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# Differences in osmolality, pH, buffering capacity, superoxide dismutase and maintenance of sperm motility in human ejaculates according to the degree of coagulation

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**Summary**
The purpose of this study was to compare various seminal plasma parameters in fresh human ejaculates exhibiting different amounts of coagulum. Poorly coagulating samples demonstrated significantly lower osmolality and buffering capacity but had a higher pH than did samples with good coagulation. However, no correlation was obtained between the activity of superoxide dismutase and the amount of coagulum. Eight hours after ejaculation, sperm motility had decreased by 15 and 80% in samples with good and poor coagulation, respectively. It is suggested that subfertility may be associated with poor coagulation of ejaculates.

**Keywords**: human semen, coagulation, osmolality, pH, buffering capacity, superoxide dismutase, sperm longevity.

## Introduction

The gelatinous coagulum characteristic of normal human seminal plasma is well established. However, we have demonstrated that the nature of human seminal coagulum varies greatly. Both the amount and the structure (under scanning electron microscope) of the coagulum have been reported to vary in ejaculates exhibiting different states of coagulation (Mandal & Bhattacharyya, 1985a). The post ejaculatory release of phosphate and choline were also shown to differ markedly in ejaculates with different extents of coagulation (Mandal & Bhattacharyya, 1985b, 1986b). Seminal vesicle activity as indicated by the levels of phosphate, choline, N-acetylamino sugar and fructose were found to be significantly lower in poorly coagulated samples than in ejaculates exhibiting good coagulation (Mandal & Bhattacharyya, 1985c, 1986b, 1987). We have also reported that the state of coagulation of human semen may be utilized to develop an effective vaginal contraceptive (Mandal & Bhattacharyya, 1986a).

In the present study, certain seminal plasma parameters of potential physiological importance, such as osmolality (which is the total concentration of active, ionized and non-ionized particles), pH, buffering capacity, activity of superoxide

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dismutase (which is believed to provide protection for spermatozoa against the toxicity of superoxide radicals through the peroxidation process: Mennella & Jones, 1980; Abramsson, Duchek & Marklund, 1985) and sperm motility, have been evaluated in relation to the amount of coagulum in human ejaculates. The interrelationships between the various parameters were also investigated.

**Materials and methods**

Ejaculates were collected by masturbation from men (mean age: 30.7 ± 0.8 years) after a period of abstinence of about 7 days. Samples were provided within 10 s of emission at the University Hospital of Calcutta. The donors were classified into the following categories: proven fertile, presumably fertile, and those from a barren union with no history of cryptorchidism, mumps, orchitis, varicocele or venereal disease. Samples were allowed to liquefy at room temperature. No specimen with an ejaculate volume outside of the range 2–6 ml, with a pus cell count $>5 \times 10^6$/ml, or with a complete liquefaction time of >30 min was included in this study. The amount of coagulum (CG) after 4 min of ejaculation was determined by subtracting the liquefied volume at that period from the total volume, and the resultant CG was expressed as a percentage of total, i.e. 4 min % CG. Details of the assay methods used for measuring the liquefaction time and the amount of coagulum of whole ejaculate using 37-μm mesh-size nylon bags have been presented elsewhere (Mandal & Bhattacharyya, 1985c, 1987).

Seminal plasma was obtained by centrifugation of liquefied semen at $8000 \times g$ for 10 min at 0°C, and was then stored at −20°C, if not used immediately. Osmolality was determined by measuring the freezing-point depression of 0.15 ml seminal plasma, using a Knauer semimicro osmometer (Herbert Knauer KG, Holzweg 2B, Oberursel, FRG). For each evaluation, the osmometer was adjusted to 0 mOsm and checked with two different standard NaCl solutions of 200 and 400 mOsm/kg. The pH and buffering capacity of seminal plasma were determined using a digital pH meter (model EM5, Scientific Instruments Co., Allahabad, India). Standard solutions used were: (i) 0.025 M $KH_2PO_4$ and 0.025 M $Na_2HPO_4$ (pH 6.86); (ii) 0.01 M sodium tetraborate (pH 9.18); (iii) 0.05 M potassium hydrogen phthalate (pH 4.01); and (iv) 0.1 N HCl (pH 1.10). Buffering capacity was determined by adding 0.4 ml of 0.1 N HCl to 0.3 ml of seminal plasma, the pH being taken before and after the addition. The resulting decrease in pH was taken as the measure of buffering capacity; the greater the decrease, the lower the buffering capacity. The activity of superoxide dismutase in seminal plasma was assessed by measuring inhibition of the autoxidation of pyrogallol (Marklund & Marklund, 1974). The reaction was performed at room temperature in a volume of 1 ml containing 50 mM tris-cacodylate buffer (pH 8.2), 1 mM diethylenetriamine-pentaacetic acid and 0.2 mM pyrogallol. One unit of activity was defined as the amount of enzyme required to inhibit the change in absorption at 420 nm by 50%.

