Journal of Andrology, Vol. 24, No. 6, November/December 2003

Quantification of Seminal Plasma Motility Inhibitor/Semenogelin in Human Seminal Plasma

KAORU YOSHIDA,* TADASHI YAMASAKI,† MIKI YOSHIIKE,* SHOICHI TAKANO,† ITARU SATO,‡ AND TERUAKI IWAMOTO*

*From the *Department of Urology, St Marianna University School of Medicine, Kawasaki, Japan; the †R&D Center, Biomedical Laboratories, Kawagoe, Japan; and the ‡Scientific Crime Laboratory, Kanagawa Prefectural Police, Yokohama, Japan.*

ABSTRACT: Semenogelin I and II (Sg I and II) are the major components of human semen coagulum. The protein is rapidly cleaved after ejaculation by the chymotrypsin-like protease prostate-specific antigen (PSA), which results in the liquefaction of the semen coagulum and the progressive release of motile spermatozoa. One of the cleavage products of the protein, a 14-kDa protein, is a sperm motility inhibitor (seminal plasma motility inhibitor [SPMI]). We developed a monoclonal antibody (mAb) that is specific to the fragment of Sgs, SPMI, and a sandwich enzyme-linked immunosorbent assay (ELISA) system for the quantification of Sgs using this mAb. Then, we measured SPMI/Sg levels in human seminal plasma from healthy male volunteers (n = 100, aged 18–24 years). The mean level of SPMI/Sg in seminal plasma was 19 ± 13 mg/mL (range, 4–68 mg/mL). Log-transformed SPMI/Sg levels were negatively correlated with the sperm motility ($r = -0.229$, $P = .0220$) and positively correlated with the total protein concentration ($r = 0.793$, $P < .0001$). This result supports that SPMI, one of the fragments of Sg, has its inhibitory effect on ejaculated spermatozoa in liquefied semen under physiological conditions.

Key words: Sperm motility inhibitor, seminal vesicles, asthenozoospermia.

J Androl 2003;24:878–884

The major structural components of human semen coagulum have been described as a disulfide-linked complex of the 52-kDa protein, known as semenogelin I (Sg I), and 2 forms of an Sg I–related protein of 71 and 76 kDa (semenogelin II [Sg II]), all originating from seminal vesicle secretions (Chaistitvanich and Boonsaeng, 1983; Lilja and Laurell, 1984, 1985). The fragmentation of Sg that occurs after ejaculation has been associated mainly with the proteolytic activity of prostate-specific antigen (PSA) (Lilja, 1985; Lilja et al, 1987, 1989; McGee and Herr, 1988). One of the fragments, which is known as a seminal plasma motility inhibitor (SPMI) (Iwamoto and Gagnon, 1988b), inhibits the sperm motility of demembranated spermatozoa and of intact spermatozoa when concentrations are 1000-fold higher than demembranated spermatozoa (Iwamoto and Gagnon, 1988a). Previously, we observed the SPMI activity on demembranated spermatozoa in human seminal plasma did not correlate with sperm motility (Iwamoto, 1999). Recently, Koistinen et al (2002) developed an immunofluorometric assay for Sg using an anti-Sg monoclonal antibody (mAb) to measure Sg levels in seminal plasma. They found no correlation between the Sg concentration and the volume of the ejaculate, sperm concentration, sperm motility, or in vitro fertilization rate. There is another fragment of Sg, the antigen recognized by the monoclonal mouse anti-human sperm antibody 5 (MHS-5). This antibody was originally raised against whole spermatozoa but was found to recognize a sperm-coating antigen originating from the seminal vesicles (Herr et al, 1986; McGee and Herr, 1987, 1988) and was used for MHS-5–enzyme-linked immunosorbent assay (ELISA) to detect human semen in forensic samples (Herr and Woodward, 1987; Keil et al, 1996). However, no study has reported on the quantification of Sg in seminal plasma with the MHS-5–ELISA system.

To evaluate the Sg quantity in liquefied seminal plasma, it is important that the stable antigen fragment is used to raise the antibody. Moreover, it is suggested that the criteria used when targeting the sperm motility region of the Sg antigen also focus on clarifying the role that it plays after the liquefaction of spermatozoa. Previously, we demonstrated that recombinant Sg I (rSg I) that lacks the SPMI region lost its inhibitory activity on demembranated spermatozoa (Miyano et al, 2003). Therefore, we produced an mAb that recognized the SPMI fragment of Sg and developed a sandwich ELISA system to quantify the original Sg concentration in seminal plasma. In the present study, the relationship between Sg levels in sem-

Supported by grants-in-aid (11694320 and 10557145) to T.I. from the Ministry of Education, Science, Culture, and Sport of Japan.

Correspondence to: Dr Teruaki Iwamoto, Department of Urology, St Marianna University School of Medicine, 2-16-1, Sugao, Miyamae-ku, Kawasaki 216-8511, Japan (e-mail: t4iwa@marianna-u.ac.jp).

