BIOLOGY OF REPRODUCTION 41, 983–989 (1989)

Stimulation of Forward Motility of Goat Cauda Epididymal Spermatozoa by a Serum Glycoprotein Factor[1]

MAHITOSH MANDAL, SASWATI BANERJEE, and GOPAL C. MAJUMDER[2]

Indian Institute of Chemical Biology
Jadavpur, Calcutta - 700 032
India

ABSTRACT

Blood sera of humans, rats, goats, and buffalo have been shown to possess a forward motility-stimulating factor (FMSF) that markedly stimulated goat cauda epididymal sperm forward motility, as assayed by a microscopic method in the presence of epididymal plasma (1.2 mg protein/ml) that had sufficient anti-sticking activity to eliminate the possibility of cell-sticking artifacts in motility assays. The specific activity of FMSF was greatest in buffalo blood serum compared to the sera of the other species. Buffalo serum at a concentration as low as 8.5 mg protein/ml induced forward motility in nearly 45% of the cells. The buffalo serum FMSF was heat-stable, nondialyzable, and sensitive to the action of trypsin. Purified proteins – casein, serum albumin, ovalbumin, myoglobin, and β-lactoglobulin – showed little or relatively low FMSF activity. FMSF is a glycoprotein, as it binds with high affinity to concanavalin A-agarose. A major portion of the serum protein (approx. 70%) did not bind to the affinity matrix, and this unretained serum protein fraction showed little FMSF activity. The FMSF activity of buffalo serum was confirmed by estimating sperm forward motility spectrophotometrically: an objective method of assessing sperm motility.

INTRODUCTION

Immotile testicular spermatozoa undergo a maturation process in the epididymis when these cells acquire forward progression and fertility. The studies of Hoskins and his associates (1978) suggest that elevated intrasperm cyclic adenosine 3′,5′-monophosphate level and an exogenous forward motility protein derived from epididymal plasma are essential for the initiation of flagellar motility during the epididymal transit. Recent studies have provided evidence showing that there is an increase of intrasperm pH during the epididymal maturation and that this change in pH may play a vital role in the maturation process (Pinto Ora et al., 1984).

The biochemical basis of the regulation of sperm motility induced in the epididymis is not well understood. Several lines of studies have demonstrated that highly motile mammalian sperm suspended in epididymal or seminal plasma lose motility upon dilution with an isotonic synthetic medium. However, this loss of flagellar motility from dilution can be reduced substantially by the addition of seminal plasma, epididymal plasma, blood serum, or their fractions (Mann, 1964; Yanagimachi, 1970; Morita and Chang, 1971; Morton and Chang, 1973; Bavister, 1975; Muller and Kirchner, 1978; Morton et al., 1979a,b; Brown and Senger, 1980). These studies indicated the presence of motility-maintenance or motility-promoting factors and sperm survival factors (SF) in the male reproductive fluids. Morita and Chang (1971) reported that the motility-maintenance activity of the secretion of the rat seminal vesicles is a high molecular weight (nondialyzable) and thermolabile factor. To the contrary, Morton and Chang (1973) provided evidence that the active principle in the hamster epididymal plasma (EP) and in human serum is a heat-stable, low molecular weight (dialyzable) factor. Muller and Kirchner (1978) demonstrated that serum albumin present in rabbit seminal plasma markedly stabilizes the motility of washed rabbit sperm derived from fresh ejaculates. Morton et al. (1979a) partially purified low and high molecular weight SF from hamster EP that prevent loss of motility due to dilution by inhibiting lysis of spermatozoa. Morton et al. (1979b) also demonstrated the presence in human serum of an SF that prevents loss of hamster epididymal sperm motility due to dilution, presumably because of its efficacy in stimulating respiration in these cells. Presence of a heat-stable dialyzable factor in human serum was reported by Yanagimachi (1970). The factor stimulates motility

Accepted June 30, 1989.
Received October 17, 1988.
[1]This work was supported in part by a research grant from the Department of Atomic Energy, Bombay.
[2]Reprint requests.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018

in hamster cauda epididymal spermatozoa that have lost motility due to dilution with an isotonic synthetic medium. Human serum has also been shown to possess a motility promoting factor with molecular weight in the range of approximately 100 – 200 (Bavister, 1975). Brown and Senger (1980) reported that ejaculated bovine spermatozoa (diluted in 2.9% sodium citrate, pH 7.0) markedly lose motility when stored at 37°C for 8 h and that motility can be regenerated in these aged cells with the addition of blood serum. The biochemical basis for this loss of spermatozoal motility during aging or because of the serum-mediated reversal of motility is not clear. Gaur and Talwar (1975) partially purified a small molecular weight factor (dialyzable) from human seminal plasma that promotes fertility in mouse sperm, presumably by improving the motility and survival of the epididymal sperm. Sheth et al. (1981) isolated a progressive motility-sustaining factor (PMSF) from human epididymal homogenate by using 40 – 60% saturation of ammonium sulfate. This factor helps to sustain forward motility of ejaculated human spermatozoa. However, none of these motility factors derived from serum and male reproductive fluids has been purified and characterized.

