Human spermatozoal motility and lipid peroxidation

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Correlation between human spermatozoal motility and lipid peroxidation is worked out following their suspension in native seminal plasma and in Biggers, Whitten and Whittengham (BWW) medium. Spermatozoa suspended in BWW showed higher motility and lesser degree of lipid peroxidation than those suspended in seminal plasma. Further, higher activities of antioxidant enzymes are recorded in the BWW suspended spermatozoa vis a vis those suspended in native seminal plasma.

Reactive oxygen species (ROS) such as the superoxide anion (O$_2^-$), the hydroxyl radical (OH$^-$) and hypochlorite radical ("OHCl) produced by spermatozoa and contaminating leucocytes damage the plasma membrane$^{1,3}$ which loses fluidity and integrity vital for their survival and sperm-oocyte fusion$^4$.

The fertilising capacity of the spermatozoa is quickly reduced when suspended in the native seminal plasma$^{5,6}$. Biggers, Whitten and Whittengham (BWW) medium, on the other hand, preserves the same for longer duration$^{9,10}$. The present studies are aimed at correlating the motility, lipid peroxidation and antioxidant potential of the human spermatozoa suspended in BWW fluid and the results compared with those suspended in the native seminal plasma.

Semen samples obtained from 10 healthy human donors were centrifuged at 1,000 g for 10 min and seminal plasma was separated. The spermatozoa in equal concentration were resuspended in (i) 2 ml of the native seminal plasma and (ii) BWW containing 0.3% (w/v) human serum albumin for working out correlation between sperm survival and the degree of lipid peroxidation. The spermatozoal percentage motility and the degree of lipid peroxidation (i.e. malondialdehyde (MDA) formation) were estimated at room temperature (25$^\circ$±2°C) after 30 min and intermittently till 8 hr. MDA formation was estimated by the reaction of thiobarbituric acid (TBA) with lipid peroxides according to the method of Buege and Aust$^{11}$.

Activities of antioxidant enzymes viz. superoxide dismutase (SOD) and catalase were estimated from the sperm fractions suspended in the native seminal plasma and BWW medium. SOD activity (EC 1.15.1.1.) was measured by the ability of the enzyme to inhibit nitroblue tetrazolium reduction by superoxide anion generated by the photo-oxidation of hydroxylamine hydrochloride$^{12}$. Catalase (EC 1.11.1.6.) activity measured represented the rate of catabolism of hydrogen peroxide and its decomposition was monitored as a change in absorbance at 240 nm$^{13}$. Protein concentrations were determined by the method of Lowry et al.$^{14}$ using bovine serum albumin as standard. The data were evaluated statistically by unpaired Student's t test. A probability value of $P<0.05$ was considered significant.

The percentage sperm motility in the BWW medium was significantly ($P<0.001$) higher at all intervals in comparison to spermatozoa suspended in the native seminal plasma; it was 23, 30, 37.8, 33.8 and 42.3% higher in the BWW medium after 1/2, 1, 2, 4 and 8 hr, respectively (Fig. 1). MDA, on the other hand, was significantly ($P<0.001$) higher in spermatozoa suspended in the native seminal plasma vis a vis those suspended in BWW medium. MDA was 50.6, 68.9, 77.5, 62.3 and 39.3% higher in the spermatozoa suspended in the seminal plasma after 1/2, 1, 2, 4 and 8 hr, respectively (Fig. 2). Activities of antioxidant enzymes viz. SOD and catalase were statistically higher in the sperm suspended in BWW medium (Table 1).
Fig. 1—Percentage motility of human spermatozoa suspended in (a) seminal plasma and (b) BWW fluid at different time intervals.

Fig. 2—MDA production by human spermatozoa suspended in (a) seminal plasma and (b) BWW fluid at different time intervals.

Table 1—Activities of superoxide dismutase and catalase in human spermatozoa (mean ± SD; n = 10)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Medium of suspension</th>
<th>P values:</th>
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<tbody>
<tr>
<td></td>
<td>Native seminal plasma</td>
<td>BWW</td>
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<tr>
<td>SOD (Inverse of the amount of mg protein causing 50% inhibition)</td>
<td>15.6±3.5</td>
<td>20.5±5.4*</td>
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<tr>
<td>Catalase (mM of H₂O₂ decomposed/min/mg protein)</td>
<td>3.6±1.0</td>
<td>8.7±2.3**</td>
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Owing to the high unsaturated fatty acid content of the plasma membrane, human spermatozoa are particularly susceptible to free radical related oxidative stress originating from contaminating leucocytes and defective spermatozoa in the semen [1,3,15,19].

The present study reveals poor sperm motility and poor antioxidant potential with concomitant rise in the rate of MDA generation in the spermatozoa suspended in seminal plasma vis a vis those in BWW medium. The present observations are in conformity with those of earlier workers who also reported loss in the percentage motility as well as in the vigour of the spermatozoa suspended in the native seminal plasma [6,7,16]. Availability of sufficient iron in the seminal plasma [17] supports the formation of extremely pernicious OH⁺ radical in a Haber-Weiss reaction. OH⁺ radical reportedly is a powerful initiator of lipid peroxidation [18].

The poor sperm motility is related with the deficient antioxidant potential viz. SOD and catalase of spermatozoa that protect them from the adverse effects of lipid peroxidation and other manifestations of oxygen toxicity [19,21].

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References
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