertilization – intra-cytoplasmic sperm injection (IVF-ICSI) pregnancy programs.

Furthermore, the semen analysis data in the pregnant group and not in each pregnancy program are shown in table 2. In all semen analysis parameters, both concentration, motility, and morphology of sperm, there were no significant differences in the pregnant group and not in natural pregnancy programs, IUI and IVF-ICSI.

In addition, the novelty of this study lies in the mean DFI of male semen samples from infertile couples who successfully conceived and who did not after undergoing natural, IUI, and IVF-ICSI programs. (Table 2) The mean DFI in semen samples of men from infertile couples who underwent a natural program was significantly lower in those who successfully conceived compared to those who did not. Similar results were also shown in the IUI and IVF-ICSI programs, which showed a significantly lower DFI compared to non-pregnant women.

Table 2. Comparison of semen analysis and sperm DNA fragmentation index (DFI) in infertile couples who successfully conceived, after undergoing natural, intra-uterine insemination (IUI) and in vitro fertilization – intra-cytoplasmic sperm injection (IVF-ICSI) pregnancy program

	Natural		IUI		IVF-ICSI		p
	+	-	+	-	+	-	
Sperm concentration (million/ml)	24.7	20.6	29.2	44.8	12.5	11.4	a*, b*, c*, d*
Motility (%)							
A.Progressive	68	67	40	42	57	55	a*, b*, c*, d*
B. Non-progressive	10	10	16	10	11	11	a, b, c, d
C. Immotile	22	23	44	48	32	34	a, b, c, d
Morphology (%)	3.5	3.4	4.7	4	3	3	a, b, c, d
DFI (%)	10.7	16.8	20.4	24.3	30.5	36.7	a*, b*, c*, d*

Information: (+) = pregnant; (-) = not pregnant. a = difference between groups who are pregnant and not in the natural program; b = difference between the groups who were pregnant and not in the IUI program and c = the difference between the groups who were pregnant and those who were not in the IVF-ICSI program; d = difference between groups who were pregnant on natural, IUI and IVF-ICSI programs;
* = significant difference

4. Discussion

In the parameters of semen analysis, both concentration, motility, and morphology of sperm, there was no significant difference in the pregnant group and not in total, nor when compared to the three types of pregnancy programs. There was only a significant difference when compared to the group that was pregnant in two types of pregnancy programs, either natural vs. IUI, natural vs. IVF-ICSI and IUI vs. IVF-ICSI. This shows that there are other markers of sperm, one of which has been investigated, namely sperm DNA damage or fragmentation.

In the population of sperm cells, both in the ejaculate, epididymis, and testes, there are sperm cells with intact or un-fragmented and fragmented DNA, with varying levels. The percentage of sperm count with fragmented DNA is not in line with the number, motility, and morphology of normal sperm. A man can have normal sperm count, motility, and morphology but have a high percentage of sperm count with fragmented DNA. Vice versa, a man can have abnormal sperm count, motility, and morphology but

have a small percentage of sperm with fragmented DNA. Fertility status is determined by the ability of the man to impregnate his partner, both from the parameters of the number of motile sperm and or intact DNA.

A study on male fertility with sperm DNA fragmentation proved and determined that the DFI threshold <15% as a good category with high fertility status, >15% DFI <30% as an intermediate category with moderate fertility status, and DFI >30% as a less category with poor fertility status. The results of this study are in line with previous studies that DFI who succeeded in getting pregnant from natural pregnancy program is 10.7%, which includes DFI in the good category and high fertility status. The results of this study also confirm the results of Spano et al. research, which showed that the probability of getting pregnant naturally through sexual intercourse was 10 times greater if DFI <30%.[10] The population of sperm cells ejaculated with DFI with good category had sperm cells with DNA which is very much intact compared to the fragmented DNA, so that the ability to fertilize the oocyte (fertility status) is high.

In addition to the DFI of those who were pregnant from the natural program, other data showed that the DFI who had successfully conceived from the IUI program was 20.4%, which included DFI in the middle category and moderate fertility status. The results of this study are in line with Bungum et al., who showed that IUI patients were 8.7 times more likely to give birth to babies with DFI <27%.[11] The population of sperm cells ejaculated with DFI in the intermediate category had more sperm cells with intact DNA than those with sperm cells. The DNA is fragmented so that the ability to fertilize the oocyte (fertility status) is moderate because the fertilization process is assisted through insemination.