It has been demonstrated that washed spermatozoa derived from caput or cauda epididymides frequently stick to the glass surface of the hemocytometer counter that is commonly used for assessing sperm motility, and this cell-sticking phenomenon may cause artifacts in motility assays (Stephens et al., 1981; Roy et al., 1985). Recent studies have demonstrated the presence of anti-sticking factors (ASF) in goat epididymal plasma that inhibit, with high affinity and specificity, sperm adhesion to glass (Roy et al., 1985; Roy, 1987). Early investigators who have reported the occurrence of types of motility-regulating factors in the male reproductive fluids and blood serum (Yanagimachi, 1970; Morita and Chang, 1971; Morton and Chang, 1973; Bavister, 1975; Gaur and Talwar, 1975; Muller and Kirchner, 1978; Morton et al., 1979a,b; Brown and Senger, 1980; Sheth et al., 1981) have not estimated the effect of cell-sticking to the hemocytometer on sperm motility. As speculated by Roy et al., (1985), the presence of variable amounts of ASF activity derived from seminal plasma, epididymal plasma, or blood serum, or their fractions in the motility assays, may lead to variable amounts of sperm sticking to glass and thus may give rise to artifacts in the motility assays. Further studies are therefore necessary to determine the presence of genuine motility-regulating factors in the biological fluids by using an experimental condition that will rule out the possibility of cell-sticking artifacts. This study demonstrates conclusively the localization of a heat-stable glycoprotein factor in blood serum that markedly stimulates the motility of cauda spermatozoa.

MATERIALS AND METHODS

Materials

Trypsin (twice crystallized), pancreatic DNase I, lipase, RNase A, penicillin, α-methyl-D-mannoside, and dialysis sacks (cut off of 12 kDa) were obtained from Sigma Chemical Co. (St. Louis, MO). Ficoll-400 was a product of Pharmacia (Sweden). Concanavalin A-agarose (ConA-agarose) was obtained from Hy-Gro Chemical Pvt. Ltd., Calcutta, India. Fresh epididymides of adult goats and fresh buffalo blood were obtained from local slaughter houses. Unless otherwise specified, goat spermatozoa were extracted from the tissue within 2 to 4 h after slaughter of the animals.

Preparation of Buffalo Blood Serum

Blood samples obtained from adult buffalo were allowed to clot at 5°C overnight. Serum (approx. 85 mg protein/ml) was obtained by centrifugation of the clotted blood at 14,000 × *g* for 15 min. Boiled serum (approx. 15 mg protein/ml) was obtained by heating serum at 100°C for 2 min prior to centrifugation at 14,000 × *g* for 15 min to remove denatured proteins.

Preparation of Spermatozoa and Epididymal Plasma

Spermatozoa were obtained from goat cauda epididymides by the procedure described earlier (Roy et al., 1985). Spermatozoa were extracted at room temperature (32 ± 1°C) from the epididymides in a modified Ringer's solution (RPS medium: 119 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM glucose, 16.3 mM potassium phosphate, pH 6.9, penicillin, 50 units/ml). Numbers of spermatozoa in the samples were determined with a hemocytometer. Freshly extracted sperm preparations contained $10 - 20 \times 10^7$ cells/ml and nearly 60 – 80% of these cells showed some form of flagellar motility. The sperm preparations were used immediately (within 15 min) for motility assays.

Goat cauda epididymal plasma (EP) was prepared by the procedure described earlier (Roy et al., 1985).

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018

Freshly extracted sperm preparations were centrifuged at 800 × g for 10 min; then, most of the spermatozoa were removed as pellet. The resulting supernatants, which were slightly turbid, were spun again at 14,000 × g for 30 min to obtain cell-free EP. A pooled sample of EP concentrated at 4 – 6°C with polyethylene glycol (12 mg protein/ml) and preserved at – 10°C was used for these studies. The concentrations of EP in the assays were expressed as its protein content.

