TURKISH JOURNAL OF UROLOGY
Turk J Urol 2020; 46(5): 348-53 • DOI: 10.5152/tud.2020.19262
ANDROLOGY
Original Article

Comparing the different methods of sperm chromatin assessment concerning ART outcomes

Mahnaz Heidari*, Mahsa Darbandi*, Sara Darbandi, Mohammad Reza Sadeghi

Cite this article as: Heidari M, Darbandi M, Darbandi S, Sadeghi MR. Comparing the different methods of sperm chromatin assessment concerning ART outcomes. Turk J Urol 2020; 46(5): 348-53.

ABSTRACT

Objective: Sperm DNA fragmentation and maturation directly interferes with reproductive efficiency. Although there are several methods for assessing sperm DNA integrity, however, many of them are laborious and require high-precision equipment in the clinics. Thus, evaluating economic and reliable methods to prepare suitable sperm for assisted reproductive technologies without DNA damage is critical.

Material and methods: A total of 114 semen samples were collected and analyzed using computer-assisted semen analysis. The DNA fragmentation index (DFI) of all samples was evaluated by two methods of sperm chromatin dispersion (SCD) and sperm chromatin structure assay (SCSA). Besides, chromatin maturation index (CMI) was assessed by three methods including aniline blue (AB)-sperm chromatin maturation assay (SCMA), fluorescence microscopic chromomycin A3 (fmCMA3), and flow cytometric CMA3 (fcCMA3).

Results: The result showed that the DFI had no statistically significant differences ($p>0.05$) between SCSA (26.98%±1.28%) and SCD (27.88%±1.278%), although SCD demonstrated a strong correlation with DNA maturity ($p<0.001$), which had not been seen in SCSA. Besides, the CMI demonstrated significant differences ($p<0.001$) when assessed by AB-SCMA (14.86%±0.65%), fmCMA3 (29.18%±1.01%), and fcCMA3 (22.45%±0.62%). Among these, only the fmCMA3 showed a significant correlation with semen parameters ($p<0.01$) and embryo development ($p<0.001$).

Conclusion: It seems that SCD and fmCMA3 were more accessible, affordable, and reliable tests for assessing DFI and CMI. It appeared these two methods may be the best choices for evaluating sperm DNA integrity in clinics.

Keywords: Chromatin maturation index; DNA fragmentation index; embryo quality; fertilization rate; semen parameters.

ORCID iDs of the authors:
M.H. 0000-0001-6075-8639;
M.D. 0000-0002-2243-9319;
S.D. 0000-0002-1868-6712;
M.R.S. 0000-0003-0401-3493

Reproductive Biotechnology Research Center, Avicenna Research Institute (ARI), ACERCR, Tehran, Iran

*Mahnaz Heidari and Mahsa Darbandi had equal contribution

Submitted:
14.12.2019
Accepted:
23.04.2020
Available Online Date:
27.05.2020

Corresponding Author:
Mohammad Reza Sadeghi
E-mail:
Sadeghi@Avicenna.ac.ir

Available online at
www.turkishjournalofurology.com

Introduction

Nowadays, the integrity of sperm DNA and chromatin maturation are being identified as reliable parameters of sperm quality and a marker of male infertility.[1,2] Studies showed that sperm DNA testing including integrity and maturity are a fertility checkpoint in men and has been a prediction for assisted reproductive technologies (ART) outcome owing to the critical role of sperm chromatin in fertilization, embryo development, and also implantation.[1,2] Sperm DNA damages can be the result of defects in chromatin remodeling during spermatogenesis, apoptosis, oxidative stress induced by environmental toxicants, chemotherapy, radiotherapy, varicocele, etc.[3-5] Although semen parameters were assessed routinely according to the World Health Organization (WHO) guideline, it needs more reliable tests to evaluate sperm function during the fertilization process in cases with repeated abortion.[6] The sperm chromatin test must be clinically useful as a possible diagnosis of infertility predictor. To decrease the volume of chromatin in somatic and sperm cells, chromatin configuration respectively is performed by DNA assessing around the histones octamer package and structuring by protamine.[7] Although alteration of this structure or the induction of DNA strand breaks during spermatogenesis may not affect the fertilizing

