

Effects of Epidermal Growth Factor on Human Sperm Cell Function

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ABSTRACT: The effects of human recombinant epidermal growth factor (EGF) on the fertilizing capacity of human sperm were investigated. At lower concentrations (0.1–10 nM) EGF did not significantly ($P > 0.05$) affect human sperm penetration of zona-free hamster oocytes (SPA). At higher concentrations (25–100 nM), EGF significantly ($P = 0.01$ to < 0.001) decreased the human sperm penetration rates in SPA. At higher concentrations (≥ 25 nM), EGF also significantly inhibited the spontaneous as well as calcium ionophore-induced acrosome reaction and release of acrosin from human sperm. There was no effect of EGF on percent sperm motility, but at higher concentrations (≥ 25 nM) it significantly affected various sperm motility characteristics especially velocity and amplitude of lateral head displacement. EGF was detected by radioimmunoassay as well as

radioreceptor assay in seminal plasma of fertile men. However, there were no statistical differences between the levels of EGF or EGF/transforming growth factor (TGF- α) in seminal plasma of fertile, infertile, and immunoinfertile men. Also, there was no significant correlation of the EGF or EGF/TGF- α levels with total sperm number, sperm motility characteristics, and penetration rates in SPA in these patients. These results indicate that EGF has either no effect or an inhibitory/negative effect on the human sperm cell capacitation and/or acrosome reaction, especially at higher concentrations (> 25 nM).

Key words: Sperm, epidermal growth factor, sperm capacitation, protein kinase.

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Protein phosphorylation has a definite role in the regulation of function of various receptors (Hunter and Cooper, 1985; Yarden and Ullrich, 1988). In addition, the receptors for several hormones and growth factors (e.g., epidermal growth factor [EGF], insulin, insulin-like growth factor [IGF]-1 and platelet-derived growth factor [PDGF]) are themselves tyrosine-specific protein kinases that are activated by ligand binding (Downward et al, 1984; O'Brien et al, 1987). In many of these signal transduction systems, receptor autophosphorylation depends upon receptor aggregation (O'Brien et al, 1987; Yarden and Ullrich, 1988).

We have started investigating the presence and role of various tyrosine kinase and related growth factors/proteins and proto-oncogene products in human sperm function. The long-term objective of this study is to search for factors/proteins that can enhance the fertilizing capacity of human sperm and thus can help us in specific diagnosis and treatment of human male infertility. Recently, we demonstrated that a synthetic thymosin peptide (T_{α}) can enhance the fertilizing capacity of human sperm

by modulation of sperm capacitation and/or the acrosome reaction (Naz et al, 1992).

The best known of the tyrosine kinase family of proto-oncogenes are the c-erbB-1 and c-erbB-2/HER2 (Adamson, 1990). The c-erbB-1 encodes a 170-kDa glycoprotein, which is a receptor for EGF (Downward et al, 1984) and transforming growth factor (TGF- α) (Todaro et al, 1980). The c-erbB-2/HER2, the human homologue of the rat proto-oncogene neu, encodes a 185-kDa glycoprotein called p185^{HER2}, which is presumed to be the receptor for a recently discovered ligand named heregulin (Holmes et al, 1992). Both the EGF receptor and p185^{HER2} have a cysteine-rich extracellular domain, a transmembrane domain, and intracellular tyrosine kinase activity (Yamamoto et al, 1986; Carpenter and Cohen, 1990).

EGF is a 53-amino acid (aa) polypeptide, first isolated from mouse submaxillary gland (Cohen, 1962; Savage et al, 1972). The peptide is a potent stimulator of growth differentiation and proliferation of numerous cells/tissues of mouse and human (Cohen and Taylor, 1974; Hirata and Orth, 1979). In humans, the human EGF (hEGF) has been detected in several tissues including the thyroid, pancreas, duodenum, submaxillary gland, and kidney, and also in plasma, saliva, milk, and urine; the blood platelets may be the source of most, if not all, hEGF found in plasma (Hirata and Orth, 1979). EGF has also been shown to have a role in both male as well as female mammalian

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reproduction (Adamson, 1990; Ahmad and Naz, 1993). Removal of the submandibular gland of mature male mice results in a significant loss of plasma EGF (not plasma testosterone), causing a significant decrease of spermatids in the testes, and mature sperm in the epididymis (Tsumi et al, 1986). Recently, it was demonstrated that EGF synergizes with $T_{\alpha 1}$ (a molecule that enhances the fertilizing capacity of human sperm cell [Naz et al, 1992]) by modulating the expression of *c-fos* proto-oncogene (Francesco et al, 1992). Recently, we demonstrated that human sperm have EGF receptors but not the expression product of *c-erbB-2/HER2* gene (Naz and Ahmad, 1992). In addition, it was further found that the incubation of human sperm with EGF increases the tyrosine phosphorylation of EGF receptors.

