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Original Research Article

Analysing the DNA integrity in hypo-osmotic swelling test types of tail curling and assessing if HOS type "B" outweighs the rest

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ABSTRACT

Background: One of the important steps in ICSI is to select a sperm with good DNA content to have a successful outcome. In case of complete asthenozoospermia the aim is to select a viable sperm using the hypo-osmotic swelling test. There has been lot of debate regarding type of tail curling and their DNA integrity. This study aimed to analyze DNA integrity among different hypo-osmotic swelling (HOS) types of tail curling and determine whether HOS type B has superior DNA quality compared to other types.
Methods: A total of 43 semen samples were collected from patients who attend a tertiary care centre in Chennai. The samples were subjected to HOS testing, followed by DNA integrity assessment using acridine orange staining.
Results: The study found that sperm with HOS type B tail curling exhibited significantly lower DNA fragmentation (24.6%) compared to HOS type A (97.7%) and other types (61.9%), suggesting that HOS type B sperm possess better DNA integrity.
Conclusions: These findings propose that selecting HOS type B sperm in cases of asthenozoospermia for intracytoplasmic sperm injection (ICSI) could enhance outcomes, as this method is cost-effective and identifies sperm with good DNA content.

Keywords: Asthenozoospermia, DNA fragmentation, HOS test, HOS type B tail curling

INTRODUCTION

One of the most significant advances in assisted reproductive technology was ICSI, which was introduced in the early 1990s (ART). Following its introduction, the method was quickly incorporated into the standard clinical procedure of fertility facilities worldwide.[1]

In ICSI, a viable sperm is chosen and injected into the cytoplasm of a mature oocyte after being judged on the basis of its appearance and motility. Choosing sperm with these two qualities will not guarantee their chromatin integrity.[2]

According to human research, DNA-damaged spermatozoa can be effectively fertilised during IVF and ICSI, allowing normal embryo development and subsequent pregnancy.[4] Because ICSI bypasses the body's natural defences and permits fertilisation with DNA-damaged sperm, there is growing concern about offspring born as a result.[3]

Intact sperm is crucial for ICSI because of the relevance of paternal DNA in embryo growth and development.[4]

The selection of sperm cells can be performed via traditional procedures, which are based on sperm motility or density, or more sophisticated techniques, which are based on membrane surface charge, high-resolution morphology, and nuclear or membrane integrity.[5] One such technique is the hypo-osmotic swelling test (HOST).

HOST, which is used in the case of asthenozoospermia, is predicated on the idea that when live spermatozoa are exposed to a hypoosmotic environment, their tails swell and bend as a result of the activity of osmo-sensitive calcium membrane channels.[6] This method enables the identification of sperm cells with improved nuclear material for ICSI treatments on the basis of the type of tail curling (a-g) via microscopy.[7]

A fluorescent cationic cytochemical stain known as acridine orange (AO) is selective for DNA in cell nuclei. AO recognises genetic material, distinguishes between DNA and RNA, and reflects sperm chromatin denaturation. When AO binds to native double-stranded and normal DNA as a monomer, it fluoresces green, and when it binds to denatured single-stranded DNA as an aggregate, it fluoresces red.[8] The AO nuclear fluorescence of sperm can therefore be used to determine nuclear maturity in mammalian sperm.

The classes with the lowest DNA fragmentation rates, according to the same researchers, were D/E (4%) and F (8%). In contrast to the findings of Stanger et al, Bassiri et al discovered HOST classes A, F, and G to be associated with the highest levels of DNA fragmentation, and B, C, and D/E were associated with the lowest amounts.[9,10]

METHODS

Study place, design, inclusion and exclusion criteria

A prospective observational study which was conducted with 43 patients who attended the department of Reproductive Medicine and Surgery at Sri Ramachandra Institute of Higher Education and Research, Chennai, from November 2021 to December 2021. The study included all the patients attending our OPD for semen analysis while excluding the once with azoospermia, surgically retrieved sperms and retrograde samples. This study was approved by the institutional ethical committees of Sri Ramachandra Institute of Higher Education and Research IEC No.-CSP/21/JUL/96/389.

Semen analysis

Semen analysis was routinely performed according to the world health organization manual (fifth edition, 2010) protocol.