Assay of FMSF

Unless otherwise specified, FMSF activity of serum was estimated by estimating forward motility (FM) of spermatozoa by the conventional microscopic method using a hemocytometer as the counting chamber. To eliminate the possibility of artifact due to sperm adhesion to glass, motility assays were carried out in the presence of EP (1.2 mg protein/ml) that contained adequate anti-sticking activity to cause nearly 100% inhibition of sperm adhesion to glass (Roy et al., 1985). Spermatozoa (0.5×10^6 cells) were incubated with EP (0.6 mg protein) in the absence or presence of 20 μl buffalo serum at room temperature (32 ± 1°C) for 1 min in a total volume of 0.5 ml of RPS medium. A portion of the cell suspension was then injected into the hemocytometer. Immediately, spermatozoa that showed well-defined FM (FM cells) (cells that moved in small or large circles were excluded) and total cell numbers were counted under a phase contrast microscope at 250 × magnification. The percentage of FM cells was then calculated. Systems lacking exogenous serum served as the blanks in all assays. A unit of activity of the FMSF was defined as the amount of the factor that induced FM in 10% of the cells under the standard assay conditions. Unless otherwise specified, the data shown are representative of at least three experiments.

Affinity Chromatography

Concanavalin A immobilized on agarose is known to have high affinity for binding D-mannose and D-glucose residues of the glycoproteins. Serum FMSF activity was subjected to ConA-agarose affinity chromatography by the procedure described earlier (Roy et al., 1985). A pasteur pipette column of ConA-agarose (0.6 × 3.4 cm: bed volume 0.5 ml) was equilibrated with 20 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.2, containing 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM $MnCl_2$ (Buffer 1). Heated buffalo serum (0.2 ml) that had been dialyzed extensively against Buffer 1 was applied to the affinity column. As the sample passed through, the column was washed with 0.8 ml of Buffer 1. The eluate from the column represented the unretained fraction (Fraction A). The column was washed further with 2.5 ml of the equilibrating buffer to remove residual unbound materials (Fraction B). Finally, the glycoproteins that bound to the affinity column were eluted with 3 ml of Buffer 1 containing α-methyl-D-mannoside (30 mg/ml) (Fraction C). All the fractions were dialyzed extensively against 10 mM Tris-HCl buffer, pH 7.4, prior to assay for FMSF activity.

Spectrophotometric Assay of Sperm Motility

FM of spermatozoa was estimated by a quantitative method reported earlier (Majumder and Chakraborty, 1984). The microscopic method of motility assay described above takes into consideration the numbers of cells with forward progression but not their velocity, whereas the spectrophotometric method is based not only on the motile cell numbers but also on their velocity. The method consists of layering 50 μl of freshly extracted cauda epididymal spermatozoa (2.6×10^6 cells) suspended in RPS medium containing 1% Ficoll-400 at the bottom of a standard optical cuvette (3-ml capacity) containing 1.3 ml RPS medium, which was sufficient to cover the entire width of the light beam. Vigorously motile spermatozoa that moved upwards into the light beam were registered continuously as an increase of absorbance at 545 nm with a Gilford spectrophotometer equipped with a recorder. After reaching maximal absorbance (A_{Eq}), the contents of the cuvette were mixed and the absorbance for all the cells (A_T) was noted. The percentage of cells that showed vigorous FM was calculated as: $A_{Eq}/A_T \times 100$.

Estimation of Protein

The protein contents of EP and buffalo serum or its fractions were estimated according to Lowry et al. (1951), with bovine serum albumin as standard.

Statistical Analysis

Unless otherwise specified, the results were reported as arithmetic means ± SEM. Differences between controls and serum-treated samples were analyzed by the Student's t-test.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018