In view of the above findings, the present study was conducted to: (1) investigate effects of EGF on human sperm penetration of zona-free hamster oocytes, (2) investigate effects of EGF on human sperm capacitation and/or the acrosome reaction using various assays (acrosomal status/acrosin distribution/sperm motility characteristics), and (3) determine the presence and concentration of EGF and TGF- α in seminal plasma of fertile and infertile men. The overall aim was to investigate the role of EGF in human sperm function and search for its utility in diagnosis and treatment of human male infertility.

Materials and Methods

EGF

EGF used in the present study was recombinant hEGF purchased from Intergen Co. (Purchase, New York; cat. #4110-80). EGF was diluted in sterile PBS, aliquoted, and stored at 5°C until used. The same batch of EGF when tested in *in vivo* phosphorylation assays caused an increase in the tyrosine phosphorylation of EGF receptors present on human sperm (Naz and Ahmad, 1992).

Sperm Penetration Assay

Human sperm penetration assay of zona-free hamster oocytes (SPA) was performed by the method of Yanagimachi et al (1976) as described elsewhere (Naz et al, 1992; Naz and Ahmad, 1992). Briefly, superovulation was induced in adult female golden hamsters by intraperitoneal (i.p.) injection of 30 IU equine chorionic gonadotropin (eCG) (Sigma Chemical Company, St. Louis, Missouri) on day 1 of the cycle. After 55–72 hours, 20 IU human chorionic gonadotropin (hCG; Sigma Chemical Company) was administered i.p. The animals were killed 15–17 hours after hCG injection, and the mature unfertilized ova were collected from the oviducts. The ova were separated from the surrounding cumulus cells by incubation with 0.2% hyaluronidase in Biggers, Whitten, and Whittingham medium (BWW) and from the zona pellucida by treatment with 0.1% pancreatic trypsin in BWW. The zona-free ova were washed twice in BWW and placed in the center of a tissue culture dish.

Semen from a fertile man was liquefied for 15–30 minutes at 37°C, and the swim-up sperm population was collected as described elsewhere (Naz et al, 1992; Naz and Ahmad, 1992). The sperm cells in the swim-up were washed with BWW supplemented with 1% BSA (fraction V, cat. #A-7906; Sigma Chemical Company), adjusted to $5-10 \times 10^6$ motile sperm/ml and then allowed to incubate for 6–7 hours at 37°C (in 5% CO₂ and 95% air mixture) with the EGF (0.1–100 nM final concentration in 100 μ l of sperm suspension) or the equivalent volume of phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) (PBS-BSA). After incubation, the sperm were washed to remove the unreacted EGF and coincubated with zona-denuded hamster oocytes (20–30 eggs/treatment in each assay) for 3–4 hours. The oocytes were removed, washed thoroughly, fixed with 3% glutaraldehyde, and stained with acetocarmine solution. Penetration was determined by the presence of a swollen sperm head with discernible tail in the cytoplasm of the ovum. Motility of sperm before and after incubation with ova was recorded. The assays were repeated at least three to five times using different fertile donors, and each sample was tested with at least 77–119 oocytes.

The percentage of ova penetrated was calculated according to the following formula:

$$\% \text{ ova penetrated} = \frac{\text{total number of sperm penetrated}}{\text{total number of ova incubated}} \times 100.$$

Using this assay (Naz et al, 1992), we obtain approximately 93–100% ova penetrated with sperm from fertile men, with an average of one sperm penetrated per oocyte.

Assessment of the Acrosome Reaction

The effect of EGF was also investigated on the human sperm acrosome reaction. The effect on the acrosome reaction was assessed by determining the acrosomal status of the sperm after EGF incubation as well as by studying acrosin distribution after the acrosome reaction.