Assessment of sperm concentration

Assessment of sperm motility

Sperm motility was assessed manually by placing a drop (10 µl) of sperm suspension on a clean glass slide a cover slip was carefully placed above. The number of motile sperms was graded and counted separately within 30 minutes from the time of the sample collection under light microscope (40X). The motility was graded into 3 categories namely progressive, non-progressive, immotile and were expressed in percentage (WHO 2010).

Figure 1: Mackler's counting chamber for sperm concentration and motility.

Total motility: total number of motile sperms per 200 sperms. Progressive motility: number of sperms moving in or in a linear fashion. Non-progressive motility: number of sperms moving in small circles or twitching at their own place or having a non-linear movement. Immotile: number of sperms showing no motility on microscopic examination.

Sperm morphology

Diff-quick staining method was performed as follows:

The semen was smeared on a glass slide and allowed to dry. After it has dried, a fixative was sprayed on the slide and again allowed to dry. After drying, the slide was dipped into stain A for 7-10 seconds followed by dipping in stain B for the same time. Then it was washed under water and viewed under 100X or oil immersion objective and morphology is calculated according to the Kruger's strict criteria as following: head defects, mid-piece defects, tail defects.

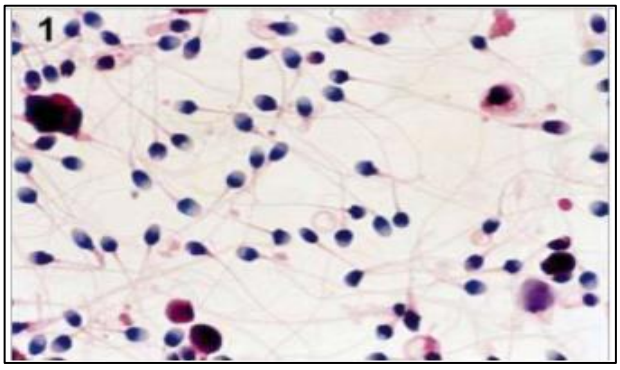

Figure 2: Diff-Quick staining for sperm morphology.

Sperm HOS test and preparation of smears

One hundred microlitres of each semen sample was diluted with 1 ml of hypo-osmotic solution (containing 0.735 gm

of sodium citrate and 1.35 gm of fructose). The mixture was then incubated at 37°C for 30 minutes.[12] Medium-thick smears were made on a clean slide and allowed to air dry for 20 minutes. The smeared slides were fixed for at least two hours and ideally incubated overnight in freshly prepared Carnoy's solution (3 parts methanol to 1 part glacial acetic acid). The slides were removed from the fixative and allowed to dry before being stained with acridine orange.[13]

Stain preparation

One gram of AO was dissolved in 1000 ml of distilled water to make a stock solution. The staining solution was made by mixing 10 ml of the stock solution with 40 ml of 0.1 M citric acid and 2.5 ml of 0.3 M $Na_2HPO_47H_2O$. The stock solution was kept at 4°C in the dark, and the AO stain was freshly prepared every day.[13]

Staining of slides

Two to three milliliters of the prepared stain was spread over each slide and left in the dark for 5 minutes. Next, the stain was washed off with deionised water and allowed to air dry. The coverslips were placed before the slides were dried completely.[13]

Assessment of DNA fragmentation among HOS types of tail curling

For each slide, a minimum of 200 sperm were randomly evaluated according to the WHO 5th edition. The semen samples were divided into groups A (HOS type A), B (HOS type B), and C (Other HOS types) on the basis of how the tails curled. The sperm DNA integrity was evaluated based on the hue of the emitted florescence. Sperm with green florescence were considered normal, whereas those with yellow, orange, or red florescence were considered aberrant.

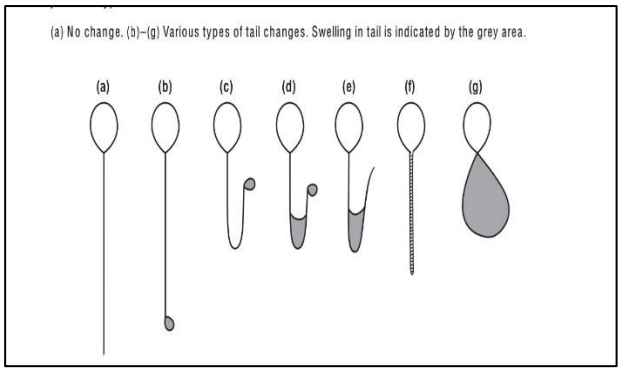

Figure 3: HOST type of tail curling.