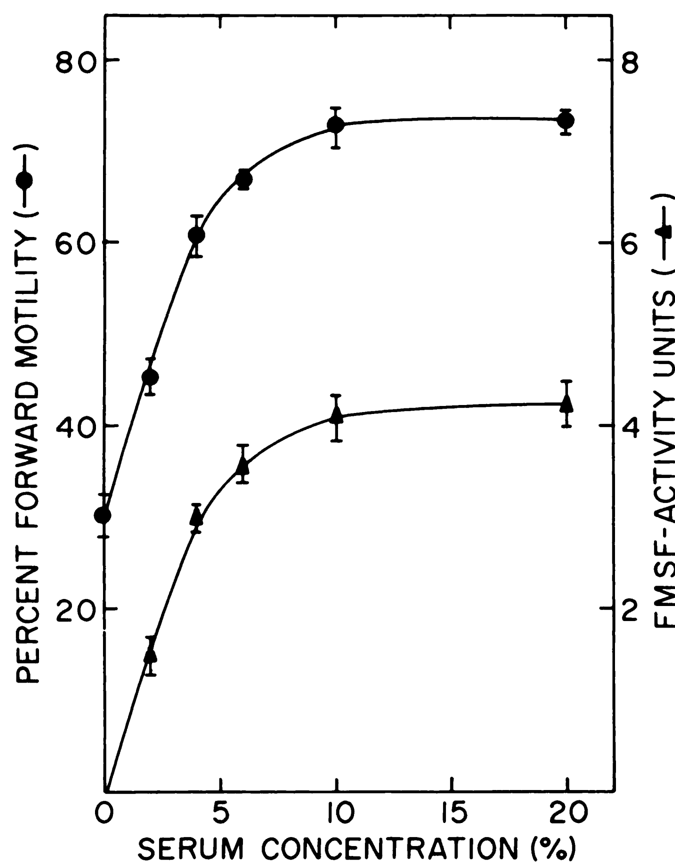

FIG. 1. Effect of varying concentrations of buffalo serum on FM of goat cauda sperm and its FMSF activity under standard assay conditions. The data shown are means ± SEM of three experiments.

RESULTS

Microscopic Method of Assay

Nearly 15 – 40% spermatozoa of the freshly extracted goat cauda sperm preparations showed forward progression when analyzed in the presence of EP (1.2 mg protein/ml) by the microscopic method of motility assay. As shown in Figure 1, numbers of FM cells increased markedly with the increase in the concentration of buffalo blood serum (p<0.01). Serum at a concentration as low as 10% caused nearly maximal stimulation of sperm flagellar motility when nearly 75% of the cells showed FM. A proportional increase in FMSF activity was observed with approximately 3 units of the factor (approx. 4% serum). Serum also showed a similar amount of FMSF activity when tested on fresher preparations of spermatozoa (extracted from goat epididymides immediately – within 30 min – after the slaughter of the animals, data not shown). Under the conditions of these assays (± serum), there was little adhesion of spermatozoa to the glass surface of the hemocytometer chamber, as estimated by the procedure described earlier (Roy et al., 1985). The data provided evidence for the occurrence of a factor (FMSF) in serum that induced FM in the goat cauda epididymal spermatozoa, and this factor was not an artifact of the motility assays since there was no appreciable adhesion of sperm cells to the counting chamber.

TABLE 1. Effect of dialysis of buffalo serum on the activity of FMSF.

Additions	Forward motility (%)	Increase of forward motility (%)	FMSF activity (units)
Nil (blank)	24	0	0
+ Serum	42	18	1.8
+ Nondialyzable serum fraction*	42	18	1.8
+ Dialyzable serum fraction*	24	0	0

*One milliliter serum (85 mg protein/ml) was dialyzed overnight in cold temperature (4 – 6°C) against 2 ml of RPS medium in a small test tube. The surrounding RPS medium contained two-thirds of the dialyzable fraction of the serum. The materials inside the dialysis tubing were then dialyzed extensively against RPS medium to obtain the nondialyzable fraction of serum.

Table 1 shows the effect of dialysis on the FMSF activity of serum. There was little loss in activity of the factor following dialysis of serum, indicating that FMSF is a nondialysable factor. Serum was heated for 3 min at 60°C, 80°C, or 100°C and then cooled to room temperature prior to assay of FMSF activity under standard assay conditions. FMSF was found to be heat stable, since there was little loss of activity when serum was subjected to these heat treatments. To elucidate the molecular nature of the factor, serum was digested with various enzymes (Table 2). Treatment of serum with DNase, RNase, and lipase had no appreciable effect on its FMSF activity, whereas trypsinization (50 μg/ml) at 37°C for 30 min inactivated the factor completely. The data showed the factor to be a polypeptide and not a nucleic acid or lipid.

TABLE 2. Effect of various enzyme treatments on FMSF activity of buffalo serum.

Treatment of serum*	FMSF Activity (units)
Nil (control)	2.26
+ DNase (50 μg/ml)	2.20
+ RNase (50 μg/ml)	1.90
+ Lipase (50 μg/ml)	1.90
+ Trypsin (25 μg/ml)	0.20
+ Trypsin (50 μg/ml)	0

*Boiled buffalo serum (1.0 ml:15 mg protein/ml) was pretreated with specified amounts of DNase, RNase, lipase, and trypsin (each in 10 μl of RPS medium) at 37°C for 30 min. After incubation, the mixtures were boiled for 2 min to inactivate the enzymes prior to estimation of the FMSF activity in 20 μl of the treated or untreated serum under standard assay conditions.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018

