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ORIGINAL ARTICLE

Importance of Seminal Hyaluronidase Activity and Hypo-Osmotic Swelling Test in Male Infertility

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Abstract: The conventional seminal parameters like sperm density, motility, morphology, etc. are not perfect indicators of seminal functional quality. Hence, Seminal Hyaluronidase Activity (SHA) and Hypo-osmotic Swelling (HOS score) of sperms that evaluate healthy sperms of fertilizing potential, were studied in male partners of infertile couples. The semen samples were obtained, by masturbation after 4 days of abstinence, from recently Fertility Proven Males (Group-I, n=30) and Male Partners of Infertile Couples [Normozoospermic (Group-II, n=30) and Oligozoospermic (Group-III, n=30)]. After performing routine seminal analysis, the semen samples were subjected for SHA and HOS score assessment. The mean ± standard deviation values of SHA (mm of ring diameter) were 9.90±1.65, 8.77±1.87 and 6.50±1.33 in Group-I, Group-II and Group-III, respectively. Similarly, the mean ± standard deviation values of HOS score (%) were 65.50±8.69, 58.77±15.95 and 39.00±9.78 in Group-I, Group-II and Group-III, respectively. The difference in values of SHA and HOS score amongst different Groups was very highly significant statistically ($p<0.001$). Also, we found significant correlation of SHA and HOS score with sperm density, % motility and % normal morphology. This study shows that Normozoospermic Infertile Males, although having normal conventional seminal parameters, have lower SHA and HOS score than Fertility Proven Males. Thus, it emphasizes the importance of SHA and HOS score assessment in infertile males.

Keywords: Hypo-osmotic swelling- Male infertility- Seminal hyaluronidase.

Introduction

For fertilization, healthy sperm swims through female genital tract to reach oocyte, penetrates through the investment of oocyte; nuclear chromatins decondenses to form male pronucleus and finally male pronucleus fuses with female pronucleus [1]. Hyaluronidase, the one of the acrosomal enzymes that causes dissolution (hydrolysis) of the cumulus oophorus matrix containing hyaluronic acid surrounding oocyte, if deficient causes infertility [2]. Similarly, the subtle defects of sperm plasma membrane can alter the functional integrity of sperms even when morphology is normal. Such defective functional integrity of sperm plasma membrane may be a contributing factor in male infertility [3-4]. The functional integrity of sperm plasma membrane can be evaluated by a simple hypo-osmotic swelling test [5]. As no single conventional parameter of seminal analysis is perfect indicator of seminal quality, we tried to compare seminal hyaluronidase activity and hypo-osmotic swelling test score of male partners of infertile couples with recently fertility proven males and emphasize the importance of above tests.

Material and Methods

Thirty semen samples from each, normozoospermic and oligozoospermic male partners of infertile couples, referred from infertility clinic, were included in this study. Equal numbers of recently fertility proven males were motivated to donate their semen samples for comparison. The informed consent was obtained from all the individuals. Semen samples were obtained by masturbation (after 4 days of sexual abstinence) and routine seminal analysis including conventional parameters like sperm density, % motility, % normal morphology, was done according to W.H.O. standards [6]. Thereafter, seminal hyaluronidase activity and hypo-osmotic swelling test score was determined for each semen sample.

Seminal hyaluronidase activity was determined by a method based on measurement of area of digestion of hyaluronic acid in agar plate and expressed as mm of ring size (i.e. area of substrate digestion) [7-8]. Hypo-osmotic swelling test was performed by diluting 0.1 ml of semen in 1ml hypo-osmotic solution (HOS). HOS was prepared by mixing equal volume (0.5 ml) each of 2.7 % fructose solution and 1.47% sodium citrate solution and then incubated at 37^0C for 30 minutes. The slide of this diluted semen examined under 40x objective of phase contrast microscope. The actual percentage of sperms showing HOS positive reaction (i.e. HOS score) was determined by subtracting the number of coiled tail sperms in untreated samples from the number of coiled tail sperms in treated sample with hypo-osmotic solution [1, 9].

Statistical analysis: The results of routine seminal analysis, seminal hyaluronidase activity and hypo-osmotic swelling test were analyzed by applying statistical tests like *ANOVA test, T test and Pierson correlation coefficient* with help of *Microsoft excel-2007*.

Results

The sperm density (10^6 /ml), % motility, % normal morphology, seminal hyaluronidase activity (SHA) in mm ring diameter and hypo-osmotic swelling score (HOS %) in different groups are presented as *mean ± standard deviation* (SD) in Table-1.

Table-1: Mean values and analysis of variance amongst different groups.

Groups	Sperm density (10^6/ml) Mean± SD	% Motility Mean± SD	% Normal morphology Mean± SD	Seminal hyaluronidase activity (mm ring diameter) Mean±SD	HOS score (%) Mean± SD
Proven fertility (n=30)	100.67±38.81	68.17±8.31	65.00±10.48	9.90±1.65	65.50±8.69
Normozoo-spermic (n=30)	81.16±45.42	53.17±18.77	63.00±11.00	8.77±1.87	58.77±15.95
Oligozoos-permic (n=30)	10.83±4.71	41.00±16.20	44.17±16.94	6.50±1.33	39.00±9.78
ANOVA test	*F=54.10, p<0.001*	*F=23.55, p<0.001*	*F=22.19, p<0.001*	*F=32.59, p<0.001*	*F=38.77, p<0.001*

Mean SHA and HOS score differed significantly in different groups when tested by ANOVA test (Table-1). Also, comparisons of these values between different groups were significant (Table-2). The correlation coefficients of seminal hyaluronidase activity and HOS score with conventional seminal parameters are as presented in Table-3**,** indicating very highly significant relationship ($p<0.001$).