Assessment of Acrosomal Status—Motile sperm were collected from fertile men by the swim-up procedure (Naz et al, 1992; Naz and Ahmad, 1992). Good quality sperm samples (5×10^6 motile sperm/ml, >75% motility, +3 to +4 forward progression on a scale of 0 to +5) were incubated with EGF (0.1–100 nM) for 7–8 hours. The controls were treated with the same amount of PBS-BSA. After incubation, the spermatozoa were centrifuged, washed with PBS-BSA, and divided into two aliquots. One aliquot was induced to acrosome react with calcium ionophore (A23187 [Sigma Chemical Company] was incubated with sperm suspension for 1 hour) (Byrd et al, 1989), and the other aliquot was studied for the spontaneous acrosome reaction without treating with the calcium ionophore. The acrosomal status was assessed by using the triple stain procedure (Jager et al, 1984) as described elsewhere (Naz et al, 1992). Briefly, the ionophore-treated sperm or ionophore-nontreated sperm (spontaneous acrosome-reacted sperm) were washed ($\times 2$) in PBS and then incubated at 37°C for 15 minutes with 2% Trypan blue (Sigma Chemical Company) (1:1, v/v) in PBS. The sperm were washed ($\times 2$), fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 60 minutes and then washed ($\times 2$) again in PBS. A drop of suspension was placed on a glass slide and allowed to air dry

overnight. The slides were stained in 0.8% Bismarck Brown (Sigma Chemical Company) in deionized water (pH 1.8 with 2 N HCl), rinsed in deionized water, and stained for 45 minutes (at room temperature) in 0.8% Rose Bengal (Sigma Chemical Company) in 0.1 M cacodylate buffer (pH 6.0). The slides were then washed in deionized water, dehydrated in an alcohol series, cleared in xylene, and mounted with permount and a coverslip. A total of 400–600 sperm per sample were evaluated and recorded as either live or dead, and the living sperm were further evaluated as acrosome-intact or acrosome-nonintact (reacted) sperm. The experiments were repeated three to four times using sperm from at least three different fertile donors.

Assessment of Acrosin Distribution—After incubation with EGF (0.1–100 nM)/PBS-BSA, and then with or without treating with calcium ionophore A23187 for 1 hour as described above, the sperm were centrifuged, and acrosin activity was determined in the supernatant and sperm by the method of Kennedy et al (1989). Briefly, 90 μ l of the supernatant or the pelleted sperm suspended in 90 μ l of PBS were mixed with 1 ml of the reaction mixture (1 mg/ml of *N*-benzoyl-DL-arginine-*p*-nitranilide [BAPNA] hydrochloride in 0.055 M NaCl, 10% [v/v] dimethyl sulfoxide, 0.1% [v/v] Triton X-100) and incubated for 3 hours at room temperature. The reaction was stopped by addition of 100 μ l of 0.5 M benzamidine, and the absorbance was measured at 405 nm. Acrosin activity was expressed as μ IU/ 10^6 spermatozoa. The daily variability of the assay was normalized using a cryopreserved partially purified human acrosin extract prepared as described elsewhere (Naz et al, 1992; Naz and Ahmad, 1992).

Sperm Motion Analysis

After incubation with EGF (0.1–100 nM)/PBS-BSA for 7–8 hours as described above, an aliquot (7 μ l) of the sperm suspension was placed into a Makler chamber (Sefi-Medical Instruments, Israel), and the sperm motion characteristics were determined using a computerized semen analyzer (CASA) (Cell Soft Cryo Resources, New York) as described elsewhere (Naz et al, 1992; Naz and Ahmad, 1992). The following parameter settings were used throughout the study: 30 frames analyzed at an image frequency of 30 Hz, 3 frames minimum sampling for motility, 15 frames minimum sampling for both velocity and amplitude of the lateral head movement (ALH) measurements, 10 μ m/second threshold velocity, minimum linearity of 2.5 for ALH measurement, and cell size range of 4–40 pixels with a magnification calibration of 0.688 μ m/pixel.

Radioimmunoassay (RIA)

The levels of EGF were assayed in the seminal plasma of fertile and infertile men by RIA. The seminal plasma samples were collected from the liquefied semen of fertile men ($n = 6$) (#1–6) and infertile men ($n = 10$) (#7–16) (30–37 years old) (Table 4) by centrifugation and stored immediately at -20°C until use. These infertile men had infertility attributed to idiopathic or male factor infertility and had abnormal semen analysis and/or defective sperm function as demonstrated by reduced (<10%) sperm penetration rates in SPA (Table 4).

