International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Tandulwadkar S et al. Int J Reprod Contracept Obstet Gynecol. 2022 Jun;11(6):1679-1684
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20221439

Original Research Article

Prevalence and clinical utility of sperm DNA fragmentation index in couples with unexplained infertility

Sunita Tandulwadkar*, Swati R. Babar, Sneha Mishra, Shruti Gupta

Department of IVF and Endoscopy, Ruby Hall Clinic, Pune, Maharashtra, India

Received: 28 March 2022
Accepted: 17 May 2022

***Correspondence:**
Dr. Sunita Tandulwadkar,
E-mail: sunitart@hotmail.com

ABSTRACT

Background: Worldwide increased burden of infertility has built up stress in reproductive age group couples. Female factor evaluation and semen analysis are carried out routinely in infertility work up. As per the recent observations, males with normal semen analysis may have abnormal sperm DNA fragmentation index (DFI). Thus, rendering semen analysis with poor diagnostic value in unexplained infertility cases. There is lack of adequate literature on prevalence of abnormal DFI in unexplained infertility. This study is directed to contribute to the literature by assessing prevalence of couples with 'unexplained infertility' having DFI>15% in male partners.
Methods: After getting approval from institutional ethical and scientific research committee, 200 couples with unexplained infertility were recruited for the study and sperm DFI using sperm chromatin dispersion (SCD) test (Halo test) was done.
Results: Out of 200 subjects, 54% were having low DFI, 32% were having moderate DFI and 14% were having high DFI.
Conclusions: Many couples diagnosed as unexplained infertility according to traditional diagnostic methods has remarkably high degrees of fragmented sperm DNA. Identification of such couples provide vital information and better therapeutic options can be offered to them to achieve best reproductive outcomes.

Keywords: Sperm DFI, Unexplained infertility, Male infertility, SCD, Infertility

INTRODUCTION

Infertility is defined as failure to achieve pregnancy after one year of unprotected sexual intercourse.[1] Infertility is estimated to globally affect as many as 186 million couples.[2] The success of a pregnancy is influenced by both men and women. Of all infertility cases, nearly 30-40% are due to male factor infertility, either as a single factor or as a combination with female factors.[3] In most cases of infertility, the workup is restricted to gynaecological evaluation and semen analysis.[4] Semen analysis is considered as key investigation in all andrology laboratories worldwide, but still it cannot differentiate fertile from infertile men. It is possible that men with normal standard semen parameters may have reduced fertility potential due to diminished sperm chromatin integrity.[5] The limitation of semen analysis is illustrated by a significant proportion of infertile couple being classified as unexplained infertility with normal semen parameters.[6]

The diagnosis of Unexplained Infertility was based on the following:[7] At least 1 year of unprotected intercourse without pregnancy, normal semen parameters according to WHO Manual, 5th edition, 2010, unremarkable andrological history (no cryptorchidism, drug abuse, cancer treatment or other iatrogenic factors), no genetic abnormalities such as Klinefelter's syndrome or Y-chromosome micro deletion and no hypogonadotropic hypogonadism, no female factors (anovulation, tubal factor or endometriosis).

Different etiologies have been ascribed to male infertility, of which, sperm DNA damage has gained the utmost attention with extensive research on the structural and functional aspects of sperm. Mounting evidence clearly indicates a crucial role of sperm DNA and mitochondrial integrity on male fertility status. The successful transmission of genome to oocyte relies heavily on the precise structural integrity of the sperm DNA.[8]

Sperm DNA integrity is crucial for fertilization and development of healthy offspring.[9] Male factors can influence not only the fertilization process but also embryonic genome expression and development. In addition, male factors may also be involved in idiopathic miscarriages, as well as autosomal dominant diseases and neuro-behavioural disorders in offspring, especially in cases of advanced paternal age.

Every man has sperm DNA imperfections, but levels are higher among sub-fertile and infertile men. High SDF correlates with poor reproductive outcomes, including lower success rates in natural pregnancy, intrauterine insemination and in vitro fertilization (IVF), as well as higher miscarriage occurrences.[10] So far, there is only limited information regarding the prevalence of high DFI in couples diagnosed with unexplained infertility. The purpose of the study is, therefore, to find out the percentage of couples with diagnosis of unexplained infertility in which the male partner has a DFI>15%.