The percentage of motile sperm at room temperature (31.9 ± 0.3°C), in their own seminal plasma, was determined indirectly from non-motile and total sperm counts at 1, 4 and 8 h after ejaculation, using a Makler Counting Chamber (Sefi-Medical Instruments, Haifa, Israel). The non-motile sperm count (NMSC) and total sperm count (TSC) were assessed at all time periods, as the total count may be reduced by disintegration of dead sperm or by their aggregation into large clumps.

Vapour from 2% osmium tetroxide solution was used to kill spermatozoa before making the total count. The percentage of motile sperm at 4 and 8 h after ejaculation was determined as follows:

$$\% \text{ motile sperm at 4h} = \frac{TSC_{1h} - [NMSC_{4h} + (TSC_{1h} - TSC_{4h})]}{TSC_{1h}} \times 100.$$

Statistical significance was evaluated by Student's *t*-test for unpaired (Table 1) or paired (Fig. 2) data, according to Richterich (1969).

## Results

Thirty-seven ejaculates were evaluated for seminal plasma osmolality, pH, buffering capacity and superoxide dismutase activity in relation to the extent of coagulation. The amount of coagulum at 4 min after ejaculation in these samples ranged from 0 to 100%. The ejaculates fell into three distinct groups, according to the amount of coagulum, and the results are summarized in Table 1. Ejaculates with 0–30% coagulum were considered as poorly coagulating (group I). Seminal plasma osmolality in this group was significantly lower than in ejaculates exhibiting both medium (group II) or good (group III) coagulation. The poorly coagulating samples also had the lowest buffering capacity and the highest pH value. The buffering capacity of human seminal plasma from groups I and III is shown in Fig. 1. In order to decrease the pH of the test samples to pH 3, the amounts of 0.1 N HCl required for ejaculates with poor or good coagulation were, respectively, 1.7 and 2.8 times that required for 0.1 M phosphate buffer. The activity of superoxide dismutase in group II ejaculates was significantly higher than in both of the other groups. The total volume of the ejaculates indicated an inverse relationship with the amount of coagulum.

**Table 1.** Seminal plasma characteristics of human ejaculates grouped according to the amounts of coagulum

| Amount of coagulum (4 min % CG) | | Osmolality (mOsm/kg) | pH | Buffering capacity (decrease in pH) | Superoxide dismutase (U/ml) | Total volume (ml) |
|---|---|---|---|---|---|---|
| 10.2 ± 3.0 | (–30) | 346 ± 8.4* | 7.69 ± 0.06 | 4.01 ± 0.17 | 35.5 ± 1.50 | 3.8 ± 0.46 |
| $n = 8$ | | | | | | |
| 58.0 ± 3.5 | (31–80) | 388 ± 6.8 | 7.43 ± 0.04 | 2.97 ± 0.09 | 53.9 ± 2.41 | 3.4 ± 0.29 |
| $n = 17$† | | | | | | |
| 91.1 ± 1.9 | | 398 ± 7.6 | 7.55 ± 0.03 | 3.30 ± 0.08 | 37.9 ± 5.41 | 3.1 ± 0.18 |
| $n = 12$ | | | | | | |
| Levels of significance between the groups | | | | | | |
| I and II | | $P < 0.005$ | $P < 0.005$ | $P < 0.001$ | $P < 0.001$ | NS‡ |
| I and III | | $P < 0.001$ | $P < 0.05$ | $P < 0.001$ | NS | NS |
| II and III | | NS | $P < 0.05$ | $P < 0.02$ | $P < 0.01$ | NS |

*Results are means ± SEM.
†$n = 14$ for buffering capacity.
‡NS = not significant.