The EGF was also measured in the seminal plasma of another group of fertile men ($n = 5$) (#1–5) and in age-matched infertile men ($n = 5$) (#6–10) (29–36 years old) (Table 5). These infertile

men had infertility attributed to immunologic reasons as evidenced by the presence of antisperm antibodies in their sperm by the immunobead technique (IBT) (Bronson et al, 1989), and these semen henceforth will be referred to as immunoinfertile. The RIA was performed using an RIA kit obtained from Biomedical Technologies Inc (Stoughton, Massachusetts; cat. #BT-450) following the supplier's instructions. The human EGF RIA kit employs a competitive binding technique in which ^{125}I -labeled hEGF competes with unlabeled hEGF for a limited number of specific antibody-binding sites (rabbit anti-hEGF antiserum). The percentage of antibody-bound ^{125}I -hEGF decreases as a function of increasing concentration of unlabeled hEGF. The antibody- ^{125}I -hEGF complex is separated from unbound ^{125}I -hEGF, after equilibrium is reached, by the addition of precipitating antiserum directed against rabbit immunoglobulin G. The radioactivity in the pellet (bound fraction) is counted after centrifuging and decanting the supernatant. Concentration of hEGF in samples is determined from a standard curve generated with a series of hEGF samples of known concentrations. Each sample was run in duplicate with appropriate controls, and the mean of the two readings was recorded.

Radioreceptor Assay (RRA)

EGF was also measured in the seminal plasma using the RRA to investigate whether or not the EGF present in seminal plasma had any biological activity. The radioreceptor kit was obtained from Biomedical Technologies Inc (cat. #BT-390). Because EGF and TGF- α (from all mammalian species) appear to react nearly identically with the receptor, this assay necessarily measures the sum of both peptides present (Tables 4 and 5).

The EGF/TGF- α RRA system is based on the competitive protein-binding principle using membrane receptor particles as the binding protein and an ^{125}I -labeled EGF peptide. The test ligand of the sample is allowed to compete with a constant amount of ^{125}I -EGF for a predetermined quantity of receptor sites. At equilibrium, the receptor-bound fraction is separated from free ligand by centrifugation, and the radioactivity in the bound fraction is determined by gamma-spectrometry. The concentration of ligand in a test sample is determined by comparison with a standard curve constructed using the highly purified EGF supplied. A nonspecific rabbit double-antibody precipitate is incorporated as a separation aid. Each sample was run in duplicate, and the mean of the two readings was recorded.

The EGF (by RIA) and EGF/TGF- α (by RRA) levels were expressed as either $\mu\text{g/ml}$ of the seminal plasma or expressed as the specific activity as ng/mg protein in the seminal plasma and correlated with the sperm concentration, various sperm motility parameters, and the penetration rates. The protein in the seminal plasma was measured by the method described by Bradford (1976).

Statistical Analysis

Significance of differences between treated (EGF) and control (PBS-BSA) and between various concentrations of EGF was calculated using paired as well as unpaired Student's *t*-test and one-way analysis of variance (ANOVA). The correlation coefficients between various sperm/seminal parameters were determined by analyzing for linear regression. The interassay and intraassay

Table 1. Effect of human recombinant EGF on human sperm penetration rates

Treatment (nM)	Ova tested (no.)	Ova penetrated % (mean ± SD)
0.1	78	92.50 ± 3.54
1.0	114	116.00 ± 5.65
10	82	100 ± 5.29
25	119	52.74 ± 3.82*
50	76	54.20 ± 10.90*
100	77	40.50 ± 7.78*
Control (PBS-BSA)	282	94.85 ± 4.89†

* Versus †, $P = 0.01$ to <0.001 ; others were insignificant vs control.

variabilities in RIA and RRA were calculated by finding out the population coefficient of variation defined as population standard deviation/population mean × 100. Correlation coefficients (r) with $P > 0.05$ were considered insignificant.

Results

Effects on Human SPA

The human recombinant EGF at lower concentrations (0.1–10 nM) did not significantly affect the penetration rates and at higher concentrations (25–100 nM) significantly ($P = 0.01$ to <0.001) decreased the penetration rates in SPA (Table 1).

There were no visible effects of the EGF at any dose on human sperm motility (percentage and progressive).

Effects on Acrosomal Reaction of Human Sperm

On incubation with EGF (0.1–100 nM) for 7–8 hours, there was a dose-dependent decrease in the percentage of acrosome-reacted sperm, though the decrease was significant ($P = 0.04$ – 0.005) only at concentrations of ≥ 25 nM (Table 2). The sperm samples treated with EGF (especially at ≥ 25 nM) showed a lower percentage of acrosome-reacted sperm whether they were tested for a spontaneous

acrosome reaction (without ionophore treatment) or investigated after an ionophore-induced acrosome reaction (Table 2).