Table-2: Comparison of Seminal hyaluronidase activity and Hypo-osmotic welling Score amongst different groups.

Comparison	Seminal Hyaluronidase Activityz	Hypo-Osmotic Swelling Score
Proven fertility Vs. Normozoospermic	t=2.45, p<0.01	t=1.97, p<0.05
Proven fertility Vs. Oligozoospermic	t=8.64, p<0.001	t=10.91, p<0.001
Normozoospermic Vs. Oligozoospermic	t=5.30, p<0.001	t=5.69, p<0.001

Table-3: Correlation of Seminal hyaluronidase activity and Hypo-osmotic swelling scores with Conventional seminal parameters.

Correlation coefficient (r)	Sperm density	% motility	% normal morphology
Seminal Hyaluronidase Activity	0.7915*	0.6499*	0.5022*
Hypo-Osmotic Swelling score	0.6704*	0.4572*	0.5401*
*Indicates p<0.001			

There was strong significant correlation between HOS score and seminal hyaluronidase activity ($r=0.5779$, $p<0.001$).

Discussion

In this study, we found significant correlation of seminal hyaluronidase activity with sperm density, % motility and % normal morphology. Similar findings were reported earlier [7-8, 10-11].

Testicular semineferous epithelium is responsible for development of hyaluronidase in newly formed spermatids and thereafter, it remains located in the acrosomal region of sperm head [12]. This spermatozoal hyaluronidase is released during acrosomal reaction in the female genital tract and cause dissolution of various envelopes of ovum, thereby, allowing penetration by sperm to initiate fertilization. Similar release of hyaluronidase from spermatozoa into the seminal plasma occurs after liquefaction of collected semen sample, depending upon the status of acrosome [7-8].

As each sperm contributes to the enzyme activity in semen, seminal hyaluronidase activity is directly proportional to the sperm density in the semen sample. Therefore, it is highest in proven fertility, slightly lower in normozoospermic and least in oligozoospermic groups. This could be due the fact that sperm acrosome becomes more resistant to hyaluronidase release in oligozoospermia and so seminal hyaluronidase activity goes on decreasing as the severity of oligozoospermia increases. Hence, both factors i.e. decreased sperm count and the decreased release of hyaluronidase may be the causative factors of infertility in oligozoospermia [7-8].

The normal functioning of axonemal proteins (dynein, actin, tubulin, etc.) in flagellum and the normal energy supply by mid-piece mitochondrial enzymes system is required to maintain normal sperm motility. Normal testicular germinal epithelium is responsible normal synthesis and functioning of sperm acrosomal proteins (acrosin, hyaluronidase, etc.) as well as axonemal and mid-piece proteins. Disordered germinal epithelium may result in abnormality of these proteins and hence lower seminal hyaluronidase activity may be associated with lower %motility [12].

The normal intratesticular sperm maturation results in development of sperms with normal morphology (including head-acrosome) and such sperms will be having normal acrosomal enzyme (hyaluronidase) content. Various acrosomal malformations are found to be associated with other morphological abnormalities such as microheads, pinheads, etc [4, 11]. Therefore seminal hyaluronidase activity is well correlated with % normal sperm morphology indicating decrease in seminal hyaluronidase activity with increase in % of abnormal sperm morphological forms that can better be understood by detailed electron microscopical study.

We observed significant correlation of HOS score with sperm density, % motility and % normal morphology. Many other workers have also found similar correlations [13-17].

HOS is a very simple and inexpensive test to evaluate functional and structural integrity of sperm plasma membrane. It is based on the principle that fluid transport occurs across the intact cell membrane under the hypo-osmotic condition until equilibrium is achieved and results in bulging of sperms, especially, in the tails where plasma membrane is loosely attached. Bulging of tails results in coiling of sperm tails [3, 5, 9]. Transport of fluid and nutrients occurs when plasma membrane is having normal pores and protein channels that can supply nutrients for normal functioning of sperm organelles [17]. Therefore, lower HOS scores which reflect abnormalities of sperm plasma membrane may be associated with dysfunctional sperm head, mid-piece and tail proteins affecting sperm density, sperm motility, sperm morphology and seminal hyaluronidase activity. All these functions are dependent primarily upon normal functioning of testicular epithelium. Affection of functions of male genital organs causes decreased seminal hyaluronidase activity and hypo-osmotic swelling scores [18-20]. Hence, HOS score has strong correlation with sperm density, % motility, % normal morphology [21] and also with seminal hyaluronidase activity.

Finally, it can be concluded that seminal hyaluronidase activity and hypo-osmotic swelling score are the direct indicators of testicular germinal epithelial activity. Thus; it emphasizes the importance of assessment of seminal hyaluronidase activity and hypo-osmotic swelling score, at least, in infertile males having normal conventional seminal parameters.

It is further recommended that detailed study should be carried out over a large sample size using electron microscope along with sperm penetration assays.

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