In addition, the pregnant women from the IVF-ICSI program showed DFI data of 30.5%, which corresponds to the DFI category with poor fertility status. These results are in line with other studies by Jun Chi H et al. and Zhang Z et al. that the pregnancy rate from IVF-ICSI was better at a high level of DFI.[12,13] The population of sperm cells ejaculated with DFI with less category has sperm cells with more intact DNA compared to fragmented DNA, so the ability to fertilize the oocyte (fertility status) is poor because the fertilization process must be assisted through in vitro fertilization

Studies by Evenson et al. and Spano et al. showed that patients were 6.5 - 10 more likely to achieve pregnancy when their DFI was <30%.[10,14] Therefore, the DFI threshold of 30% for humans has been confirmed in recent studies with respect to in vivo or natural program procedures and IUI. When IVF-ICSI data were taken into account, it was shown that patients were 2 to 9.5 more likely to achieve pregnancy when their DFI was <30%.

These results clearly demonstrate that sperm DNA fragmentation is an important component of infertility testing. This study confirms the results of previous studies and suggests that if a man has a DFI >30%, IUI may not be considered and switch to IVF-ICSI. More specifically, this study recommends a natural program with a high pregnancy success rate if DFI <16.8%, an IUI program if DFI <24.3%, and an IVF-ICSI program if DFI <36.7%. However, if the DFI is greater than these values for each of the pregnancy program choices, then it should be treated first so that the DFI decreases and is in accordance with the selected pregnancy program. In other words, DFI also plays a role in infertility management, especially in sperm quality selection for pregnancy program type selection.

In addition, sperm in the semen that underwent washing before ART in IUI and IVF-ICSI programs underwent a selection process to obtain sperm with more intact DNA or less fragmented DNA than before washing, so sperm of higher quality was used for IUI and IVF-ICSI programs.[15]

5. Conclusion

The DNA fragmentation index (DFI) complements the pre-existing sperm selection marker for the selection of pregnancy programs in the management of infertility.

6. References

1. Kumar N and Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reproduction Sci. 2015; 8(4): 191-196
2. Agarwal A, Mulgund A, Hamada A and Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015; 13: 37
3. Zegiraj A, Beadini S, Beadini N, Aliu H, Gashi Z, et al. Male infertility and sperm DNA fragmentation. Open Access Maced J Med Sci. 2018; 6(8): 1342-1345
4. Junior EB, Setti AS, Braga DPAF and Zanetti BF. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. Fertility and Sterility 2019; 112(3).
5. Zini A, Boman JM, Belzile E dan Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Human Reproduction 2008; 23(12): 2663-2668.
6. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Human Reproduction 2012; 27(10): 2908-2917.
7. Wiweko B dan Utami P. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. Basic and clinical andrology. 2017; 27: 1-7.
8. WHO, WHO laboratory manual for the examination and processing of human semen. 5th ed. WHO Press, Geneve. 2010.
9. Fernandez JL, L. Muriel V. Goyanes E. Segrelles and J. Gosálvez, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertility Sterility 2005; 84: 833-842.
10. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, et al. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril. 2000; 73: 43–50
11. Bungum M. Sperm DNA integrity assessment: A new tool in diagnosis and treatment fertility. Obstet Gynecol Int. 2012; 2012: 531042.
12. Jun Chi H, Gi Kin S, Young Kim Y, Young Park J, Seok Yoo C, et al. ICSI significantly improved the pregnancy rate of patients with a high sperm DNA fragmentation index. Clin Exp Repro Med. 2017; 44(3): 132-140
13. Zhang Z, Zhu L, Jiang H, Chen H, Chen Y, et al. Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: a meta-analysis
14. Evenson DP, Jost LK, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay (SCSA) as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999; 14(4): 1039–49.
15. Lestari SW, Sari T dan Pujianto DA. Sperm DNA fragmentation and apoptosis levels: A comparison of the swim-up and the density gradient centrifugation methods for sperm preparation. Online Journal of Biological Sciences. 2016; 16(4): 152-158.