Effects on Acrosin Distribution

Again, on incubation with 25–100 nM of EGF, a significantly ($P = 0.04$ – 0.005) lesser quantity of acrosin concentration was released in the supernatant with a higher quantity of acrosin concentration in the sperm cells, compared to PBS-BSA-treated sperm (Table 2). At lower concentrations (0.1–10 nM) of EGF, the effects were insignificant ($P > 0.05$). At concentrations of ≥ 25 nM, the effects were apparent whether investigated with or without the ionophore treatment, though the effects were more predominant after the ionophore treatment.

Effects on Sperm Motility Characteristics

EGF at concentrations of 0.1, 1.0 and 10 nM did not significantly ($P > 0.05$) affect percent motility nor any motility characteristics of the human sperm cells as compared to PBS-BSA-treated sperm (Table 3). However, at concentrations of 25–50 nM, EGF significantly ($P = 0.02$ to <0.01) affected the velocity and ALH of sperm cells. At a concentration of 100 nM, EGF significantly (<0.01) affected all the motion parameters, namely the velocity, ALH, linearity, and beat frequency of sperm cells.

Presence of EGF in Seminal Plasma of Fertile and Infertile Men

EGF was detected in seminal plasma of both fertile as well as infertile men (mean ± SD, fertile men: 3.27 ± 1.16 ng/mg, 30.84 ± 12.45 ng/ml; infertile men: 4.00 ± 1.24 ng/mg, 42.40 ± 14.47 ng/ml; differences insignificant, $P > 0.05$) as detected by RIA (Table 4). EGF/TGF- α was also detected by RRA in seminal plasma of both fertile as well as infertile men (mean ± SD, fertile men: 3.18 ± 2.47 ng/mg, 15.23 ± 13.55 ng/ml; infertile men: 5.09 ± 2.74 ng/mg, 27.08 ± 15.59 ng/ml; differences insignificant, $P > 0.05$) (Table 4). Neither the EGF nor

Table 2. Effects of human recombinant EGF on acrosome reaction of human sperm*

Treatment (nM)	Without ionophore treatment			With ionophore treatment		
	Acrosome-reacted sperm (%) (mean ± SD)	Acrosin activity†		Acrosome-reacted sperm (%) (mean ± SD)	Acrosin activity†	
		Cells (mean ± SD)	Supernatant (mean ± SD)		Cells (mean ± SD)	Supernatant (mean ± SD)
0.1	12.75 ± 2.06	55.80 ± 9.67	16.30 ± 1.68	67.00 ± 2.95	20.12 ± 1.56	64.27 ± 6.18
1.0	13.82 ± 2.58	59.85 ± 10.41	14.62 ± 1.72	65.75 ± 2.50	22.80 ± 2.56	59.52 ± 6.23
10	11.92 ± 1.29	60.12 ± 10.71	14.50 ± 2.10	65.50 ± 3.10	23.05 ± 3.18	58.75 ± 5.72
25	9.04 ± 1.41‡	61.30 ± 7.56	13.02 ± 1.80‡	59.24 ± 3.31‡	26.20 ± 4.50‡	56.20 ± 4.45‡
50	8.60 ± 1.92‡	63.18 ± 8.43‡	12.50 ± 1.90‡	56.72 ± 3.84‡	27.81 ± 2.50‡	54.75 ± 6.71‡
100	7.58 ± 2.16‡	64.12 ± 9.20‡	11.24 ± 2.50‡	51.62 ± 4.23‡	29.32 ± 3.28‡	51.20 ± 6.28‡
Control (PBS-BSA)	14.00 ± 4.18	58.75 ± 8.29	16.27 ± 2.69	67.02 ± 4.36	19.57 ± 2.34	64.12 ± 6.6

* Assays (3–5) were performed on various days using sperm collected from at least three different fertile men.

† Acrosin activity was expressed as μ l of acrosin/ 10^6 sperm cells.

‡ Versus control, $P = 0.04$ – 0.005 ; others were insignificant vs control.