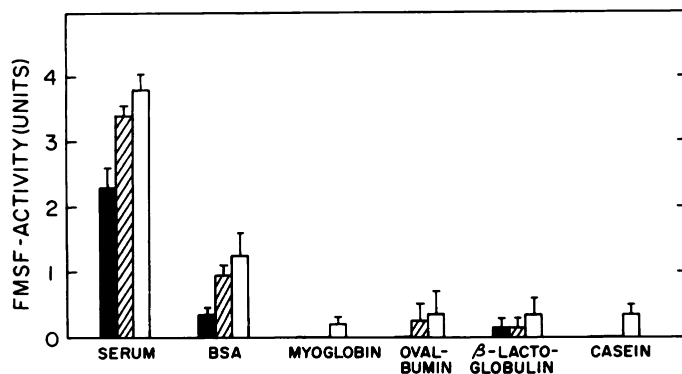

FIG. 2. Effect of different proteins on the sperm FM under standard assay conditions. *Solid*, *hatched*, and *open bars* represent 0.6, 1.5, and 3.0 mg protein/ml, respectively.

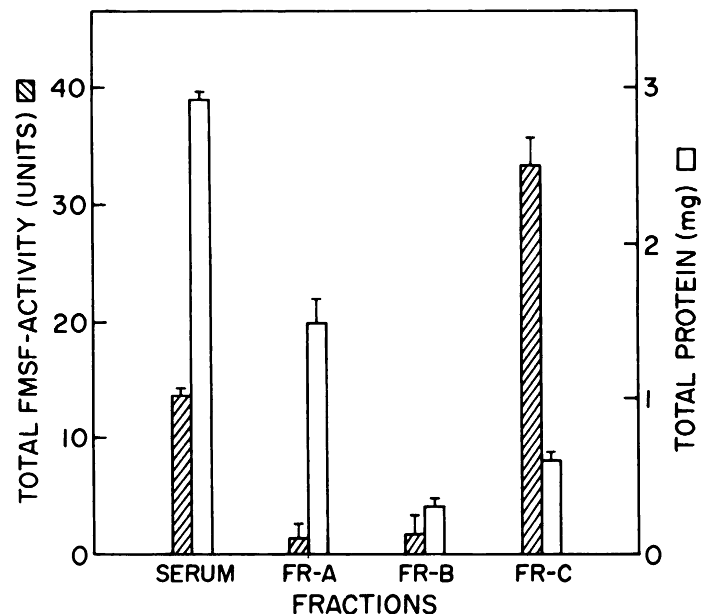

FIG. 3. ConA-agarose affinity chromatography of serum FMSF activity. A portion of dialyzed boiled serum (3.00 mg protein) was subjected to ConA-agarose affinity chromatography. Aliquots of the boiled serum and the chromatographic fractions (FR-A:unretained, FR-B:wash, FR-C:glycoprotein eluate) were assayed for the FMSF activity under standard assay conditions. The data shown are means ± SEM of three experiments.

It is possible that the observed motility-promoting activity of serum may be due to nonspecific action of the proteins. Studies were therefore carried out to estimate the FM-promoting activity of several commercially available purified proteins (Fig. 2). Casein, myoglobin, β-lactoglobulin, and ovalbumin had little motility-promoting activity, whereas bovine serum albumin (BSA) was capable of stimulating sperm motility to a small extent. The specific FMSF activity of the boiled serum (7.8 ± 1.0 Units/mg protein) was nearly six times greater than that of BSA (1.3 ± 0.3 Units/mg protein). FMSF specifically bound to the ConA-agarose column and could then be eluted with α-methyl-D-mannoside (Fig. 3). Nearly 70% of the serum proteins did not bind to the affinity column, and these proteins had little FMSF activity. By this affinity chromatography, the recovery of the FMSF activity was nearly 250%. Recovery of FMSF activity much greater than the initial value (100%) suggests that during the chromatography some interfering substance(s) that directly inhibit sperm FM and/or inactivate the serum FMSF was removed. The data revealed that serum FMSF was a glycoprotein with at least one D-mannose or D-glucose residue at the sugar side chain.

Blood sera from several species were evaluated for their FMSF activity (Table 3). The activity of the factor was demonstrated in the sera of all the species tested; however, the concentration, as well as the specific activity of the factor, was maximal in buffalo serum.