METHOD

After getting approval from institutional ethical and scientific research committee prospective observational study was carried out on 200 couples with unexplained infertility.

Study type

This was prospective observational study conducted from Sept 2020 to August 2021 at IVF department, Ruby Hall clinic, Pune.

Selection criteria

The inclusion criteria were male partners with normal semen analysis and semen culture.

Exclusion criteria were male partners with abnormal semen analysis and semen culture, couples with female factor infertility.

Procedure

After taking written informed consent from all participants, SDF testing using SCD test (Halo test) was done. Participants were informed about all prerequisites to be taken for SDF testing. 3 days abstinence was asked to follow. Ejaculated semen sample collection was done by participant at the IVF centre in semen collection room in a sterile, nontoxic 100 ml plastic container. DFI testing was started within 30 min of semen sample collection.

The SCD uses fluoroscopy to dye intact DNA, and unlike the other assays, SCD quantifies the normal DNA rather than the DNA fragmentation. The primary advantage to SCD is that it comes in an inexpensive, accessible kit.[11]

SCD test (Halo sperm test)

SCD test was proposed by Fernández in 2003, and was subsequently improved to make the sperm Chroma kit (Cryo lab international, Chennai, India). The optical microscope is used to observe the results and maintains the integrity of the sperm tail. It is a detection technology that is easy to operate, cheap, and highly accurate.[12]

The evaluation of DNA dispersion after denaturation was carried out using a sperm Chroma kit following the manufacturer's guidelines: (1) preparation of a mixture containing sperm cells (≤ 20 mi/mL) and melted agarose (1:2), (2) placement of the sperm suspension (10 µL) on the center of a super-coated slide and (3) denaturation, lysis, dehydration and staining of sperm cells with cosine and thiazine.

This procedure results in DNA loops spreading out into the inert matrix, producing halos of chromatin. In comparison, human sperm with fragmented DNA don't produce halos or produce halos that are very small. This procedure has been validated in situ since only those spermatozoa without or with small halos are tagged by other sequential DNA breakage labelling assays.[13]

Hamilton Throne computer assisted semen analysis (HT-CASA) software is used with green filter for DFI reporting.

DFI outcome is measured under 3 categories as follows: High DFI (>30), moderate DFI (15-30) and low DFI (<15).[14]

Thus, the SCD test, is a powerful and versatile methodology that not only facilitates easy assessment of sperm DNA quality in the clinic but also can be used in both basic and applied research related to human sperm DNA damage and organization.

Ethical approval received.

Statistical methods for data analysis (plan)

The data on categorical variables will be presented as n (% of cases) and the values on continuous variables will be presented as Mean ± SD. The statistical significance of difference of distribution of categorical variables will be tested using Chi-Square test or Fisher's exact probability test for 2×2 contingency table if more than 50% cells have expected count less than 5. The statistical significance of difference of distribution of means of continuous variables

will be tested using independent sample t test. The underlying normality assumption will be tested before subjecting the study variables to t test.

P values less than 0.05 will be considered to be statistically significant. All hypotheses will be formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data will be statistically analysed using statistical package for social sciences (SPSS version 22.0, IBM corporation; NY, USA) for MS windows.

RESULTS

Age wise distribution among study subjects

Mean age of the 200 male study sample was 32.08 years (standard deviation-4.616 years), with the highest 42 years and lowest 23 years.

84 samples (42%) were from the 26-30 years age group, 54 subjects (27%) in the 36-40 years age group, shown in Figure 1.

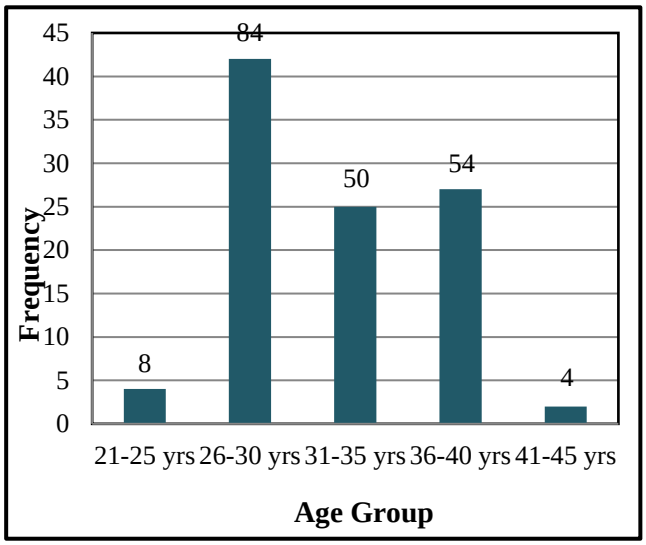

Figure 1: Age distribution.

