Original Article

A New Simple Test for Checking Vitality of Spermatozoa

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Abstract

Objective: Hypo-osmotic swelling (HOS) test was done to assess the vitality of spermatozoa. Sterile water is easily available and inexpensive when compared to HOS reagent. Efficacy of sterile water is checked in comparison with Hypo-osmotic swelling (HOS) Reagent for assessing the vitality of human spermatozoa.

Setting: Prospective study conducted at a tertiary referral centre and Research Institute.
Patient Criteria: Study includes 30 patients who had come for routine semen analysis. Consent forms had been taken from the patients.

Methodology: All samples were included except azoospermic samples. Semen sample was subjected to HOS test, sterile water test, Eosin - Nigrosin test (EN) after routine semen analysis.

Results: Average number of vital spermatozoa of HOS test,sterile water test and EN test are 55.83%,58.10% and 59% respectively. Average number of vital spermatozoa of HOS test were compared with sterile water test (p= 0.468), average number of vital spermatozoa of HOS test were compared with EN test (p= 0.286) and average number of vital spermatozoa of sterile water test were compared with EN test (p= 0.847). The p-value for all the three tests together (HOS test, sterile water test, Eosin nigrosin test (EN)showed 0.567 which is statistically insignificant thereby indicating that there was no major difference between the three tests.

Conclusion: Sterile water can replace HOS reagent for vitality test of spermatozoa.It might prove beneficial to the patient as it is less expensive and cost- effective.

Key Words: Human spermatozoa, HOS test, Vitality, Sterile water

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Introduction

Vitality is defined as presence of structural and functional intact membrane of the cell. Hypo- osmotic swelling test (HOS test) is a vitality test done for spermatozoa. It is an additional sperm function test, normally used to identify the percentage of vital spermatozoa within semen groups for asthenozoospermia, Immotile Cilia syndrome, occasionally motile spermatozoa and spermatozoa retrieved from testicular biopsies, where the motility of the spermatozoa is diminished.A simple new test using sterile water can be used to assess the vitality thereby making it simpler and much more cost effective.

HOS test was developed by Jeyendran et al, in 1984[1]. The basic HOS reagent is 150 mOsm. It contains 0.735g of sodium citrate dihydrate and 1.351g of D-fructose in 100ml of purified water[1]. The basic principle of HOS test is that solution enters the spermatozoa under hypo-osmotic conditions due to osmosis. The sperm tail then expands and coils depending on the sperm membrane function and integrity. Sperm tail does not coil if the sperm membrane is damaged. Sterile water test works on the same basic principle as HOS test. Sterile water is 0 mOsm which is hypo-osmotic. The principle of Eosin Nigrosin test is that spermatozoa with intact membrane integrity do not take up the stain and therefore are colourless. Spermatozoa in which membrane integrity has been lost take up the stain, therefore they are pink in colour[2]. The aim of our study was to check whether sterile water can be used to assess the vitality of spermatozoa.

Methodology

Patient criteria: Prospective study was done at Chettinad Hospital & Research Institute. All types of semen samples were included except azoospermic samples. HOS reagent, sterile water and eosin nigrosin reagent were used for this study. Samples were subjected to HOS test, sterile water test and eosin nigrosin test after the initial semen analysis had been done according to WHO manual 2010. All the semen samples which were about to be discarded after initial semen analysis were used for this study. Thirty samples were included in the study.

HOS test: HOS reagent which was used belongs to Cell Life Company, Vishakhapatnam. Protocol was followed as per instructions given in the HOS test kit.

1. 1 ml of HOS reagent (150 mOsm/L) was incubated for normozoospermic samples in an incubator at

37°C for 20minutes. 0.5ml of HOS reagent was incubated for oligozoospermic samples in an incubator at 37°C for 20minutes.

2. One drop(5µl) of semen was mixed with HOS reagent and incubated for 10minutes.

3. One drop(5µl) of well mixed semen was taken on the glass slide and a cover slip was placed on it. Hundred spermatozoa were counted randomly. Spermatozoa with coiled tails were considered as vital and uncoiled tails were considered as dead (figure 1).

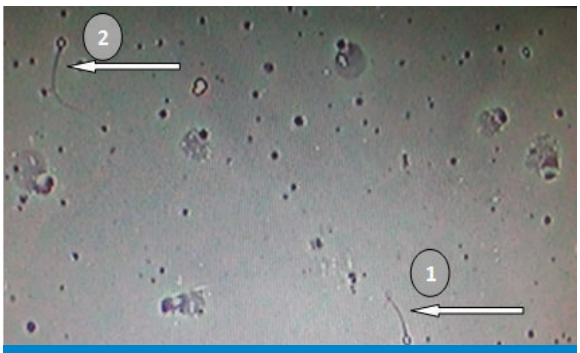

Fig 1 - (1)Coiled tail- vital. (2).Straight tail– Non vital

Sterile Water Test Protocol

1. 1 ml of sterile water (0 mOsm/L) was incubated for normozoospermic samples in an incubator at 37°C for 20minutes. 0.5ml of sterile water was incubated for oligozoospermic samples in an incubator at 37°C for 20minutes.