Spectrophotometric Assay

Although the microscopic method of motility assays as described above ruled out the possibility of cell-sticking artifacts, this method provides a subjective assessment of sperm motility. We also analyzed the serum FMSF activity by quantitating vigorous FM of spermatozoa with a spectrophotometric method (Fig. 4). The addition of 20% boiled serum (3 mg protein/ml) markedly stimulated (nearly 3-fold) the FM of spermatozoa; it also caused a profound increase in the initial slope of the curve as well as in the number of spermatozoa displaying vigorous forward motility. The control cells showed approximately 25% vigorous FM whereas the addition of serum enhanced the proportion of FM cells to nearly 70%. As shown in Figure 5, the serum

TABLE 3. FMSF activity in the sea of different species.

Source of serum	Concentration of FMSF activity* (units/ml) Means ± SEM	Specific activity of FMSF* (units/mg protein) Means ± SEM
Buffalo	105 ± 10[a]	1.23 ± 0.12[a]
Goat	53 ± 2	0.47 ± 0.04
Rat	47 ± 1	0.59 ± 0.02
Human	43 ± 2	0.47 ± 0.03

[a]Significantly higher ($p<0.01$) than the other species.

*The data shown are for three samples of each serum.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907 by guest on 17 March 2018

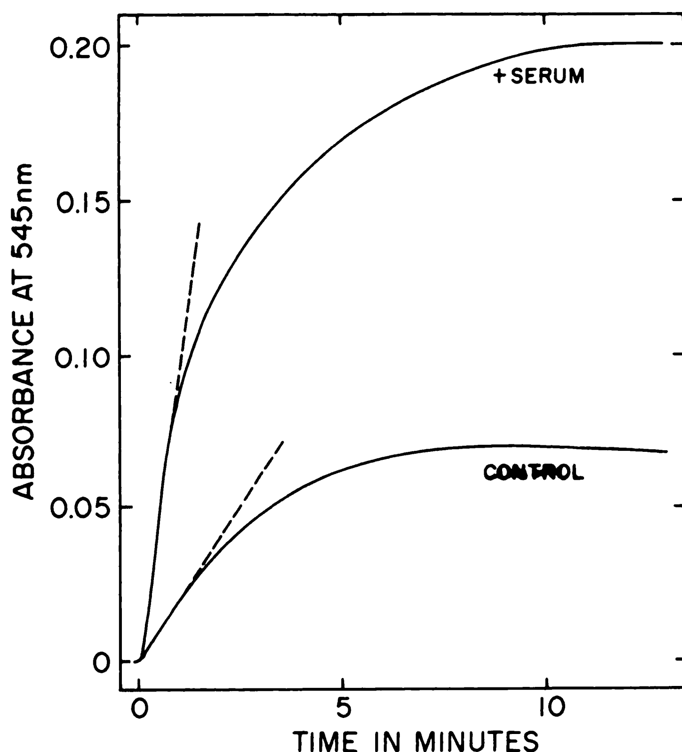

FIG. 4. A representative spectrophotometric tracing showing the effect of 20% boiled serum (3 mg protein/ml) on sperm FM assayed spectrophotometrically. Absorbance for all the cells after mixing the contents of the cuvette (A_T) was 0.275.