Association between age and sperm DFI among study sample

There was significant association found between age more than 35 years and raised DFI value shown in Table 1.

Table 1: Age and DFI.

Age and DFI	Normal	Raised	Total	P value
≥35 years	92	50	142	0.0007
More than 35 years	16	42	58	
Total	108	92	200	

Sperm DFI among study sample

Out of 200 subjects, 108 subjects (54%) were having normal DFI (DFI <15%) and 92 subjects (46%) with abnormal DFI (DFI >15%) shown in Figure 2.

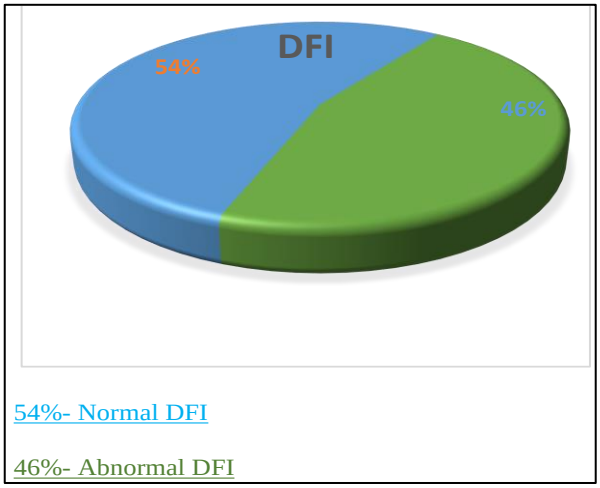

Figure 2: DFI distribution (Normal and abnormal).

In 200 subjects, 108 male subjects (54%) were having low DFI (<15%), 64 subjects (32%) with moderate DFI (15-30%) and 28 subjects (14%) with high DFI (>30%), as shown Figure 3.

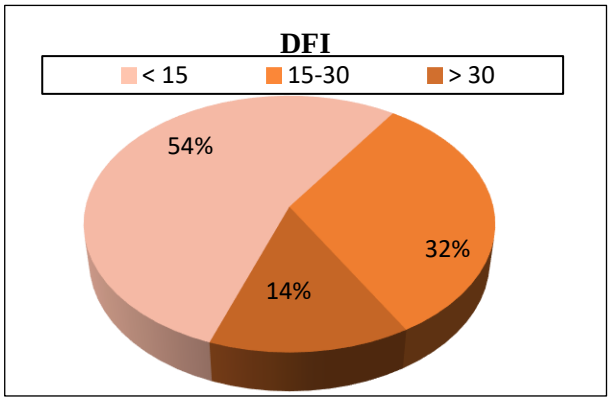

Figure 3: DFI distribution (Low, moderate and high).

Association between smoking and sperm DFI among study sample

There was significant association found between smoking addiction and raised DFI value, shown in Table 2.

Table 2: Smoking and DFI.

Smoking and DFI	Low	Raised	Total	P value
Present	8	28	36	0.0028
Absent	100	64	164	
Total	108	92	200	

Association between BMI and sperm DFI among study sample

There significant association found between BMI more than 25 kg/m^2 and raised DFI value shown in Table 3.

Table 3: BMI and DFI.

BMI and DFI	Low	Raised	Total	P value
Less than 25	104	34	138	0.000
More than 25	4	58	62	
Total	108	92	200	

Association between exercise and sperm DFI among study sample

There was significant association found between lack of exercise and raised DFI value, as shown in Table 4.

Table 4: Exercise and DFI.

Exercise and DFI	Low	Raised	Total	P value
Present	72	28	100	0.0003
Absent	36	64	100	
Total	108	92	200	

Association between alcohol and sperm DFI among study sample

There was no association found between consumption of alcohol and DFI value, as shown in Table 5.

Table 5: Alcohol and DFI.