DNA fragmentation index calculation

The spermatozoa with damaged DNA fluoresced red or orange, whereas normal spermatozoa with intact double-stranded DNA stained green. By calculating the percentage of spermatozoa with fragmented DNA, the DFI was determined.[14]

Groups A, B, and C were the three groups into which the sperm were divided.

The sperm in group A had tail curling of type A, those in group B had tail curling of type B, and those in group C had tail curling of different types.

Assessment of stained slides

The curled tails were further assessed for DNA denaturation based on the fluorescence emitted using fluorescent microscopy at 450-490 nm.[13] The sperm heads that emitted green fluorescence were considered normal while the ones with yellow and red fluorescence were considered denatured. The ratio between the number of (yellow to red)/(green + yellow to red) was given as the DFI percentages (Figure 4 represents the sperms with and without denaturation).[13]

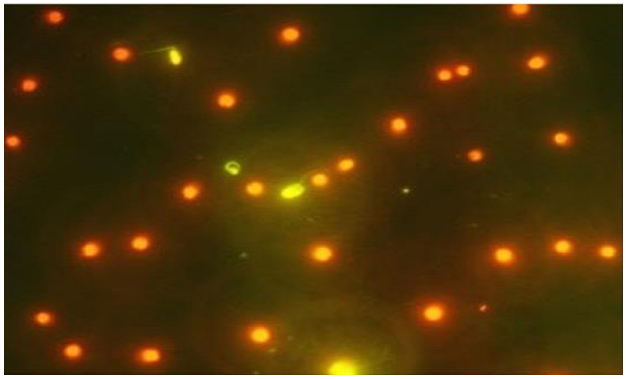

Figure 4: Acridine orange-stained sperms under fluorescent microscope.

Statistical analysis

The collected data were analysed with IBM SPSS Statistics for Windows, version 23.0. A percentage analysis was used for categorical variables, and the means and SDs were used for continuous variables (Armonk, NY: IBM Corp.). To identify significant differences in the multivariate analysis, one-way ANOVA with Tukey's post hoc test was used. To assess the relationships among the variables, Pearson's correlation was used. For all the above statistical tools, a probability value of 0.05 was considered significant

RESULTS

The age of the males in this study ranged from 26-46 years, with a mean age of 33.5±4.4 years. In the present study, the sperm concentration ranged from 3 to 90 million/ml (mean: 29.15±23.1 million/ml), the percentage of motility ranged from 15% to 87% (mean: 52.2±16.7), and the percentage of normal morphology ranged between 1% and 13% (mean: 3.3±2.8).

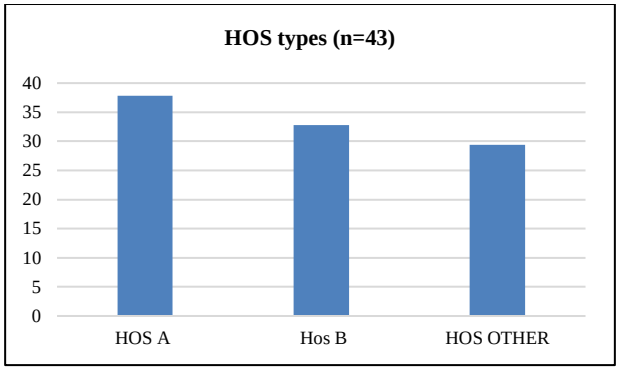

Figure 5: Distribution of HOS types of tail curling in the study.

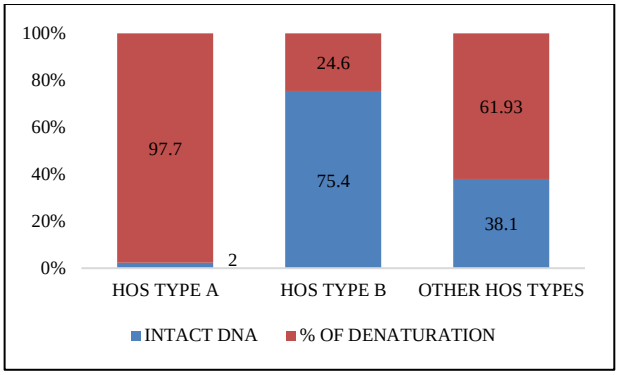

Figure 6: Comparison of intact and denatured sperm among the groups.