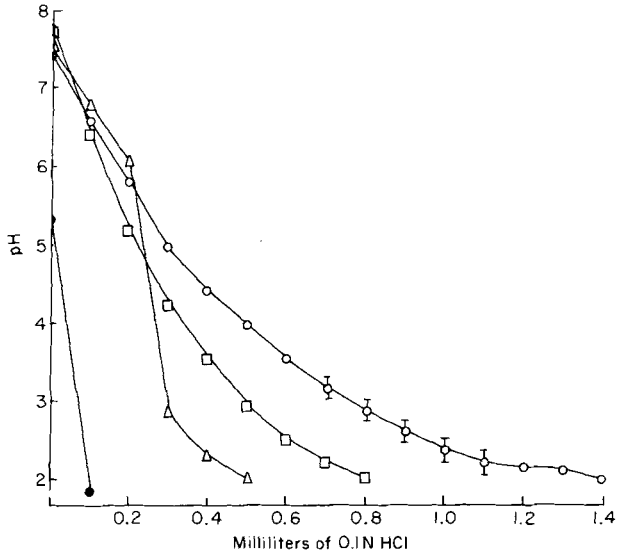


**Fig. 1.** pH-titration curves for 0.3 ml of 0.1 M phosphate buffer (△——△) and for 0.3 ml human seminal plasma from ejaculates exhibiting good (○——○, $n$ = 4, 4 min % CG = 82 ± 5; group III) or poor coagulation (□——□, $n$ = 5, 4 min % CG = 12 ± 2; group I). Titration was against 0.1 N HCl (pH 1.1); (●——●), titration curve for water.

Maintenance of sperm motility in their own seminal plasma was studied in five ejaculates showing good coagulation (4 min % CG = 89 ± 5), and in seven showing poor coagulation (4 min % CG = 25 ± 7) (Fig. 2). The mean ± SEM values for motile sperm at 1, 4 and 8 h after ejaculation in samples with good coagulation were 60 ± 8, 58 ± 6 and 51 ± 6%, respectively. Those for samples with poor coagulation were 55 ± 8, 28 ± 6 and 11 ± 4%, respectively. The decrease in the percentage of

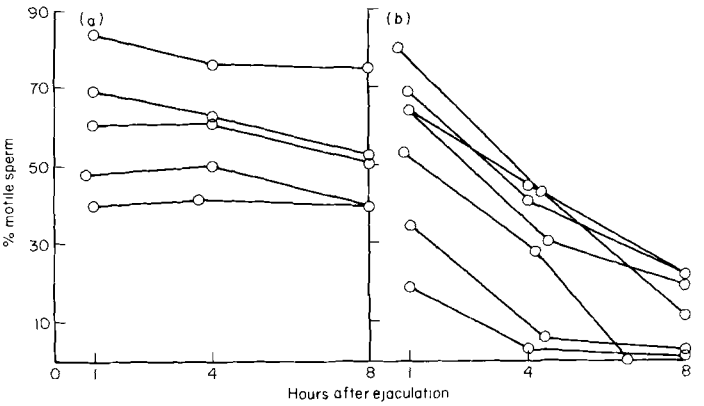


**Fig. 2.** Maintenance of sperm motility in their own seminal plasma according to whether the samples derived from ejaculates with good (a: $n$ = 5, 4 min % CG = 89 ± 5, sperm count = 108 ± 18 × $10^6$/ml) or poor coagulation (b: $n$ = 7, 4 min % CG = 25 ± 7, count = 73 ± 17 × $10^6$/ml) characteristics.

motile sperm in the poorly coagulating ejaculates were statistically significant (1 and 4 h, $P<0.001$; 4 and 8 h, $P<0.01$).

Table 2 shows the values obtained when a correlation matrix was constructed using the various parameters measured. An increased amount of coagulum was accompanied by a significant increase in osmotic pressure in buffering capacity, and by a decrease in total volume. However, the activity of superoxide dismutase and the pH were not correlated significantly with the degree of coagulation. Seminal plasma osmolality showed a positive relationship with the buffering capacity and a negative relationship with volume. Higher pH values were found to be associated with lower buffering capacity and vice versa. The activity of superoxide dismutase was correlated negatively with pH and positively with the buffering capacity.