Table 3. Effects of human recombinant EGF on human sperm motility parameters*

Treatment (nM)	Percent motility	Motility characteristics (mean ± SD)			
		Velocity	Linearity	ALH†	Beat frequency
0.1	77.75 ± 6.02	57.00 ± 5.69	4.30 ± 0.24	4.05 ± 0.53	12.75 ± 0.76
1.0	74.00 ± 6.00	57.65 ± 5.19	4.37 ± 0.38	4.03 ± 0.57	12.70 ± 0.88
10	75.25 ± 5.50	57.30 ± 4.32	4.37 ± 0.33	4.02 ± 0.51	12.48 ± 0.79
25	72.00 ± 2.82	53.10 ± 4.10‡	4.28 ± 0.05	3.40 ± 0.42‡	12.85 ± 0.78
50	72.10 ± 5.25	52.05 ± 5.80‡	4.18 ± 0.32	3.32 ± 0.40‡	12.79 ± 0.76
100	71.60 ± 4.75	51.29 ± 4.21‡	4.01 ± 0.08‡	2.98 ± 0.59‡	13.12 ± 0.82‡
Control (PBS-BSA)	76.50 ± 5.45	57.35 ± 5.65	4.40 ± 0.38	4.02 ± 0.47	12.52 ± 0.91

* Assays ($n = 3-5$) were performed on various days using sperm collected from three different fertile donors.

† ALH means amplitude of lateral head displacement.

‡ Versus control, $P = 0.02$ to <0.01 ; others were insignificant vs control.

the EGF/TGF- α , whether expressed as specific activity (ng/mg protein) or ng/ml of seminal plasma, correlated ($r = -0.006$ to 0.45) significantly ($P > 0.05$) with total sperm concentration or with various sperm motility parameters or penetration rates in SPA.

Similarly, the EGF or EGF/TGF- α was also detected in seminal plasma of immunoinfertile patients (Table 5). However, these concentrations were statistically insignificant ($P > 0.05$) as compared to those of fertile men (mean ± SD, EGF—fertile men: 3.6 ± 1.7 ng/mg, 39.8 ± 17.3 ng/ml; infertile men: 4.0 ± 1.1 ng/mg, 37.4 ± 15.0 ng/ml; EGF/TGF- α_1 —fertile men: 2.46 ± 1.71 ng/mg, 25.3 ± 2.5 ng/ml; infertile men: 2.23 ± 1.41 ng/mg, 21.1 ± 15.1 ng/ml). Neither the levels of EGF nor EGF/TGF- α correlated ($r = -0.025$ to 0.54) significantly ($P > 0.05$)

with total sperm concentration or with various sperm motility parameters when expressed as specific activity or ng/ml of seminal plasma.

For the RIA and RRA, the coefficient of variations were 2.6% and 4.3% for the intraassay variability, and 3.8% and 5.1% for the interassay variability, respectively.

Discussion

Our results demonstrate that incubation with EGF does not increase the penetration rates of SPA. At lower concentrations (<25 nM) there was no significant effect, but at higher concentrations (≥ 25 nM) there was a dose-dependent decrease in penetration rates.

Table 4. EGF and EGF/TGF- α levels in seminal plasma of fertile and infertile men

Patient (#)	Semen analysis						SPA ova penetrated (%)	EGF*		EGF/TGF- α †	
	Total sperm ($\times 10^6$)	Motility (%)	Velocity	Linearity	ALH	Beat frequency		ng/mg	ng/ml	ng/mg	ng/ml
Fertile men											
1	167	47	36.4	4.4	1.8	13.1	70	2.4	26	1.9	10.1
2	94	50	32.2	4.5	1.5	11.6	58	2.2	20	1.6	6.9
3	155	61	37.4	4.1	2.2	13.0	100	3.6	29	2.9	11.5
4	132	64	40.5	4.5	2.3	12.0	61	5.2	55	8.0	42.4
5	166	80	33.2	1.8	1.2	8.4	57	2.4	24	1.4	7.0
6	85	70	47.3	3.5	2.8	10.8	67	3.8	31	3.3	13.5
Infertile men											
7	133	56	35.7	3.3	2.1	12.0	0	5.3	59	9.3	51
8	20	20	47.6	3.8	2.8	11.6	0	5.1	55	6.8	38
9	55	22	35.7	2.7	1.8	11.1	0	5.9	64	9.4	50.8
10	76	27	31.8	4.2	1.5	13.0	4	1.6	18	1.2	6.3
11	78	8	37.0	2.9	1.1	9.2	0	4.1	33	4.3	17.1
12	55	22	41.0	4.1	2.2	13.0	0	3.4	42	2.3	14.0
13	85	15	33.5	4.8	1.7	14.6	3	4.3	47	5.4	29.9
14	113	9	36.0	2.3	2.1	5.7	0	3.5	44	4.8	30.2
15	2	22	49.0	1.0	2.0	10.1	0	3.7	28	4.3	16.3
16	138	16	39.7	4.2	2.3	12.0	0	3.1	34	3.1	17.2

* EGF was measured by RIA; expressed as specific activity (ng of EGF/mg seminal plasma protein) or ng of EGF/ml of seminal plasma.