2. One drop(5µl) of semen was mixed with sterile water and incubated for 10minutes.

3. One drop(5µl) of well mixed semen was taken on the glass slide and a cover slip was placed on it. Hundred spermatozoa were counted randomly. Spermatozoa with coiled tails were considered as vital and uncoiled tails were considered as dead (figure 2).

Fig 2 - (1)Coiled tails . (2).Straight tail– Non vital

Eosin Nigrosin Test Protocol

Eosin nigrosin kit which was used belongs to Cell Life Company, Vishakapatnam. Protocol was followed as per instructions given in the Eosin nigrosin kit.

1. One drop(5µl) of eosin nigrosin stain was mixed with one drop(5µl) of semen in an eppendorf tube and kept for 25 seconds.

2. A drop(5µl) of well mixed sample was taken from the eppendorf tube and placed on the glass slide and thin smear was made on the glass slide. Slide was incubated at 37°C in an incubator for 10minutes. Hundred spermatozoa were examined. Pink coloured heads of spermatozoa were considered as non-vital and colorless heads were considered as vital.

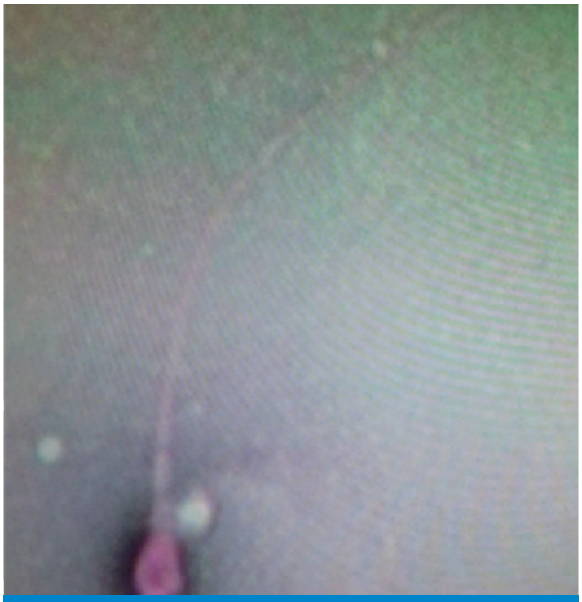

Fig 3 - Pink Coloured Head - Nonvital

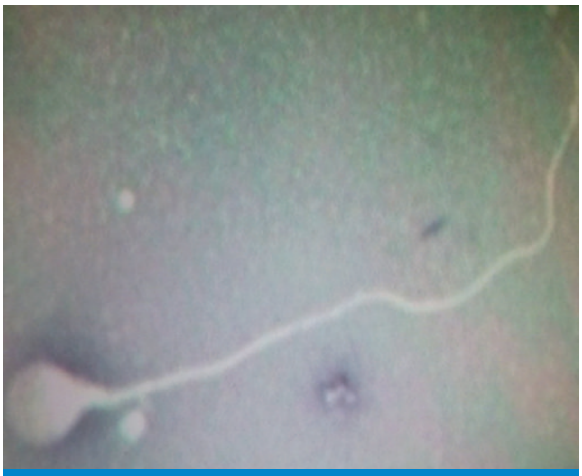

Fig 4 - Colourless Head – Vital

Statistical Analysis

Statistical analysis was done with Mann-Whitney U test and Kruskal -Wallis H test. Both the tests were done by comparing the mean of the variables of HOS test, Sterile water test and Eosin-nigrosin test.

Results

Thirty samples were included in the study. Mean age of the patients was 33.6 years. Mean spermatozoa concentration was 78.16 million per ml. Mean total motility of spermatozoa was 58.8 % (table 1).

Patient's criteria (n= 30)	Mean
Age(years)	33.6
Concentration(million/ml)	78.16
Motility (%)	58.8

Table 1 - Mean criteria of patients

VARIABLES	MIN	MAX	MEAN	SD	Mean difference		P VALUE
					MEAN	SD	
HOST (%)	30	89	55.83	14.66	2.27	0.48	0.468
Water (%)	30	80	58.10	14.18			
HOST (%)	*30*	*89*	*55.83*	*14.66*	*3.17*	*0.34*	*0.286*
EN (%)	30	80	59.00	14.32			
Water(%)	30	80	58.10	14.18	0.90	0.14	0.847
EN (%)	30	80	59.00	14.32			

Table 2 - Comparison between mean of the variablesof vital spermatozoa of HOS Test, Sterile Water Test, Eosin – Nigrosin Test(En)