Alcohol and DFI	Low	Raised	Total	P value
Present	30	28	58	0.77
Absent	78	64	142	
Total	108	92	200	

DISCUSSION

In our study we observed that out of 200 subjects, 108 male subjects (54%) were having low DFI, 64 subjects were having moderate DFI and 28 subjects (14%) with high DFI. Comparable results were seen in previous studies conducted by Oleszczuk et al, Faduola et al and Vinnakota et al.[4,7,14]

A study conducted by Oleszczuk et al evaluating prevalence of high DFI in male partners of unexplained infertile couples showed 17.7% of subjects had DFI 20-30% and 8.4% of subjects had DFI >30%.[7]

Faduola et al studied "Sperm chromatin structure assay results in Nigerian men with unexplained infertility" and observed that 63% of the men had a DFI greater than 20% and 15.2% of the subjects had a DFI greater than 30%.[4]

Chitra Vinnakota et al study entitled "Incidence of high sperm DNA fragmentation in a targeted population of sub-fertile men" showed the distribution of men with low, moderate and high SDF (<15, 15-30 and >30%) was 74.8%, 19.4% and 5.8%, respectively.[14]

Approximately 46% of all unexplained infertility cases have been reported to have a deranged DFI (15%). The variable incidence of high DFI in the literature may be population specific and the rates of moderate and high DFI reported here may not apply to other patient populations.[14] Our study has biological and clinical implications. From a biological point of view, it is interesting that sperm DNA fragmentation can, at least partly, explain as many as 46% of previously unexplained cases. Clinically, our data indicate that SCD testing may help in management of couples with unexplained infertility.[7]

Aging is a natural and inevitable process that affects every individual and introduces a series of physiological changes in bodies. One of the changes associated with aging is reduced reproductive capacity.[15,16] We found that men with age >35 years had higher DFIs than younger men. We believe this is new information related to a general adoption of SDF and that this finding will help guide our practice, limiting the application of this test to cases where it has a good chance to identify an affected individual.[14]

Mean age of the population under study was 32.08 years with the highest 42 years and lowest 23 years.

There was significant association found between age more than 35 years and raised DFI value.

As per the study by Gunes, sperm DFI is positively correlated with paternal aging ($p<0.00001$).[16] Pino et al studied the effects of aging on semen parameters and sperm DNA fragmentation in 2020 and observed that Males above the age of 50 presented a statistically significant increase in DNA damage and were 4.58 times more likely to present sperm DNA fragmentation than men aged 21-30.[15] Systematic review and meta-analysis by Johnson et al showed that age related decline in semen volume, percentage motility, progressive motility, normal morphology and unfragmented cells was statistically significant.[17]

There was significant association found between smoking addiction and raised DFI value ($p=0.0028$). As per study by Xiangrong et al the smoking group exhibited a significantly higher DNA fragmentation rate, compared with the non-smoking group.[18]

There was significant association found between lack of exercise and raised DFI value ($p=0.0003$).

Recently, new guidelines for male oxidative stress infertility suggest that lifestyle management against oxidative stress should be provided, including exercise. Exercise intervention favourably attenuated inflammation as indicated by seminal cytokines (IL-1β, IL-6, IL-8 and TNF-α), oxidative stress (SOD, MDA and 8-isoprostane) and enhanced antioxidants (SOD and catalase) ($p<0.05$). These changes correlate with favourable improvements in semen parameters, sperm DNA integrity and pregnancy rate in this cohort of infertile patients ($p<0.05$).[19]

There was significant association found between BMI more than 25 years and raised DFI value (p=0.000). Over the past few years, there has been a growing interest on the link between male nutrition and infertility. It is important to evaluate the potential effect of overweight or obesity on DNA integrity. As per the study conducted by Charlotte et al the DNA fragmentation rate was significantly higher in obese men compared with men with normal BMI but not in overweight men.[20]

There was no association found between consumption of alcohol and DFI value. On the contrary, a review by Pourmasumi et al concluded that alcohol consumption may not increase the rate of sperm residual histones and protamine deficiency, but it causes an increase in percentage of spermatozoa with DNA fragmentation and apoptosis.[21]

A meta-analysis conducted by Santi et al evaluating 28 studies, observed that SDF levels were significantly higher in infertile men ($p<0.001$), independently of the SDF testing method applied. These meta-analyses demonstrate the SDF relevance in male infertility, suggesting a higher accuracy in detecting sperm function than conventional semen parameters. Although larger prospective trials are needed, SDF represents a promising tool for clinical and research practice.[22]