ability of spermatozoa, it may induce definitive changes in the genomic information transmitted to the progeny.[8] Therefore, sperm chromatin quality is an important factor that can influence not only the fertility of an individual but also the general health of future generations.[8] Currently, the sperm chromatin integrity is carried out by the assessment of sperm DNA fragmentation index (DFI) with sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) test, terminal transferase dUTP nick-end labeling (TUNEL assay), the alkaline comet assay, DNA breakage detection fluorescence in situ hybridization, and toluidine blue stain.[9-11] Studies showed that SCD and SCSA are two simple and inexpensive methods for the determination of sperm DNA fragmentation.[1,2,12] Besides, the sperm chromatin maturation index (CMI) is checked by chromomycin A3 (CMA3) and aniline blue (AB)-stain sperm chromatin maturation assay (SCMA) tests.[11,13] Although some of these are laborious and require high-precision equipment, selecting the methods that can easily and inexpensively assess chromatin status in the routine seminal analysis as a screening fertility test is required. Therefore, this study aimed to evaluate and compare a) two of the most available, accessible, and inexpensive methods of DNA integrity including SCSA and SCD and b) three methods of DNA maturation including AB-SCMA, flow cytometric (FC) CMA3, and fluorescence microscopic (FM) CMA3 to assessing the efficacy of these methods in evaluating sperm chromatin status in men.

Material and methods

Study groups

This study included the semen specimens of 114 men who visited the Avicenna infertility clinic (Iran, Tehran) after 48-72 h of sexual abstinence and were allowed to liquefy for at least 30 min at 37°C. None of the subjects had any history of radiotherapy, chemotherapy, varicocele, azoospermia, leukocytospermia, genital inflammation, chronic diseases, endocrine abnormality, chromosomal aberrations, and Y-chromosome microdeletion. Informed consent was approved by the Medical Ethics Committee of Avicenna Research Institute obtained from all subjects. Seminal volume, pH, and sperm vitality (eosin-nigrosin staining) were evaluated by direct macroscopy, and sperm concentration, motility, and morphology were assessed using computer-assisted semen analysis according to WHO.[6]

Main Points:

- Two of the most available, accessible, and inexpensive methods of DFI including SCSA and SCD.
- Three methods of CMI including AB-SCMA, fcCMA3, and fmCMA3 to assess the efficacy of these methods in evaluating sperm chromatin status. It seems that SCD and fmCMA3 were more accessible, affordable, and reliable tests for asDFI and CMI. It appeared these two methods may be the best choices for evaluating sperm DNA integrity in clinics.

Sperm processing was done by density gradient centrifugation. Then, the pellet was washed and the aliquot containing sperm was used for chromatin assays and injection to the oocyte of the men's partner. Sperm DNA fragmentation was estimated by SCSA and SCD and sperm chromatin maturation was assessed by AB-SCMA, fcCMA3, and fmCMA3.

Assessment of DFI

The sperm DNA integrity was assessed by SCSA and SCD tests according to protocols.[14]

SCD assay

Initially, 50 µL sperm sample, which contained one million spermatozoa per milliliter after dilution in Hams F10, was mixed with 50 µL agarose (6.5%). Then, 20 µL was loaded onto a pretreated glass slide, cooled (5 min, 4°C), treated with a denaturating (7 min) and a lysing solution (15 min), and after that, the slide was washed (5 min) and dehydrated (70%, 90%, 100% ethanol, 2 min), air dried, and stained. At least 200 sperms were assessed under 1000× magnification using a light microscope. Sperm with large or medium halo were reported as intact chromatin and those with no halo or small halo were described as sperm with fragmented DNA.[15]

SCSA

The SCSA procedure included two steps of Acridine orange (AO) staining and, subsequently, FC measurement.[14] All steps were performed at 4°C. Sample was diluted with TNE buffer (0.15 mol/L NaCl, 0.01 mol/L Tris, 0.001 mol/L EDTA, pH 7.4) to obtain the concentration of $<2 \times 10^6$ sperm/mL. A 200 µL aliquot was removed and admixed with 400 µL of a low pH detergent solution (0.15 mol/L NaCl, 0.08N HCl, 0.01% Triton X-100, pH 1.4). After 30 s, 1.2 mL of the staining solution (6 µg/mL AO, chromatographically purified in 0.2 mol/L Na2HPO4, 1 mmol/L disodium EDTA, 0.15 mol/L NaCl, 0.1 mol/L citric acid monohydrate, pH 6.0) was added, and the stained sample was placed in the flow cytometer chamber. Abnormal chromatin structure, defined as increased susceptibility to acid or heat-induced denaturation *in situ*, was quantitated by flow cytometric measurement of the metachromatic shift from green (native DNA) to red (denatured, single-stranded DNA) fluorescence. The final result was presented as DFI (%).