According to the WHO guidelines, Figure 5 depicts the distribution of HOS types of tail curling in 43 samples. The mean proportion of HOS type A cases was 37.8±9.1%, the mean percentage of HOS type B cases was 32.8±0.7%, and the mean percentage of all other HOS types was 29.4±9.1%.

AGE versus HOS types

The mean percentages of HOS type A, type B, and other types were 39.3±10.6, 32.2±7.8, and 28.4±4.2, respectively, in patients <30 years (p value <0.05). The mean HOS type A was 37.7±10.8, the mean HOS type B was 31.7±10, and the mean HOS type was 30.3±5.6 (p value <0.05) in the 31-35 age group. Males older than 35 years had mean HOS types of 36.9±12.03, 34.4±8.2 for HOS type B, and 28±7.3 for all other HOS types (p=0.05).

AGE versus DFI and viability

The mean DFIs for those under 30 years of age and between 31 and 35 years and above 35 years of age were 61.3%, 61.05% and 61.8%, respectively (p=0.9). Age groups under 30 years of age had a viability of 60.6%, those between 31-35 years of age had a viability of 62.1%, and those above 35 years of age had a viability of 62.5%. (p=0.9).

DFI percentages and comparisons among the groups

The percentage of DFIs in the groups was as follows: HOS type A had 97.7% DFI, HOS type B had 24.6%, and the other HOS types had 61.9% DFI, indicating that HOS type A had increased DNA intact sperm. (Figure 6). A comparison of the DFIs among HOS type A and type B patients (97.7±2.6 and 24.69±9.4, respectively) revealed that the DFI was significantly greater in type A patients, with a p value of <0.05, and the DFIs among HOS type B patients and other types (24.69±9.4 and 61.9±11.4, respectively) revealed that the DFI was significantly lower in HOS type B patients than in other types of HOS patients, with a p value of <0.05.

DISCUSSION

The goal of semen analysis is to better understand the physiological features of the sperm that are responsible for fertilisation. One such test is the test for vitality, which assesses the functional and structural integrity of the sperm. The dye exclusion test used in conventional semen analysis identifies sperm with intact plasma membranes but does not consider functional integrity. This test is purely diagnostic, as sperm stained with eosin and nigrosine cannot be used for ICSI procedures.[18,19] The HOS test is another test for vitality that can assess both the functional and structural integrity of the sperm plasma membrane without staining.[20]

Since the discovery of ICSI in 1990, various attempts have been made to improve the method of sperm selection; however, selecting sperm with low DNA fragmentation remains a challenge. The accumulation of single- or double-strand breaks in DNA can be explained by fragmentation, which appears to have a deleterious effect on human fertility.[15] During development, sperm DNA has a special structure that protects it against potential insults, including heat, free radicals, medications, radiation, and infections. Nevertheless, failure to maintain this structure due to lifestyle alterations could result in DNA breaks.[16,17] Although newer devices have been developed to replicate the natural selection of sperm, they fall short in addressing difficulties such as cost and abnormal/low-volume semen samples.

As age increased, the degree of DNA fragmentation increased, which was similar to the findings of our study. Researchers have shown that HOST type A have increased levels of DNA fragmentation, as they are considered dead.[21,22] Bloch et al, in their cell-level analysis of HOS classes, concluded that HOS type B is the best-quality spermatozoa with low DNA fragmentation and good nuclear architecture. Our study confirmed the hypothesis that HOS type B sperm have better DNA content than other HOS types of tail curling. The novelty of the study is that, individual sperm were assessed for type of tail curling and DNA denaturation simultaneously via phase contrast

and fluorescence microscopy, which revealed that HOS type B sperm had a better DNA content than other types.

Further research with a large sample size is required to prove the same. Also, more research is required to analyse the ICSI out comes using B-type of HOS tail curling sperms used for fertilization.