A critical review for clinicians, reproductive professionals and researchers by Agarwal et al concluded an expert opinion suggesting the potential role of SDF testing in specific clinical scenarios. This would expand the horizon of SDF testing globally as a prognostic and diagnostic tool in various male infertility scenarios and their treatment management.[8]

A systematic review by Cho et al observed the significant role of SDF in male factor infertility is supported by current evidence. SDF testing has a beneficial role in selection of varicocelectomy candidates, evaluation of patients with unexplained infertility and recurrent pregnancy loss, selection of the most appropriate assisted reproductive technique with highest success rate for infertile couples, and assessment of infertile men with modifiable lifestyle factors or gonadotoxin exposure.[6]

A scientometric analysis by Baskaran et al revealed an increasing trend in SDF publications over the past 20 years. Currently, a substantial increase in research is essential to establish SDF as a prognostic/ diagnostic parameter in the evaluation of clinical scenarios and ART outcomes.[23]

A review article by Ashok Agarwal et al compared the two recent clinical practice guidelines published by Agarwal et al and Esteves et al.[9,24,25] Both guidelines recommended SDF testing for idiopathic male infertility (IMI), unexplained male infertility (UMI), and RPL. They also both reviewed the adverse impact of lifestyle and exposure risk factors.[24] The European association of urology (EAU) recommend SDF testing only for men with unexplained infertility or after RPL.[26]

Recently, the American urological association (AUA) and American society for reproductive medicine (ASRM) published a guideline on male infertility and they recommend against SDF testing in initial evaluation of fertility, but advocate its use and importance in couples experiencing RPL.[27]

As per the recent ASRM guidelines (Oct, 2020), in a patient with high sperm DNA fragmentation, a clinician may consider using surgically obtained sperm in addition to ICSI. Therefore, DNA fragmentation testing may be advantageous for men in couples undergoing IVF with repeated IVF failure. Physicians should be aware that there are some data to suggest that men with very high levels of DNA fragmentation in ejaculated sperm typically have lower levels of DFI in surgically extracted testicular sperms. Thus, improving fertility outcomes. Therefore, a clinician might consider using testicular sperm as opposed to ejaculated sperm for IVF/ICSI. In a prospective cohort study of over 100 couples with high DNA fragmentation, testicular sperm yielded substantially higher live birth rates than ejaculated sperm.[27]

The incidence and impact of high DNA fragmentation is poorly understood, making it difficult to know which patients would benefit from being tested.[14] In most cases of infertility, the workup is restricted to gynaecological evaluation and semen analysis. Men are often categorized as fertile if their semen analysis is within the WHO normal range. A man classified as fertile by these poor predictive parameters may attribute the cause of their childlessness to the female partner. It is now becoming clear that high sperm DNA fragmentation may be the responsible factor in most couples with a history of unexplained infertility.[4]

CONCLUSION

With SDF test results in hand, reproductive specialists can recommend necessary lifestyle changes and guide patients toward the fertility treatment options that offer the best opportunities for success. Though the clinicians cannot provide a particularly effective treatment, they can give patients some appropriate symptomatic treatments, such as antioxidant drugs and essential trace element supplementation treatments and guide patients to avoid harmful environments and toxic exposure. These