† EGF/TGF- α was measured by RRA; expressed as specific activity (ng of EGF/TGF- α /mg of seminal plasma protein) or ng of EGF/TGF- α /ml of seminal plasma.

Table 5. EGF and EGF/TGF- α levels in seminal plasma of fertile and immunoinfertile men

Patient (#)	Semen analysis													
	Total sperm ($\times 10^6$)	Motility (%)	Semen analysis				Beat frequency	Immunobead technique*			EGF†		EGF/TGF- α ‡	
			Velocity	Linearity	ALH	IgG		IgA	IgM	ng/mg	ng/ml	ng/mg	ng/ml	
Fertile men														
1	125	52	37	4.4	2.9	13.1	0	0	0	6.6	66	6.7	67.4	
2	312	40	33	4.7	1.7	11.8	0	0	0	2.8	22	0.8	6.2	
3	295	54	36	4.9	2.0	12.1	0	0	0	2.5	28	1.0	10.7	
4	390	55	49	4.6	2.9	12.8	0	0	0	2.9	36	1.0	12.6	
5	307	73	35	5.3	1.7	13.6	0	0	0	3.0	47	1.9	29.5	
Immunoinfertile men														
6	150	65	41	5.0	2.0	12.1	65 (TT)	0	0	3.5	25	1.4	10.2	
7	95	39	46	4.0	2.1	12.9	0	71 (H&TT)	0	5.4	52	4.6	43.8	
8	225	42	37	4.7	2.9	13.0	96 (H&TT)	0	0	4.4	48	2.1	22.2	
9	160	64	32	4.4	2.7	11.9	0	60 (H&TT)	0	2.5	18	0.7	4.7	
10	170	57	42	4.6	2.6	12.0	52 (H&TT)	0	0	4.2	44	2.4	24.4	

* Expressed as percent sperm bound with the class of antibody; H means head bound; TT means tail tip bound.

† EGF was measured by RIA; expressed as specific activity (ng of EGF/mg of seminal plasma protein) or ng of EGF/ml of seminal plasma.

‡ EGF/TGF- α was measured by RRA; expressed as specific activity (ng of EGF/TGF- α /mg of seminal plasma protein) or ng of EGF/TGF- α /ml of seminal plasma.

The above findings may suggest that EGF at higher concentrations is affecting the capacitation and/or acrosome reaction, as the SPA has been reported to be a measure of capacitation of human sperm (Gould et al, 1983). Indeed, the sperm samples treated with EGF especially at concentrations of ≥ 25 nM that significantly decreased the penetration rates in SPA, demonstrated a decrease in the percentage of acrosome-reacted sperm when allowed to spontaneously acrosome react or induced to acrosome react in the presence of calcium ionophore. These results were further confirmed by a decrease of acrosin activity in the supernatant and an increase of acrosin activity in sperm in both spontaneously acrosome-reacting or ionophore-induced acrosome-reacting sperm. These results indicate that EGF at higher concentrations may be inhibiting capacitation and/or the acrosome reaction. Because capacitation is a prerequisite for sperm to undergo the acrosome reaction, EGF may be inhibiting the capacitation process. Incubation of sperm with EGF did not affect percent motility of sperm at any concentration tested (0.1–100 nM) and did not affect any of the motility characteristics namely the velocity, linearity, ALH, and beat frequency at 0.1, 1, and 10 nM. However, at 25–50 nM, EGF significantly affected velocity and ALH of sperm with concomitant slight but insignificant decreases in linearity and beat frequency of sperm cells. At 100 nM, EGF significantly affected all the motion parameters. These motion parameters have been reported to be important contributors toward the estimation of hyperactivation of

sperm, which is considered to be an integral part of capacitation preceding the acrosome reaction and sperm binding to the zona pellucida (Mortimer et al, 1984; Robertson et al, 1988).

These results agree with our previous findings that incubation of sperm with various concentrations of neutralizing antibodies against the EGF receptors did not affect human sperm penetration rates (Naz and Ahmad, 1992). The present findings are surprising because EGF has been shown to have mitogenic (stimulatory) effects on various somatic cells and cancer cell lines (Adamson, 1990). We have previously shown that human sperm have EGF receptors, and on incubation with EGF (same batch of human recombinant EGF and at concentrations of 25 and 50 nM, which causes reduced penetration rates and acrosome reaction) causes an increase in tyrosine phosphorylation of EGF receptors (Naz and Ahmad, 1992). The effects of EGF were specific, as absorption with the antibody abolished these effects (Naz and Ahmad, 1992).