CONCLUSION

These findings suggest that in the case of complete astheno-zoospermia, HOS-type sperm selection can be employed as a method of selection for ICSI, as it is cost effective and helps identify sperm with good DNA content.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research IEC No.-CSP/21/JUL/96/389

REFERENCES

1. Rubino P, Viganò P, Luddi A, Piomboni P. The ICSI procedure from past to future: a systematic review of the more controversial aspects. Hum Reprod Update. 2016;22(2):194-227.
2. Celik-Ozenci C, Jakab A, Kovacs T, Catalanotti J, Demir R, Bray-Ward P, et al. Sperm selection for ICSI: shape properties do not predict the absence or presence of numerical chromosomal aberrations. Hum Reprod. 2004;19(9):2052-9.
3. Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, Sanchez-Martin M, Ramirez MA, Pericuesta E, et al. Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. Biol Reprod. 2008;78:761-72.
4. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, et al. Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod. 2004;19:1409-17.
5. Vaughan DA, Sakkas D. Sperm selection methods in the 21st century. Biol Reprod. 2019;101:1076-82.
6. Rossato M, Di Virgilio F, Foresta C. Involvement of osmo-sensitive calcium influx in human sperm activation. Mol Hum Reprod. 1996;2:903-9.
7. Bassiri F, Tavalaee M, Shiravi AH, Mansouri S, Nasr-Esfahani MH. Is there an association between HOST grades and sperm quality? Hum Reprod. 2012;27:2277-84.
8. Ichimura S, Zama M, Fujita H. Quantitative determination of single-stranded sections in DNA using the fluorescent probe acridine orange. Biochem Biophysiol Acta. 1971;240:485-95.
9. Stanger JD, Vo L, Yovich JL, Almahbobi G. Hypo-osmotic swelling test identifies individual spermatozoa with minimal DNA fragmentation. Reprod BioMed Online. 2010;21:474-84.
10. Bassiri F, Tavalaee M, Shiravi AH, Mansouri S, Nasr-Esfahani MH. Is there an association between HOST grades and sperm quality? Hum Reprod. 2012;27:2277-84.
11. WHO laboratory manual for the examination and processing of human semen-5th ed. ISBN 9789241547789.
12. Ramu S, Jeyendran RS. The hypo-osmotic swelling test for evaluation of sperm membrane integrity. In: Spermatogenesis: Methods and protocols. Springer Nautre; 2013:21-25.
13. Tejada RI, Mitchell JC, Norman A, Marik JJ, Friedman S. A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil Steril. 1984;42(1):87-91.
14. Zwamel AH, Fakhrildin MB, Hassani HH. Impact of exposure period to liquid nitrogen vapor on criteria of human spermatozoa cryopreserved in new cryopreservation technique. Indian J Forens Med Toxicol. 2020;14(3):2379-84.
15. Simon L, Emery B, Carrell DT. Sperm DNA Fragmentation: con- sequences for reproduction. Adv Exp Med Biol. 2019;1166:87-105.
16. Aitken RJ, Jones KT, Robertson SA. Reactive oxygen species and sperm function- in sickness and in health. J Androl. 2012;33:1096-106.
17. Saleh RA, Agarwal A, Nelson DR, Nada EA, El-Tonsy MH, Alvarez JG, et al. Increased sperm nuclear DNA damage in normo-zoospermic infertile men: a prospective study. Fertil Steril. 2002;78:313-8.
18. Neild D, Chaves G, Flores M, Mora N, Beconi M, Aguero A. The hypo-osmotic swelling test in equine spermatozoa. Theriogenology 1999; 51:721-7.
19. Herak M. Artificial insemination of domestic animals, domestic animal reproduction. In: Veterinary Manual. 7th edn. Merck Veterinary Manual; 1991;181-209.
20. Madeja Z, Waroczyk M, Strable T, Lechniak D. Use of the hypo-osmotic swelling test for evaluating bull and boar semen quality. Medycyna Weterynaryjna. 2003;59:1115-8.
21. Bollendorf A, Check JH, Kramer D. The majority of males with subnormal hypoosmotic test scores have normal vitality. Clin Exp Obstet Gynecol. 2012;39:25-6.
22. Bloch A, Rogers EJ, Nicolas C, Martin-Denavit T, Monteiro M, Thomas D, Morel H, Lévy R, Siffroi JP, Dupont C, Rouen A. Detailed cell-level analysis of sperm nuclear quality among the different hypo-osmotic swelling test (HOST) classes. J Assist Reprod Genet. 2021;38(9):2491-9.

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