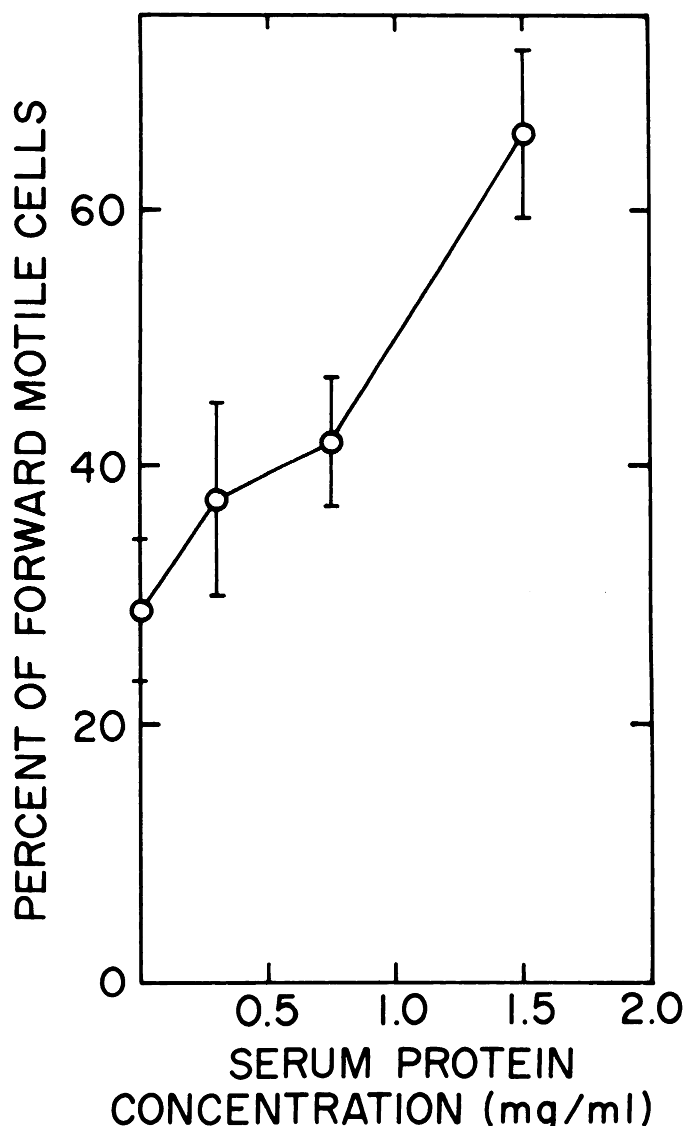

FIG. 5. Effect of varying concentrations of serum (boiled) on sperm FM as estimated spectrophotometrically by the procedure described in *Materials and Methods*. Serum protein concentration of 1.5 mg/ml corresponds to 10% of the boiled serum. The values shown are means ± SEM of three experiments.

FMSF caused a dose-dependent increase in FM of spermatozoa ($p<0.01$).

Comparison of the two methods of FMSF assays shows that addition of serum (native or boiled) in the concentration range of 2 – 10% to the sperm preparations caused a dose-dependent increase in forward progression of sperm (Figs. 1 and 5). By the microscopic method, serum (2 – 10%) was found to induce forward FM in nearly 15 – 41% of the sperm (Fig. 1), whereas the same concentration range of the serum induced FM in approximately 8 – 37% of the cells as analyzed by the spectrophotometric motility assay (Fig. 5).

DISCUSSION

Although earlier investigators reported different types of motility factors in serum and male reproductive fluids (Yangimachi, 1970; Morita and Chang, 1971; Morton and Chang, 1973; Bavister, 1975; Gaur and Talwar, 1975; Muller and Kirchner, 1978; Morton et al., 1979a,b; Brown and Senger, 1980; Sheth et al., 1981), the validity of these findings remains to be proved in view of the recent observations that washed spermatozoa extensively stick to the glass surface of the hemocytometer; that this phenomenon of cell-sticking may give rise to artifacts in motility assays (Stephens et al., 1981; Roy et al., 1985; Roy, 1987). The present study has demonstrated conclusively the presence of a heat-stable glycoprotein factor (FMSF) in buffalo serum that induces FM in the weakly motile and immotile cauda spermatozoa. Both microscopic and spectrophotometric motility assays (Figs. 1 and 4) supported the view that serum FMSF is not an artifact of cell-sticking to glass.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018

Although albumin, the major, heat-labile serum protein, showed FM-stimulating activity (Fig. 2), the serum FMSF cannot be attributed to albumin, because serum FMSF activity remains unchanged after removal of albumin by heat treatment of the serum. Specific FMSF activity of BSA was markedly lower compared to that of serum (Fig. 2), suggesting that albumin serves as a less potent or nonspecific FMSF. The activity of FMSF is not due to the nonspecific actions of proteins, since most of the serum proteins (Fig. 3) and several purified proteins showed low or little motility-promoting activity (Fig. 2). The serum FMSF is a glycoprotein (Fig. 3), but the motility-promoting activity is not a general characteristic of all glycoproteins, since purified glycoproteins such as casein and ovalbumin did not show any FMSF activity (Fig. 2).

It is now well documented that specific serum proteins filter into the rete testicular and epididymal fluids (Muller and Kirchner, 1978; Setchell, 1970). It is thus possible that serum PMSF may be present in male reproductive fluids. The widespread distribution of FMSF activity in the sera of different species suggests that the factor may have a physiological role in activating FM in the mature cauda epididymal spermatozoa.

Buffalo are milch animals of considerable economic importance in several countries. It has been recognized for a long time that buffalo semen possesses a much greater problem than cattle semen with respect to its preservation for artificial insemination (Ganguli et al., 1973; El-Kafrawi and Barrada, 1974). To increase milk production, it is essential to make full use of the semen from the best buffalo bulls by devising highly effective methods of preserving buffalo semen. Although it would have been most appropriate to investigate the effect of buffalo serum on the motility of spermatozoa of the same species, it was not possible to use a buffalo sperm model at the present time because of the unavailability of fresh buffalo semen as well as unavailability of mature epididymides from comparative species. Nevertheless, the data from this study raised the possibility that the serum FMSF may be extremely useful as a diluent for the preservation of not only buffalo semen but also semen from other species, since FMSF may enhance fertility of spermatozoa by activating FM in these cells. Serum FMSF may also be useful in eliminating certain infertility problems in humans that are due to poor sperm motility. Studies are in progress to purify the factor from buffalo serum.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S.C. Pakrashi, the Director of the Institute, for his interest in this study. The authors also acknowledge the technical assistance of Nabina Roy, Miss K. Chakraborty, and Mrs. S. Banerjee.