Received for publication April 14, 2003; accepted for publication July 21, 2003.

inal plasma and semen parameters, which were obtained from semen analysis, was examined to elucidate the function of SPMI under physiological conditions.

Materials and Methods

Sample Preparations

Semen samples were collected from human male volunteers (n = 100, aged 18–24 years) by masturbation in a sterile container after at least 48 hours of abstinence. Each semen sample was liquefied for 15–30 minutes at 37°C before assessment of sperm parameters. After an assessment of sperm parameters according to World Health Organization guidelines (1999) for seminal plasma preparations, liquefied semen was centrifuged to eliminate the solid materials. The supernatants as seminal plasma were placed in aliquots and frozen at −80°C until analysis.

The tissue specimens of the seminal vesicle and prostate were obtained from patients (n = 2) who were undergoing a prostatectomy for prostate cancer. The epididymis and testis were from a patient who was undergoing an orchiectomy for a testicular tumor. These specimens were fixed with 10% formalin in phosphate-buffered saline (PBS) for 12 hours at 25°C and embedded in paraffin. The 5- to 7-μm-thick sections were cut with a microtome and mounted on glass slides (Mitsubishi Biochemical Laboratory, Tokyo, Japan). These sections were provided for immunohistochemistry after deparaffinization and dehydration.

Anti-Sg polyclonal antibody (pAb) was prepared by immunizing rabbits with human rSg II and purified by affinity-column chromatography with protein G-sepharose (HiTrap Protein G, Amersham Biosciences, Uppsala, Sweden). Mice were also immunized with human rSg II to produce an mAb. The mAb F11 was purified from mouse ascitic fluid by affinity-column chromatography with protein G-sepharose (HiTrap Protein G, Amersham).

The Ethical Committee/Institutional Review Board of St Marianna University approved the study. Informed consent was obtained from volunteers before the use of their tissue or semen for research.

Western Blotting

rSg proteins rSg I (aa 23–462), rSg I, which lacks the SPMI region (aa 108–159 were deleted from rSg I; rSg I [−SPMI]), and rSg I, which lacks the repeat region (aa 320–379 were deleted from rSg I; rSg I [−repeat]) were produced by the baculovirus system in *Spodoptera frugiperda* (Sf21) cells. A recombinant SPMI fragment (aa 108–159; rSPMI) was produced by *Escherichia coli*. Then, these recombinant proteins were purified with the QIA express Ni-NTA Protein Purification System (QIAGEN, Hilden, Germany) as previously reported (Murakami et al, 1998; Miyano et al, 2003). Seminal plasma and purified recombinant protein samples were solubilized in sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, and 0.0125% bromophenol blue), after the measurement of each protein concentration by the bicinchoninic acid assay (Smith et al, 1985) using bovine serum albumin (BSA) as a standard. Molecular-mass separations were carried out on 10%–20% (wt/vol) gradient polyacrylamide SDS gels. These gels were electroblotted onto polyvinylidene difluoride membranes, and the membranes were stained with Coomassie brilliant blue R-250 or blocked for 1 hour at 37°C with a solution of 5% (wt/vol) nonfat dried milk in Tris-buffered saline (20 mM, pH 7.8) supplemented with Tween 20 (0.05%; TBS-T). The membranes were incubated either with anti-human rSg mAb (F11) or with mouse normal immunoglobulin G (IgG) at 1 μg/mL in TBS-T containing 5% (wt/vol) nonfat dried milk for 1 hour at 37°C. After 3 washes in TBS-T for 5 minutes, the membranes were incubated with goat anti-mouse IgG conjugated with alkaline phosphatase (BioRad, Richmond, Calif) at a dilution of 1:3000 in TBS-T for 30 minutes at 37°C. Following several washes in TBS-T, detection was performed with 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium (BCIP/NBT; Sigma Chemical Co, St Louis, Mo).

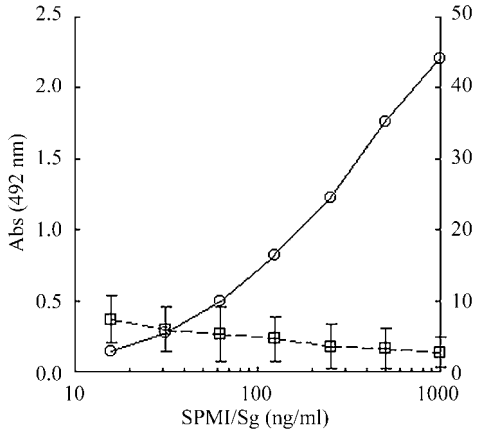

Figure 1. A typical standard curve (circles) and the mean ± standard deviation of the coefficient of variation (n = 5) (squares) obtained with the enzyme-linked immunosorbent assay (ELISA) system of seminal plasma motility inhibitor/semenogelin (SPMI/Sg).