Assessment of Sperm CMI

AB staining (SCMA)

First, each sample was diluted to reach the concentration of one million sperm/mL and centrifuged (300 g, 5 min). Then, thin smear was prepared and processed with 3% buffered glutaraldehyde in phosphate buffer 0.2 M (pH=7.2) for 30 min at 25°C. Each slide was stained with AB at room temperature. A minimum of 200 sperms were assessed in the different fields of each slide using a light microscope with 1000× magnifica-

Turk J Urol 2020; 46(5): 348-53
DOI: 10.5152/tud.2020.19262

tion. The pink and the blue sperms were, respectively, reported as mature and immature sperms. The percentage of immature sperm chromatin was reported as CMI (%).

CMA3 staining

First, 1×10^6 sperm/mL of each sample was centrifuged (300 g, 5 min) and processed with a fixed Carnoy's solution for 5 min at 4°C and then the pellets were stained with 100 µL of 0.25 mg/mL CMA3 solution at room temperature. For FM assessment, the thin smears with CMA3 solution were prepared and 200 sperms in each sperm slide were observed. FC-based samples, which were exposed to CMA3, were washed twice with phosphate-buffered saline and then assessed by particle analyzing system flow cytometer, using an argon laser with an excitation wavelength of 488 nm using fluorescence detector-2 (FL-3) with a 585/42 nm bandpass filter. A minimum of 10,000 sperms were examined for each assay and analyzed using Flowjo software. The incubating spermatozoa (37°C for 10 min) with 200 mmol dithiothreitol was used as a positive control.

Assessing the fertilization and embryo quality

Ovarian hyperstimulation was conducted according to the long luteal suppression protocol, which uses a Gonadotrophin-releasing hormone agonist (Superfact, Germany) and a combination of human menopausal gonadotropin. Ovulation was triggered by the administration of human chorionic gonadotropin (hCG). Oocytes were collected 36 h post hCG, using a simple lumen aspiration needle. Oocytes were retrieved by transvaginal ultrasound-guided follicle aspiration. The cumulus oocytes were collected from the follicular fluid. Granulosa cells were detached from collected oocytes using enzymatic and mechanical digestion for all samples. Sperm were injected (ICSI: Intracytoplasmic sperm injection) to their respective partner's (clinically fertile) metaphase II oocytes to evaluate fertilization rate and embryo quality. The fertilized oocytes indicated two pronuclear (PNs) and the embryo quality was estimated by morphological principles documented according to the fragmentation degree and the regularity of blastomeres on 48-72 h post-ICSI technique. The embryos were considered as grade A (without fragmentation), grade B (fragmentation<20%), and grade C (fragmentation>20%) based on their quality.[16]

Statistical analysis

Statistical analyses were performed using the IBM Statistical Packag for the Social Sciences version 19 (IBM SPSS Corp.; Armonk, NY, USA). Data were checked for normality test. The result was reported as mean±SD. Statistical significance in samples was calculated using analysis of variance and paired *t* test with $p<0.05$. Correlations between groups were evaluated using Pearson correlation coefficients.

Results

A total of semen samples of 114 men with a mean age of 32.89±0.42 years and semen volume of 4.21±1.89 mL were used for semen analysis. DNA integrity for all samples was assessed by SCSA and SCD and chromatin maturity was evaluated by AB-SCMA assay, fmCMA3, and fcCMA3. Sperm of all samples were intracytoplasmic injected to the partner's oocytes. The mean of the partner ages was 32.03±0.45 years. Subsequently, the fertilization rate and embryo feature were