These findings suggest that EGF may have a negative rather than a positive role in human sperm function. EGF was detected in seminal plasma both by RIA as well as RRA. There were no statistical differences between the levels of EGF or EGF/TGF- α in seminal plasma of fertile, infertile, and immunoinfertile patients, indicating that the presence of male factor or idiopathic/immunological factors in seminal plasma do not affect the EGF concentrations. There was no significant correlation of the EGF or EGF/TGF- α levels with total sperm number, sperm mo-

tility characteristics, and penetration rates, probably indicating that these parameters are not affected by the physiological concentrations of EGF. The EGF concentration range (ng/ml) varied from 4.7 to 67.4 ng/ml in various fertile/infertile samples by RIA or RRA (Tables 4 and 5), which correspond to approximately 0.67–10 nM. At this concentration range EGF did not affect human sperm function *in vitro*.

The extracellular domain of the EGF receptor is characterized by its capacity to bind EGF and EGF-like ligands (TGF- α) with high affinity, and the cytoplasmic portion of this receptor is the sequence defining the tyrosine kinase domain (Carpenter and Cohen, 1990). In other cell systems, it has been known that the formation of EGF-receptor complexes on the cell surface is followed by rapid internalization and degradation of ligand and receptor (Stoscheck and Carpenter, 1984), and the mitogenic signaling is enhanced if internalization is slowed (Chen et al, 1989). A short segment of the EGF-receptor residues 973–991 has been identified as responsible for mediating internalization of ligand/receptor complexes (Chen et al, 1989). It is possible that lack of internalization of ligand/receptor complexes especially due to absence/defect in the 973–991-aa epitope of EGF receptor in sperm cells may be causing an enhanced mitogenic response at higher concentrations of EGF, resulting in an inhibition of capacitation and/or acrosome reaction. It is also possible that there may be some additional mechanism(s)/factor involved in EGF-induced signal transduction pathways responsible for this inhibition.

In conclusion, our data indicate that incubation of sperm with EGF causes either no significant effect or inhibition of capacitation and/or acrosome reaction, depending upon the concentration of EGF. It was further found that human seminal plasma from fertile men had EGF or EGF-like activity, and these levels were not affected in seminal plasma of infertile men by the presence of male/idiopathic/immunologic factors in the semen. Thus, though EGF has been shown to have a stimulatory/positive effect on a number of somatic/cancer cell lines, including the development of preimplantation embryos (Adamson, 1990; Paria and Dey, 1990; Ahmad and Naz, 1993), it seems to have an inhibitory/negative signaling effect on the sperm capacitation and/or acrosome reaction, especially at higher concentrations. The exact mechanisms/molecules responsible for this differential effect need to be investigated.

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Student Awards

American Society of Andrology Annual Meeting 1993

New Investigator Award

Michael A. Palladino—Multiple Epididymal Glutamyl Transpeptidase mRNAs Are Differentially Regulated By Androgens And Testicular Factors In A Region-Specific Manner (Abstract #87). Department of Anatomy and Cell Biology, University of Virginia.

Merit Awards

Robert P. DeMott—Specific Inhibition Of Hamster Sperm Binding To Oviductal Epithelium (Abstract #90). College of Veterinary Medicine, University of Florida.

Linda R. Johnson—Sperm From Mice Carrying A t Haplotype Have Less Progressive Motility And Are Dysfunctional in Fertilization *In Vitro* (Abstract #8). Department of Anatomy and Cell Biology, Temple University School of Medicine.

Hossein Najmabadi, MD—A Phorbol Ester (PMA) Augments 3'–5' Cyclic Adenosine Monophosphate (cAMP) Induction of Inhibin Alpha Gene Transcription in JEG.3 Cells (Abstract #3). Department of Medicine Harbor–UCLA Medical Center.

Debra D. Ricker—Regional Specificity of Luminal Protein and Histologic Changes Following Partial Sympathetic Denervation Of The Rat Epididymis (Abstract #96). Department of Urology, Johns Hopkins Medical School.

Behrouz Salehian—Pituitary–Testicular Axis During HIV Infection: A Prospective Study (Abstract #9). Department of Medicine, Harbor-UCLA School of Medicine.

Richard V. Clark, MD, PhD
Chairman, Awards Committee