CORRELATION OF SEMINAL STEROID LEVELS WITH IMPAIRED SPERM ACROSOMAL ENZYME ACTIVITY IN SEMEN OF UNEXPLAINED INFERTILITY CASES

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Received: May 8, 2011; Accepted: July 25, 2011

Abstract: Alteration in seminal steroid levels is known to induce changes in functional status of sperm, a major cause of unexplained infertility world wide. The present study was carried out on a specific group of fifty males with mean age range 36.5 years, referred with unexplained infertility and poor sperm in vitro fertilizing ability of Ahmedabad region. The study revealed that there was a significant decrease in sperm count and viability in this group as compared to control. The hypo-osmotic swelling test suggested altered membrane permeability. Spermatozoa from the infertile samples showed fewer halos in the gelatin slide test. The activities of acrosomal enzymes indicates prematurely reacted spermatozoa that led to loss of membrane integrity and breakdown of sperm acrosome, resulting in impaired sperm function and fertilizing ability. The results of altered acrosomal activity confirmed low androgen levels further indicating decreased levels of testosterone and elevated levels of estradiol resulting in an imbalance of T/E$_2$ ratio. These findings suggest that the functional status of sperm depends upon testosterone levels.

Key words: Impaired fertility, Acrosomal enzymes, Seminal steroids.

INTRODUCTION

The causes of unexplained infertility are not well understood and it is also known that in several cases the causes are difficult to identify. In addition, there is evidence of repeated failure of sperms fertilizing the oocyte in-vitro, in the cases of unexplained infertility studies. The present study was focussed on a specific group of males referred with unexplained infertility.

The aim of the study was to determine the acrosomal status of the spermatozoa and to evaluate the alteration in sperm function in correlation with indicators of testicular steroids. Acrosin is an acrosomal enzyme, which is a trypsin like serine protease that is a vital enzyme required by the spermatozoa to penetrate the oocyte zona pellucida during the process of fertilization [1]. In addition hyaluronidase plays a key role in sperm penetration through cumulus oophorus layers of the oocyte. The activity of these enzymes therefore largely influences the success of sperm fertilizing ability [2]. The most notable steroids found in human seminal plasma are testosterone (T) and estradiol (E$_2$). Testosterone is known to be the most important testicular steroid [3,4] and exerts significant control over libido, behaviour, accessory sex organs, epididymal function and spermatogenic processes.

Estradiol appears to be a potent germ cell survival factor in human testis. It has been suggested that suppression of estradiol production in a subpopulation of subfertile men may improve the semen quality [5]. Thus, it appears that a specific T/E$_2$ ratio should be maintained for sperm survival and function.

MATERIALS AND METHODS

Male unexplained infertility cases (n=50) were studied...
with all other diagnostic reports recorded in these cases showed parameters which were within normal range. Normal, age matched volunteers of proven fertility were selected as control (n=65).

**Collection and analysis of semen samples:** - The samples were collected by masturbation after sexual abstinence for 3-4 days. The samples provided were analysed soon after liquefaction for appearance, volume, consistency and pH according to the WHO criteria [6].

Sperm density was measured using the Neubauer chamber according to the WHO method [6a] and was expressed in million spermatozoa/ml. Sperm motility was determined according to the WHO method [6b] by scoring the motile spermatozoa in 20 separate non-touching fields and expressed as percent motility. Sperm viability was determined using a 0.1% Trypan blue stain as described by Talbot and Chacon [7]. The unstained spermatozoa were scored as live and observations were recorded in 30 separate high power fields and the sperm viability was expressed in percent live spermatozoa.

Sperm function test to evaluate membrane integrity by scoring the number of spermatozoa that swell under hypo osmotic conditions was carried out by the method of Jayendran et al. [8]. A mixture of equal parts of fructose and sodium citrate (150mosmols) with ionic strength 0.15 resulted in a maximal number of identifiable swollen sperms. When placed again in iso-osmotic solution the spermatozoa regain their original form, indicating normal functional integrity and good semi-permeability of the sperm membranes. The final result was expressed as percent of spermatozoa showing swelling under hypo-osmotic condition (% swelling).

Gelatin slide test for acrosome intactness was carried out based on the principle that acrosomal enzymes dissolve gelatin, coated on the slide to form halos according to the procedure of Gopalkrishna et al. [9] and expressed as % sperm with positive acrosomal integrity.

Acrosin was estimated from the sperm pellet after removing the seminal plasma by repeated washing in phosphate buffered saline. The acrosome was then disrupted using a mild detergent extraction procedure of Polakoski and Zaneveld [10]. Free acrosin activity was measured by the change in absorption, which occurs when acrosin hydrolyzes benzoyl arginine ethyl ester (BAEE). The reaction was followed spectrophotometrically at 253 nm where the maximum absorption difference between benzoyl arginine occurs. Acrosomal activity was expressed in micromoles/minute.

Hyaluronidase hydrolyses the bond between N-acetyl hexosaminase and D-glucuronate residues in hyaluronic acid and forms a glucorozoline intermediate which then reacts with p-dimethylamino benzaldehyde (DMAB) in an acid medium to yield a blue chromophore having a maximum absorbance at 585nm which was assayed as described in the method by Polakoski and Zaneveld [10].