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05

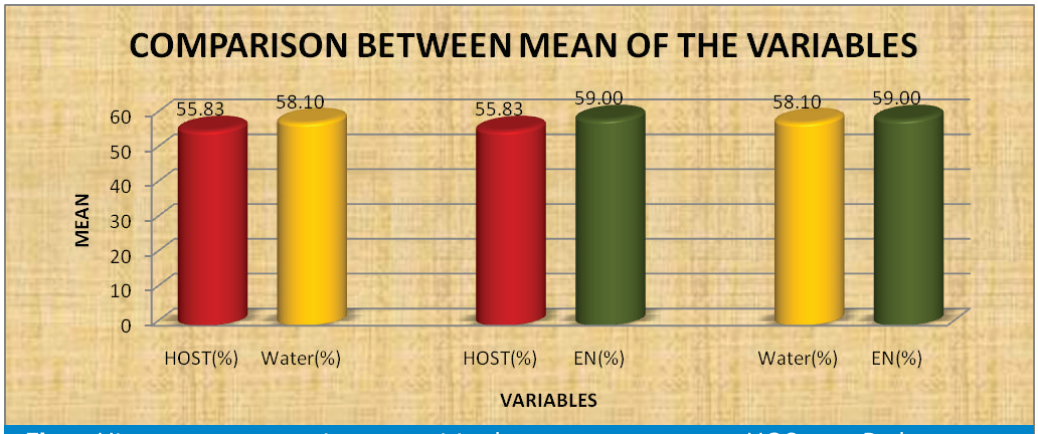

Fig 5 - Histograms representing comparision between any two tests . HOS test – Red, Sterile water test – Yellow and eosin nigrosin test – Green.

VARIABLES	MIN	MAX	MEAN	SD	P VALUE
HOST(%)	30	89	55.83	14.66	0.567
Water(%)	30	80	58.10	14.18	
EN(%)	30	80	59.00	14.32	

Table 3 - Comparison between mean of the variables of vital spermatozoa of HOS test, sterile water test, eosin – nigrosin(en) test

Statistical Analysis: Kruskal-Wallis H test. Statistically significant if P<0.05

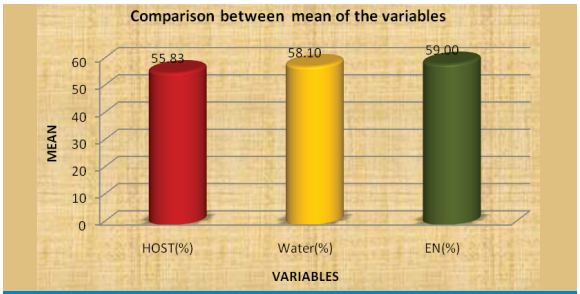

Fig 6 - Histograms representing comparision of all the three tests. HOS test – red, sterile water test – yellow and eosin nigrosin test – green.

Discussion

Sterile water test (0 mOsm), HOStest (150 mOsm) and Eosin nigrosin tests were compared to check the vitality of spermatozoa. All the three tests gave similar results. The vital spermatozoa of the three tests have showed correlation with total motility of spermatozoa.Eosin nigrosin test checks the membrane integrity of the spermatozoa but they cannot be used for Intracytoplasmic Sperm Injection(ICSI) once stained. However, Spermatozoa assessed through HOS test can be used for ICSI[4]. Similar study comparing HOS test and sterile water test was done on Drone spermatozoa[3]. There was no difference in results of HOS test and sterile

water test in Drone spermatozoa[3]. Aneuploidy rates are similar in morphogically normal vital immotile spermatozoa selected through HOS test and normal motile spermatozoa[5] .

Conclusion

The degree of hypo-osmolarity of sterile water (omOsm) to spermatozoa is more when compared to HOS reagent(150 mOsm). Coiling of tail takes place faster when compared to HOS test.Sterile water is less time consuming and cost effective. Therefore sterile water can be used as alternative to HOS reagent.

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Authors declare no conflict of interest.

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Maternal Diabetes and Offspring's Intelligence

According to a new study from Denmark, pregnant women with type I diabetes should keep the blood glucose level under control; otherwise, the offspring's cognitive development may be adversely affected. In that study, which is published in the latest edition of Diabetes Care, academic performance of 707 primary school children of mothers with type I diabetes, was correlated with blood glucose levels of their mothers during and before pregnancy and also with academic performance of 60000 children of non-diabetic mothers. It was found that children of mothers with good control of blood sugar performed better than average academically when compared to their peers in general population.Women with the best diabetes control in pregnancy had offspring that performed better academically than offspring of women without diabetes! Conversely, children of mothers with poorly controlled diabetes had lower grades. This correlation was found to be independent of parental education. As no such studies have been conducted with other types of diabetes, the results cannot be extrapolated.

- Dr. K. Ramesh Rao