**Table 2.** Correlation matrix of the various parameters measured in 37 human ejaculates*

| | 4 min % CG | Osmolality | pH | Buffering capacity | Superoxide dismutase |
|---|---|---|---|---|---|
| Osmolality | $r=0.551$ †<br>$P<0.001$ | | | | |
| pH | $r=-0.204$<br>NS | $r=-0.312$<br>NS | | | |
| Buffering capacity | $r=0.468$<br>$P<0.01$ | $r=0.458$<br>$P<0.01$ | $r=-0.852$<br>$P<0.001$ | | |
| Superoxide dismutase | $r=0.030$<br>NS | $r=0.198$<br>NS | $r=-0.352$<br>$P<0.05$ | $r=0.440$<br>$P<0.01$ | |
| Volume | $r=-0.343$<br>$P<0.05$ | $r=-0.486$<br>$P<0.005$ | $r=-0.291$<br>NS | $r=-0.007$<br>NS | $r=-0.166$<br>NS |

*$n$ = 34 for buffering capacity; NS = not significant.
†$r$ = correlation coefficient.

## Discussion

The extent of seminal coagulation in terms of the amount of coagulum has been determined after 4 min of ejaculation, as we have shown previously that significant liquefaction of vesicular coagulum occurs after 4 min of emission (Mandal & Bhattacharyya, 1985c, 1987). We have also shown that for evaluation of coagulation-liquefaction properties in relation to other seminal plasma characteristics, either the amount of coagulum or the liquefaction time can be used. In contrast to our observations, Polak & Daunter (1984) found no correlation between osmolality and liquefaction time, and this may be due to the use of less precise methodology. These authors used a small fraction of semen (0.2 ml), which was passed through a nylon mesh (size: 400) instead of employing the whole ejaculate and 37-μm mesh, as in our studies, as this has been shown to allow only the liquefied fraction and the sperm to pass through (Mandal & Bhattacharyya, 1985b). The significance of using the whole ejaculate is that both the coagulum volume and the consistency differ in fractions of split ejaculate. The highly significant positive correlation between osmolality and the degree of coagulation, possibly indicates that coagulation plays

an important role in the control of osmotic pressure in seminal plasma as well as in seminal vesicles, as the volumes of the ejaculates were higher in samples showing poor coagulation (Tables 1 and 2), and the phenomenon of coagulation is known to be characteristic of human seminal vesicles (Oettle, 1954; Tauber *et al.*, 1980). Unlike that of other body fluids, the osmotic state of seminal plasma depends, to a high degree, not on the inorganic components but upon organic constituents such as fructose, phosphorylcholine, N-acetylamino sugar, etc. (Mann & Lutwak-Mann, 1981). The above assumption is further strengthened by the fact that these vesicular products occur at lowest concentrations in the poorly coagulating samples (Mandal & Bhattacharyya, 1985c, 1986b), and coagulation fails to occur in ejaculates showing decreased vesicular activity (Mandal & Bhattacharyya, 1985a). When subjected to variations of pH (5.25–9.75) and osmolality (150–1000 mOsm/kg), sperm demonstrate maximum velocity at pH 7.65 and 400 mOsm/kg (Makler *et al.*, 1981). It is therefore of interest that the mean pH and osmolality of ejaculates with good coagulation (group III, Table 1) are almost identical to those for optimum sperm velocity, and to the pH value found in the normal female reproductive tract (Feo, 1955; Moghissi, 1966; MacDonald & Lumbley, 1970).

Samples with relatively low osmolality have been found to be associated with high pH and poor buffering capacity. Seminal plasma buffering capacity is considered to be an important determinant of fertility (Searcy & Simms, 1967), since vaginal fluid is highly acidic (pH 4.0–4.9) with lowest pH values occurring at midcycle (Wagner & Levin, 1978), and as sperm are immobilized completely at pH 5.0 or below (Makler *et al.*, 1981). In bull seminal plasma, Anderson (1946) indicated a similar relationship between buffering capacity and pH, and specimens that maintained good motility on storage also had better buffering capacity. Maintenance of sperm motility in samples with good coagulation was found to be similar to that obtained by Dahlberg (1978) for proven fertile men. The rapid decrease in the percentage of motile sperm in poorly coagulating samples may be attributed to their relative hypotonicity, poor buffering capacity and low content of fructose (Mandal & Bhattacharyya, 1985a) in seminal plasma. Although the level of activity of superoxide dismutase in seminal plasma revealed no correlation with their coagulation properties, this enzyme demonstrated a significant positive relationship with buffering capacity and with the concentration of sperm ($r = 0.487$, $P<0.005$, $n = 37$), which are also known to contain significant amounts of this enzyme (Mennella & Jones, 1980). It is therefore possible that the poor coagulational characteristics of ejaculates may be related to the occurrence of subfertility among men.

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