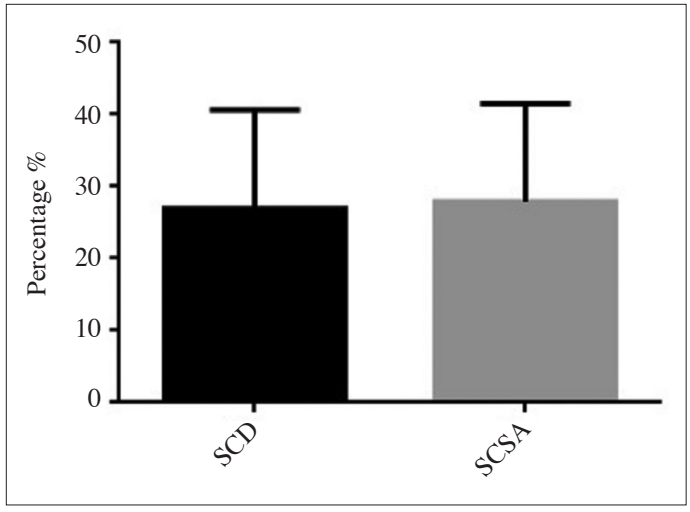

Figure 1. Comparison of sperm DNA fragmentation assays with SDFA and SCD
The difference between sperm DNA fragmentation (%) was not statistically significant when compared with SCD and SCSA (p=0.25)

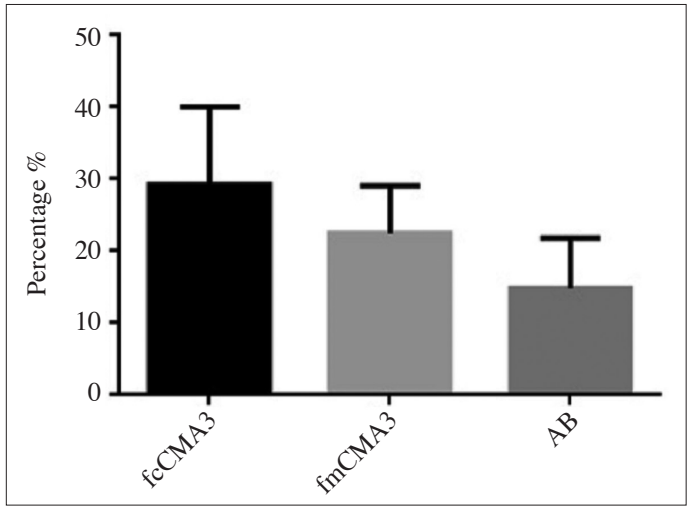

Figure 2. Comparison of sperm chromatin maturation assay with fcCMA3, fmCMA3, and AB
The difference between sperm chromatin (%) was statistically significant when compared with fcCMA3, fmCMA3, and AB-SCMA (p<0.05)

Table 1. Mean and correlation of parameters

		DFI		CMI		
n=114	**Mean±SE**	**SCD**	**SCSA**	**AB-SCMA**	**fmCMA3**	**SCD**
Concentration	37.25±1.12	r=-0.18* p=0.049	r=-0.20* p=0.032	r=-0.10 p=0.276	r=-0.23* p=0.015	r=-0.16 p=0.087
Mophology (%)	3.67±0.18	r=-0.36** p=0.000	r=-0.38** p=0.000	r=-0.38** p=0.022	r=-0.10 p=0.000	r=-0.22* p=0.077
Prograsive (%)	36.51±1.40	r=-0.32** p=0.000	r=-0.30** p=0.001	r=-0.32** p=0.001	r=-0.30** p=0.001	r=-0.09 p=0.323
Vitality (%)	70.70±1.03	r=-0.23* p=0.015	r=-0.21* p=0.025	r=-0.10 p=0.286	r=-0.24* p=0.011	r=-0.08 p=0.402
Total embryo (%)	67.00±2.38	r=-0.50** p=0.000	r=-0.47** p=0.000	r=-0.02 p=0.816	r=-0.47** p=0.000	r=0.01 p=0.911
Grade A (%)	36.53±2.93	r=-0.70** p=0.000	r=-0.66** p=0.000	r=--0.02 p=0.771	r=0.64** p=0.000	r=0.09 p=0.305
Grade B (%)	13.12±1.48	r=-0.16 p=0.084	r=-0.08 p=0.376	r=-0.18 p=0.056	r=-0.17 p=0.065	r=0.04 p=0.655
Grade C (%)	6.10±1.24	r=0.58** p=0.000	r=0.48** p=0.000	r=0.03 p=0.746	r=0.51** p=0.000	r=-0.10 p=0.249
SCD (%)	26.98±1.28	r=1.00** p=0.000	r=0.76** p=0.000	r=0.05 p=0.561	r=0.79** p=0.000	r=-0.16 p=0.089
SCSA (%)	27.88±1.28	r=0.76** p=.000	r=1.00** p=0.000	r=0.03 p=0.698	r=0.84** p=0.000	r=-0.05 p=0.561
AB-SCMA (%)	14.86±0.65	r=0.06 p=0.561	r=0.04 p=0.698	r=1.00** p=0.000	r=0.11 p=0.210	r=0.21* p=0.023
fmCMA3 (%)	29.18±1.01	r=0.79** p=0.000	r=0.12 p=0.210	r=0.12 p=0.210	r=1.00** p=0.000	r=-0.05 p=0.628
fcCMA3 (%)	22.45±0.62	r=-0.16 p=0.089	r=-0.06 p=0.561	r=0.21* p=0.023	r=-0.05 p=0.628	r=1.00** p=0.000