symptomatic treatments can help some patients overcome unexplained infertility to achieve a successful pregnancy outcome.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Khamar T, Narooie-Nejad M, Kordi L, Farzaneh F. Frequency of Sperm DNA Fragmentation Index in Infertile Couples with Male Factor Infertility. Crescent J Med Biolog Sci. 2020;7:4.
2. Gill K, Rosiak-Gill A, Jakubik J. The higher risk for sperm DNA damage in infertile men. Ginekologia Polska. 2019;90(12):684-91.
3. Wiweko B, Utami P. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. Basic Clin Androl. 2017;27:1.
4. Faduola P, Kolade CO. Sperm chromatin structure assay results in Nigerian men with unexplained infertility. Clin Exp Reproduct Med. 2015;42(3):101.
5. Gill K, Jakubik J, Rosiak-Gill A. Utility and predictive value of human standard semen parameters and sperm DNA dispersion for fertility potential. Int J Environmental Res Public Health. 2019;16(11):2004.
6. Cho CL, Agarwal A. Role of sperm DNA fragmentation in male factor infertility: A systematic review. Arab J Urol. 2018;16(1):21-34.
7. Oleszczuk K, Augustinsson L, Bayat N. Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples. Andrology. 2013;1:357-60.
8. Agarwal A, Panner Selvam MK, Baskaran S, Cho CL. Sperm DNA damage and its impact on male reproductive health: a critical review for clinicians, reproductive professionals and researchers. Expert review of molecular diagnostics. 2019;19(6):443-57.
9. Agarwal A, Majzoub A, Baskaran S. Sperm DNA fragmentation: A new guideline for clinicians. World J Men's Health. 2020;38(4):412.
10. Palmer WJ. Sperm DNA Fragmentation - The New Frontier of Fertility Testing. AACC.org. Clinical Laboratory News. 2019.
11. Fatool SK, Harlev A. Clinical uses and implications of sperm DNA fragmentation assays. Urol Nephrol Open Access J. 2018;6(3):108-10.
12. Qiu Y, Yang H, Li C, Xu C. Progress in Research on Sperm DNA Fragmentation. Medical science monitor: Int Med J Exp Clin Res. 2018;26:e918746.
13. Fernández JL, Johnston S, Gosálvez J. Sperm Chromatin Dispersion (SCD) Assay. In: Zini A., Agarwal A. (eds) A Clinician's Guide to Sperm DNA and Chromatin Damage. Springer, Cham. 2018.
14. Vinnakota C, Cree L, Peek J, Morbeck DE. Incidence of high sperm DNA fragmentation in a targeted population of subfertile men. Systems Biol Reproduct Med. 2010;65(6):451-7.
15. Víctor P. The effects of aging on semen parameters and sperm DNA fragmentation. JBRA Assisted Reproduction. 2020;24(1):82-6.
16. Gunes S, Hekim GN, Arslan MA, Asci R. Effects of aging on the male reproductive system. J Assist Reprod Genet. 2016;33:441-54.
17. Johnson SL, Dunleavy J, Gemmell NJ, Nakagawa S. Consistent age-dependent declines in human semen quality: a systematic review and meta-analysis. Ageing Res Rev. 2015;19:22-33.
18. Xiangrong C. Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. Molecular Med Rep. 2016;14(1):753-61.
19. Hajizadeh Maleki B, Tartibian B. Resistance exercise modulates male factor infertility through anti-inflammatory and antioxidative mechanisms in infertile men: a RCT. Life Sci. 2018;203:150-60.
20. Charlotte D. Obesity leads to higher risk of sperm DNA damage in infertile patients. Asian J Androl vol. 2013;15(5):622-5.
21. Pourmasumi S, Sabeti P, Rahiminia T. The etiologies of DNA abnormalities in male infertility: An assessment and review. Int J Reprod Biomed. 2017;15(6):331-44.
22. Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management-meta-analyses. Reproduct Biomed online. 2018;37(3):315-26.
23. Baskaran S, Agarwal A, Selvam MK. Tracking research trends and hotspots in sperm DNA fragmentation testing for the evaluation of male infertility: a scientometric analysis. Reproduct Biol Endocrinol. 2019;17(1):1-3.
24. Agarwal A, Farkouh A, Parekh N. Sperm DNA Fragmentation: A Critical Assessment of Clinical Practice Guidelines. World J Mens Health. 2021;39:e21.
25. Esteves SC, Zini A, Coward RM. Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. Andrologia. 2021;53(2):e13874.
26. Tharakan T, Bettocchi C, Carvalho J. EAU Working Panel on Male Sexual Reproductive Health. European Association of Urology guidelines panel on male sexual and reproductive health: a clinical consultation guide on the indications for performing sperm DNA fragmentation testing in men with infertility and testicular sperm extraction in non-azoospermic men. Eur Urol Focus. 2021;8(1):339-50.
27. Schlegel PN, Sigman M, Collura B. Diagnosis and Treatment of Infertility in Men: AUA/ASRM Guideline. 2020;15:21-2.

Cite this article as: Tandulwadkar S, Babar SR, Mishra S, Gupta S. Prevalence and clinical utility of sperm DNA fragmentation index in couples with unexplained infertility. Int J Reprod Contracept Obstet Gynecol 2022;11:1679-84.