The hormone assay was carried out by the method of Soos et al. [11] on the principle of competitive solid phase enzyme immunoassay, using the hormone horseradish peroxide conjugate in the anti-rabbit IgG coated well. The concentrations of estradiol and testosterone in the seminal plasma were expressed as pg/ml and ng/ml respectively. The estimation of 17α-ketosteroids was carried out by the method of Dorfman [12]. The final colour was read on the wavelength at 510nm and expressed as μU/ml. The data obtained is subjected to statistical analysis, using Student’s “t” test and the level of significance is expressed below the tabulated data.

**RESULTS**

The mean age of patients evaluated was 36.5 years with age range of 30-40 years. The result of the present study revealed that these individuals had sperm count comparable to the normal, fertile males whereas the sperm motility was insignificantly less. Sperm viability was found to be significantly decreased (p<0.001). The percentage of spermatozoa showing swelling under hypo-osmotic condition was also lower than normal (p<0.001). In addition, the gelatinolysis where the halo dimensions determined from the gelatin slide test was also found to be significantly reduced in the infertile samples. A decreased percentage of sperm with positive acrosomal enzyme activity was observed as compared to the control samples. It was observed that the acrosomal enzyme (acrosin and hyaluronidase) activity also low indicating acrosomal dysfunction (p<0.001). Seminal testicular steroid level was reduced whereas estradiols levels were significantly increased (p<0.001) indicating hindrance in
function and membrane integrity of spermatozoa from altered steroid levels on the acrosomal and membrane integrity of spermatozoa from the samples of cases with unexplained infertility showed poor activity of acrosomal enzymes and failed to cause dissolution of the gelatin-coated slides. Hence fewer halos were scored in the cases studied, suggesting that the sperms had possibly lost the acrosomal function or had undergone the acrosomal reaction prematurely. It has been stated that the ability of viable sperm to undergo the acrosomal reaction, was significantly reduced or absent in subfertile men indicating acrosomal dysfunction as a likely cause of fertilization failure [13]. Correlated with these observations, the activities of specific acrosomal enzymes, acrosin and hyaluronidase were significantly lower in the infertile group as compared to the normal, fertile samples. Acrosomeless spermatozoa that are almost devoid of acrosin are infertile, even if they are motile and possess an intact plasma membrane. Moreover the hypo-osmotic swelling test revealed that in the infertile cases the sperm showed altered membrane function with absence of swelling under hypo-osmotic conditions. Jayendran et al. [8] had used the test to distinguish non-motile viable spermatozoa from non-viable sperm which had lost their membrane integrity.

From the present data, it is evident that spermatozoa in the infertile samples analysed, had altered membrane integrity, which resulted in poor sperm viability. The study revealed that the spermatozoa from the samples of cases with unexplained infertility showed poor activity of acrosomal enzymes and failed to cause dissolution of the gelatin-coated slides. Hence fewer halos were scored in the cases studied, suggesting that the sperms had possibly lost the acrosomal function or had undergone the acrosomal reaction prematurely. It has been stated that the ability of viable sperm to undergo the acrosomal reaction, was significantly reduced or absent in subfertile men indicating acrosomal dysfunction as a likely cause of fertilization failure [13]. Correlated with these observations, the activities of specific acrosomal enzymes, acrosin and hyaluronidase were significantly lower in the infertile group as compared to the normal, fertile samples. Acrosomeless spermatozoa that are almost devoid of acrosin are infertile, even if they are motile and possess an intact plasma membrane [8]. Hyaluronidase enzyme is one of the acrosomal enzymes which plays an important role in gamete interaction and fertility in mammals [14]. In our study as well, the testosterone levels were reduced in semen of men with unexplained infertility while seminal estradiol levels were increased suggesting an imbalance in T/E₂ ratio. Similar to our findings Micic and Dotlic [15] found that seminal testosterone levels in infertile men were decreased, but this was only significant in cases with severe oligozoospermia, and this was further confirmed by Davidson et al.

**DISCUSSION**

The present study was aimed at determining the effect of altered steroid levels on the acrosomal function and membrane integrity of spermatozoa from semen of infertile males. Semen analysis of the cases investigated revealed that though the sperm density was significantly lower than normal, the sperm motility was within the normal range. Hence, an attempt for fertilization could be achieved based on the motility, provided the spermatozoa are functionally stable. It was also observed that the sperm viability was significantly lower than normal, which is an indication of loss of selective semi-permeability of the sperm membrane. Moreover the hypo-osmotic swelling test revealed that in the infertile cases the sperm showed altered membrane function with absence of swelling under hypo-osmotic conditions.

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In the present study 17α-ketosteroids levels were also reduced from the sample of infertile patients which confirmed low androgen level and could be correlated with low sperm count, motility and altered acrosomal enzyme activity. Hence, from the results obtained it is evident that spermatozoa of unexplained infertility studied, showed altered membrane and acrosomal integrity, impaired function. In addition, it was found that the effect of reduced testicular steroids could be correlated with low sperm density and spermatogenic arrest. These changes could also account for impaired sperm in vivo and in vitro fertilizing ability.

REFERENCE