DFI: DNA fragmentation index. CMI: chromatin maturation index. SCD: sperm chromatin dispersion. SCSA: sperm chromatin structure assay. AB-SCMA: aniline blue-sperm chromatin maturation assay. fmCMA3: fluorescence microscopic chromomycin A3. r: indicates the Pearson correlation coefficient. *, ** and *** means p<0.05, p<0.01, p<0.001, respectively.

considered through visualizing of two PN and cleavage stages in one, two, and three days after ICSI. The result showed that the sperm DFI values were not statistically significant between SCSA (26.98%±1.28%) and SCD (27.88%±1.278%) assays (p>0.05) (Figure 1); whereas, the value of sperm CMI showed significant differences (p<0.001) when assessed by AB-SCMA (14.86%±0.65%), fmCMA3 (29.18%±1.01%), and fcCMA3 (22.45%±0.62%) assays (Figure 2). Besides, correlations between semen parameters and DFI and CMI values were investigated in all samples. The degree of correlation was assessed with the coefficient correlation (r) and p-value (Table 1). It seems that both sperm DFI methods (SCD and SCSA) showed significant correlations with sperm concentration (p<0.05), percentage of normal morphology (p<0.001), progressive motility (p<0.001), and vitality (p<0.05), and a strong correlation with the total embryo (p<0.001), grade A embryo (p<0.001), and grade C (p<0.001), which seems needs more samples to confirm. Correlations between sperm CMI evaluating methods (fmCMA3, fcCMA3, and AB-SCMA) and semen parameters showed that AB-SCMA assay had a significant correlation with the percentage of normal morphology (p<0.05) and progressive motility (p<0.001), which is concerning. Additionally, fmCMA3 showed significant correlation with sperm concentration (p<0.05), percentage of progressive motility (p<0.001), vitality (p<0.01), total embryo (p<0.001), grade A embryo (p<0.001), and grade C (p<0.001).

Turk J Urol 2020; 46(5): 348-53
DOI: 10.5152/tud.2020.19262

However, the fcCMA3 method had no significant correlation with semen and embryo parameters. With the comparison of both DFI methods, it was detected that SCD had a strong positive correlation with SCSA ($p<0.001$), fmCMA3 ($p<0.001$), but the SCSA method just has statistical correlation with SCD ($p<0.001$). With the evaluation of the three CMI methods, it was demonstrated that AB-SCMA assay had a positive correlation with fcCMA3 ($p<0.05$), also fmCMA3 had a positive correlation with SCD ($p<0.05$) and SCSA ($p<0.001$).

