Partial purification of components from fasting human blood serum which stimulate the forward migration of human spermatozoa[1]

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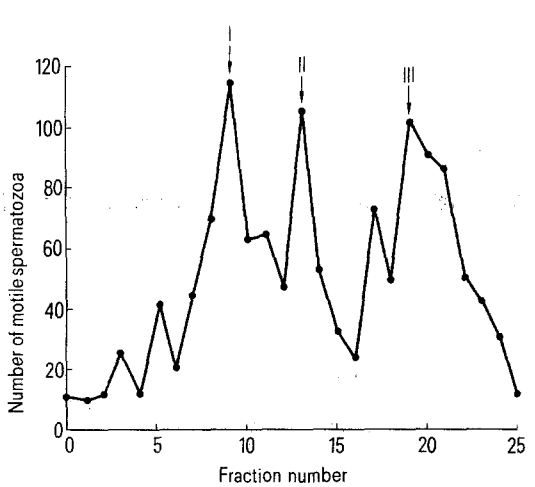
Summary. Components from fasting human serum that could stimulate the forward migration of human spermatozoa were isolated by gel filtration on Sephadex G-25. The results suggest that these components may be used to enhance sperm forward migration and, hence, pregnancy rate in artificial insemination with husband's semen, (AIH) especially in cases where the sperm forward migration is not optimal.

The presence of a component in human blood serum which can stimulate the in vitro motility of hamster epididymal spermatozoa was first demonstrated in 1970[2]. This component has a mol.wt of 100–200, is sensitive to mild acid treatment, and is effective even after excretion from the body[3]. Recently, we have reported that such a component(s) in human serum can activate human sperm forward migration in vitro[4]. Its concentration appears to be higher in blood serum after fasting.[4] Since the active forward migration of spermatozoa plays an important role in their transport through the female genital tract[5], it can be assumed that improving sperm migration rate prior to AI should improve chances for conception, especially in cases where the semen samples have a sub-optimal sperm migration rate. This would be of particular value in AIH since, with AID, a donor with good sperm forward migration is selected. Thus, it was decided to undertake a study to isolate the components from fasting human blood serum which can increase the forward migration rate of human spermatozoa.

Materials and methods. Fasting human blood sera used for gel filtration were obtained from normal healthy volunteers (2 female and 1 male) who had fasted for at least 16 h. The blood samples were allowed to clot at 4 °C overnight and the cells were separated by centrifugation at 500 × g for 15 min. The sera were decomplemented by heating at 56 °C for 30 min and divided into 1 ml aliquots and frozen at −20 °C until use.

1 ml of serum was chromatographed on a column (0.8 × 45 cm) of Sephadex G-25 (Medium grade, Pharmacia, Sweden) (bed volume 30 ml) which had been equilibrated and washed with 100 ml of modified Tyrode's solution[6]. The column was pumped under positive pressure at a flow rate of about 40 ml/cm^2/h. Fractions of 3 ml were collected. Protein concentration of each fraction was monitored with a spectrophotometer by measuring absorption at 280 nm.



Fractionation of fasting human blood serum on Sephadex G-25 to isolate components which increase the forward migration of human spermatozoa. All points are the means of 2 replicates of an experiment. 2 additional experiments gave similar results.

To test the effects of the fractions on human sperm forward migration, the eluate in each fraction (undiluted) was drawn into a capillary tube (micropipettes, 60 mm × 0.1 mm outer diameter; Drummond Scientific Co., Broomall, Pa., USA). One end of the capillary tube was sealed with modelling wax and the other end was inserted into the reservoir of the sperm penetration meter[7] which contained 100 µl of ejaculated human semen (sperm concentration 50×10^6 sperm/ml) from normal healthy donors. After the sperm penetration meter was incubated at room temperature (22 °C) for 20 min, the number of motile sperm at the 10-mm migration distance was determined at 125 × magnification with phase contrast microscopy.

Results and discussion. 3 distinct sperm forward migration-stimulating components were isolated from fasting human blood serum by gel filtration (fig.). The peaks of activities were eluted at approximately fractions 8–10 (fraction I), fraction 13 (fraction II) and fractions 19–21 (fraction III). 3 separate experiments gave similar observations. The majority of proteins (over 95%) in the serum was eluted between fractions 5 and 8. These results show that the fasting human serum contains at least 3 components which can strongly enhance sperm forward migration in vitro. The chemical nature of these components, however, remains to be established.

It has been suggested that these components may be endogenous steroids present in the fasting serum, since it has been reported that estradiol-17β can activate human sperm forward migration in vitro[4,8,9]. However, observations made during the current studies indicate that these purified components lost their ability to activate sperm forward migration after they had been stored at −20 °C for 3 days or more. In addition, it has been reported that these components are sensitive to mild acid treatment[3]. These observations provide evidence suggesting that these components are not steroidal in nature. Work is now in progress to determine the chemical nature of these components and to study their effects on sperm forward migration in semen collected from infertile men with poor sperm motility.

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