REFERENCES

Bavister BD, 1975. Properties of the sperm motility-stimulating component derived from human serum. J Reprod Fertil 43:363 – 66

Brown DV, Senger PL, 1980. Influence of homologous blood serum on motility and head-to-head agglutination in non-motile ejaculated bovine spermatozoa. Biol Reprod 23:271 – 75

El-Kafrawi SS, Barrada MS, 1974. Effect of deep freezing techniques on the preservation of buffalo and bovine semen for long periods at – 196°C. Agric Res Rev 52:9 – 16

Ganguli NC, Bhosrekar M, Stephan J 1973. Milk whey as a diluent for buffalo semen. J Reprod Fertil 35:355 – 58

Gaur RD, Talwar GP, 1975. Further studies on the fertility-promoting factor from human seminal plasma. Int. J Fertil 20:133 – 36

Hoskins DD, Brandt H, Acott TS, 1978. Initiation of sperm motility in the mammalian epididymis. Fed Proc 37:2534 – 42

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with Folin phenol reagent. J Biol Chem 193:265 – 75

Majumder GC, Biswas R, 1979. Evidence for the occurrence of an ecto-(adenosine triphosphatase) in rat epididymal spermatozoa. Biochem J 183: 737 – 43

Majumder GC, Chakraborty CK, 1984. A simple spectrophotometric method of assay of forward motility of goat spermatozoa. J Reprod Fertil 70: 235 – 41

Mann T, 1964. The Biochemistry of Semen and of the Male Reproductive Tract. London: Methuen, p. 343

Morita Z, Chang MC, 1971. Maintenance of the motility of rat epididymal spermatozoa in the presence of male accessory secretions. J Reprod Fertil 24:247 – 54

Morton BE, Chang TSK, 1973. The effect of fluid from the cauda epididymidis, serum components and caffeine upon the survival of diluted epididymal hamster spermatozoa. J Reprod Fertil 35:255–63

Morton BE, Fraser CF, Albagli L, 1979a. Studies on factors in hamster cauda epididymal plasma and other sources which inhibit sperm dilution damage. Fertil Steril 32:99 – 106

Morton BE, Fraser CF, Sagadraca R, 1979b. Inhibition of sperm dilution damage by purified factors from hamster cauda epididymal plasma by defined diluents. Fertil Steril 32:107 – 14

Muller B, Kirchner C, 1978. Influence of seminal plasma proteins on motility of rabbit spermatozoa. J Reprod Fertil 54:167 – 72

Pinto Ora Amir D, Schindler H, Hurwitz S, 1984. Effect of pH on the metabolism and fertility of turkey spermatozoa. J Reprod Fertil 70:437 – 42

Roy N, 1987. Studies on anti-sticking factors of epididymal plasma that prevent adhesion of spermatozoa to glass. Ph.D. Thesis, University of Calcutta, Calcutta, India

Roy N, Majumder GC, Chakrabarti CK, 1985. Occurrence of specific glycoprotein factor(s) in goat epididymal plasma that prevent adhesion of spermatozoa to glass. Andrologia 17:200 – 06

Setchell BP, 1970. Testicular Blood Supply. Lymphatic Drainage, and Secretion of Fluid. In Johnson AD, Gomes WR, Vandemark NL (eds.) The Testis. New York and London: Academic Press, pp. 101 – 239

Sheth AR, Gunjikar AN, Shaha GV, 1981. The presence of progressive motility-sustaining factor (PMSF) in human epididymis. Andrologia 13: 142 – 46

Stephens DT, Acott TS, Hoskins DD, 1981. A cautionary note on the determination of forward motility protein activity with bovine epididymal spermatozoa. Biol Reprod 25:945 – 49

Yanagimachi R, 1970. In vitro capacitation of golden hamster spermatozoa by homologous and heterologous blood sera. Biol Reprod 3:147 – 53

Downloaded from https://academic.oup.com/biolreprod/article-abstract/41/6/983/2763907
by guest
on 17 March 2018