Discussion

This study evaluated and compared a) two of the most available, accessible, and inexpensive methods of DFI including SCSA and SCD and b) three methods of CMI including AB-SCMA, fcCMA3, and fmCMA3 to assess the efficacy of these methods in evaluating sperm chromatin status in 114 men. Besides, the correlation of these results with semen parameters and embryo development was evaluated. Previous studies reported that sperm concentration, motility, vitality, and morphology had a correlation with fertilization rates in ART.[1,2] It has been shown that normal sperm genetic is required for successful fertilization, as well as for further embryo and fetal development.[1,2] Sperm with abnormal DNA could lead to disorders in the reproductive process.[1,2] Some sperm with abnormal morphology in the head was associated with poor chromatin packaged and an increase in the incidence of chromosomal aneuploidy.[17,18] Therefore, spermatozoa with abnormal heads were also observed to have a higher incidence of failed fertilization rates post-ICSI due to chromatin abnormalities.[19] Additionally, the high incidence of DNA damage has been frequently observed among infertile couples with unexplained and high abortion rates.[20-22] In this study, the significant correlation between DFI and CMI with semen parameters, fertilization rate, and embryo quality, similar to the results previously shown by some authors was demonstrated.[23,24] In general, sperm DNA maturation and fragmentation assay can be met with the use of several methods.[23,25] Despite many standard protocols reported previously, many of them are laborious and require expensive equipment. As DNA fragmentation and maturation assessment become routine soon, an economic and reliable method that can be incorporated into the routine seminal analysis as a screening fertility test is required to evaluate DFI and CMI from semen.[1,2] In this study, with a comparison of some simple and utilized techniques, we not only recommended the simple, economical, and reliable protocols for each of them, but also showed their relation with semen parameters and fertilization rate for selecting the most applied technique. The SCD and SCFA protocols are widely used for evaluating sperm DFI and the AB-SCMA and CMA3 for sperm CMI.[1,2] Studies showed that SCD yields different information than SCSA and there are conflicting results in choosing the most accurate of these two methods.[26,27] Despite the differences in methodology of the SCD and SCSA, some investigators showed that both of them use similar threshold levels to determine the extent of sperm DNA damage.[28] According to our results, these two techniques had no significant differences in their DFI reports, but SCD demonstrated a strong correlation with DNA maturity, which had not been seen in SCSA results. Additionally, the SCD technique was less expensive than SCDA and was capable to be performed in all clinical laboratories with a simple optical microscope. In a comparison of AB-SCMA, fmCMA3, and fcCMA3 methods for assessing sperm CMI, it was shown that as these three techniques were reliable but had significant differences in CMI values. Among them, AB-SCMA had a significant correlation with the percentage of normal sperm morphology and progressive motility, vitality, total grade A embryo, and grade C embryo, although fcCMA3 method had no significant correlation with semen and embryo parameters. With an evaluation of the three CMI methods, it was confirmed that fmCMA3 had a positive correlation with DFI. According to our comparison, it was shown that fmCMA3 had a strong correlation with semen parameters, DNA fragmentation, and embryo quality, which was not observed in other methods. In addition, the fmCMA3 technique is less expensive than AB-SCMA and fcCMA3 and could be performed in all clinical laboratories with simple optical microscopes. Thus, it seems that SCD and fmCMA3 were more accessible, cheaper, and reliable methods to assess DFI and CMI. It appeared that these two methods may be the two best choices for evaluating sperm DNA fragmentation and maturation.

Ethics Committee Approval: Ethics committee approval was received for this study from the Medical Ethics Committee of Avicenna Research Institute (93/4886).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.H., M.D., S.D., M.R.S.; Design - M.H., M.D., S.D., M.R.S.Supervision - M.R.S.; Resources - M.H., M.D., S.D.; Materials - M.H., M.D., S.D., M.R.S.; Data Collection and/or Processing - M.H., M.D., S.D.; Analysis and/or Interpretation - M.H., M.D., S.D.; Literature Search - M.H., M.D., S.D.; Writing Manuscript - M.H., M.D., S.D.; Critical Review - M.H., M.D., S.D., M.R.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This project was financially supported by Avicenna Research Institute (Tehran, Iran)

References

1. Shamsi MB, Imam SN, Dada R. Sperm DNA integrity assays: diagnostic and prognostic challenges and implications in management of infertility. J Assist Reprod Genet 2011;28:1073-85. [Crossref]
2. Panner Selvam MK, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab J Urol 2018;16:65-76. [Crossref]
3. Darbandi S and Darbandi M. Lifestyle Modifications on Further Reproductive Problems. Cresco J Reprod Sci 2016;1:1-2.
4. Darbandi M, Darbandi S, Agarwal A, Baskaran S, Dutta S, Sengupta P, et al. Reactive oxygen species-induced alterations in H19-Igf2 methylation patterns, seminal plasma metabolites, and semen quality. J Assist Reprod Genet 2019;36:241-53. [Crossref]
5. Darbandi M, Darbandi S, Agarwal A, Baskaran S, Sengupta P, Dutta S, et al. Oxidative stress-induced alterations in seminal plasma antioxidants: Is there any association with keap1 gene methylation in human spermatozoa? Andrologia 2019;51:e13159. [Crossref]
6. The World Health Organization. Examination and processing of human sperm. World Health Organization 2010;
7. Darbandi M, Darbandi S, Khorshid HRK, Akhondi MM, Mokarram P, Sadeghi MR. A simple, rapid and economic manual method for human sperm DNA extraction in genetic and epigenetic studies. Middle East Fertility Society J 2017;23:216-9. [Crossref]
8. Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA damage in male infertility: etiologies, assays, and outcomes. J Assist Reprod Genet 2010;27:3-12. [Crossref]
9. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, Abad C, Amengual M, Prada E, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. Andrology 2013;1:715-22. [Crossref]
10. Oliveira H, Spanò M, Santos C, Pereira M. Flow cytometric analyses of sperm functions and genetic integrity. Male and Female Infertility: Genetic Causes, Hormonal Treatments and Health Effects, Edition 2011;1:1-32.
11. Cortés-Gutiérrez E, Dávila-Rodríguez M, Fernández J, López-Fernández C, Gosálvez J. DNA breakage detection-fluorescence in situ hybridization in buccal cells. Eur J Histochem 2012;56:e49. [Crossref]
12. Fernández JL, Muriel L, Rivero MT, Goyanes V, Vazquez R, Alvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 2003;24:59-66.
13. Oehninger S, Franken DR, Ombelet W. Sperm functional tests. Fertil Steril 2014;102:1528-33. [Crossref]
14. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25-43. [Crossref]
15. Sivanarayana T, Krishna R, Jaya Prakash G, Krishna K, Madan K, Sudhakar G, et al. Sperm DNA fragmentation assay by sperm chromatin dispersion (SCD): correlation between DNA fragmentation and outcome of intracytoplasmic sperm injection. Reprod Med Biol 2014;13:87-94. [Crossref]
16. Kim JY, Kim JH, Jee BC, Lee JR, Suh CS, Kim SH. Can intracytoplasmic sperm injection prevent total fertilization failure and enhance embryo quality in patients with non-male factor infertility? Eur J Obstet Gynecol Reprod Biol 2014;178:188-91. [Crossref]
17. Carrell DT, Emery BR, Hammoud S. Altered protamine expression and diminished spermatogenesis: what is the link? Hum Reprod Update 2007;13:313-27. [Crossref]
18. Tsuribe PM, Lima Neto JF, Golim Mde A, Dell'Aqua Cde P, Issa JP, Gobbo CA. Assessment of sperm DNA in patients submitted the assisted reproduction technology procedures. JBRA Assist Reprod 2016;20:17-22. [Crossref]
19. Sadeghi MR, Hodjat M, Lakpour N, Arefi S, Amirjannati N, Modarresi T, et al. Effects of sperm chromatin integrity on fertilization rate and embryo quality following intracytoplasmic sperm injection. Avicenna J Med Biotechnol 2009;1:173-80.
20. Avendaño C, Franchi A, Duran H, Oehninger S. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril 2010;94:549-57. [Crossref]
21. Lewis SE, Aitken RJ, Conner SJ, De Iuliis G, Evenson DP, Henkel R, et al. The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 2013;27:325-37. [Crossref]
22. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? Syst Biol Reprod Med 2011;57:78-85. [Crossref]
23. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl 2017;19:80-90.
24. Cissen M, van Wely M, Scholten I, Mansell S, de Bruin JP, Mol BW, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. PLoS One 2016;11:e0165125. [Crossref]
25. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online 2015;30:120-7. [Crossref]
26. Martínez-Pastor F, Del Rocío Fernández-Santos M, Domínguez-Rebolledo ÁE, Esteso M, Garde J, Group BoR. DNA status on thawed semen from fighting bull: a comparison between the SCD and the SCSA tests. Reprod Domest Anim 2009;44:424-31. [Crossref]
27. Pérez-Cerezales S, Miranda A, Gutiérrez-Adán A. Comparison of four methods to evaluate sperm DNA integrity between mouse caput and cauda epididymidis. Asian J Androl 2012;14:335-7. [Crossref]
28. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online 2006;12:466-72. [Crossref]