Immunohistochemistry

The tissue sections were blocked for endogenous peroxidase activity with 3% hydrogen peroxide in methanol for 15 minutes. Nonspecific antibody binding was blocked in PBS containing 10% normal goat serum for 1 hour at room temperature. The sections were incubated with the mAb F11 at 5 μg/mL or with normal mouse IgG as a negative control at 5 μg/mL in PBS containing 10% normal goat serum for 1 hour at 37°C. Each section was treated with peroxidase-conjugated anti-mouse IgG (Histofine Simple Stain MAX PO, Nichirei, Tokyo) for 30 minutes at room temperature and developed with 3-amino-n-ethylcarbazole (Nichirei) for visualization. Sections were counterstained with Mayer hematoxylin for 10 seconds and mounted in aqueous permanent mounting solution (Nichirei).

The Quartz-Crystal Microbalance

The fragment peptides of SPMI that were chemically synthesized and purified by reverse-phase high-pressure liquid chromatography (HPLC) were purchased from the manufacturer (QIAGEN K. K., Tokyo, Japan). Detection of peptides binding to the mAb F11 was carried out using a quartz-crystal microbalance (QCM) technique. A QCM is a very sensitive mass-measuring device.

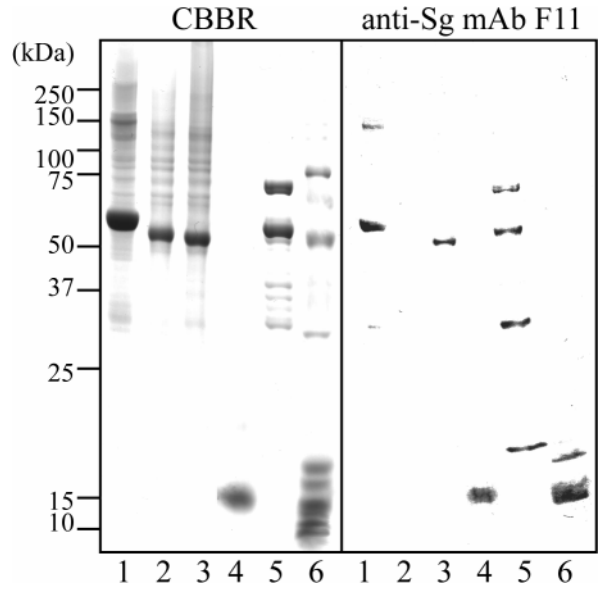

Figure 2. Western immunoblot analysis of seminal plasma motility inhibitor/semenogelin (SPMI/Sg). The left panel shows the membrane stained with a Cbbr protein, and the right panel shows the membrane stained with anti-Sg F11 monoclonal antibody (mAb). Lane 1: rSg I. Lane 2: rSg I (−SPMI). Lane 3: rSg I (−repeat). Lane 4: SPMI fragment. Lane 5: urea-treated seminal plasma. Lane 6: liquefied seminal plasma.

Its resonance frequency decreases linearly upon the increase of the mass on the QCM electrode at nanogram levels (Okahata et al, 1999). Previously, this technique was applied for protein–DNA interactions using a 27-MHz QCM (Okahata et al, 1998); however, there has been no study of protein–protein interactions. First, the mAb F11 (0.8 μg) was immobilized on a gold electrode surface of a 27-MHz QCM with hydrophobic interactions, and the frequency of the electrode was detected and recorded with a frequency counter–equipped computer (Affinix Q System, Initium Inc, Tokyo, Japan) in the mixing chamber containing PBS at 25°C. After stabilization, each peptide or rSPMI was added to the chamber at 10 μg/mL, and the changes in frequency were recorded. We confirmed the QCM result with Western blotting as described above. Purified Sg proteins were separated using 10% SDS gel. For immunoblot, mAb F11 (0.3 μg/mL) and mAbF11 (0.3 μg/mL) preabsorbed with each peptide (5 μg/mL) were used.

Determination of SPMI/Sg in Seminal Plasma

Seminal plasma levels of Sg were measured using a sandwich ELISA system that we developed for this study. This was an ELISA system with a quantitative sandwich technique using microtiter plates. A human seminal plasma sample diluted with a dilution buffer (0.1 M phosphate buffer, pH 7.5, containing 0.9% NaCl, 0.1% Tween 20, and 1% BSA) was placed into the wells of a microtiter plate precoated with the mAb F11 at 400 ng/well. As the detecting antibody, the anti-Sg pAb was added to the wells at 100 ng/well in the dilution buffer. The secondary antibody, horseradish peroxidase–conjugated goat anti-rabbit IgG (Zymed Laboratories Inc, San Francisco, Calif), was added to the wells at 1:4000 diluted with the dilution buffer. The resulting absorbance with *o*-phenylendiamine was proportional to the amount of bound Sg, and was measured at 492 nm; concentrations were estimated using a standard curve. HPLC-purified Sg II was prepared by a modified method previously reported (Robert and Gagnon, 1996) and was used as the standard. Briefly, semen samples treated with 8 M urea were reduced with dithiothreitol and treated with iodoacetamide; then, the centrifuged supernatant was loaded onto an SP-Sepharose column (Amersham Biosciences, Tokyo, Japan). Among the fractions of Sg that were eluted with a linear gradient of NaCl (0–400 mM), the former fractions containing Sg II were pooled and loaded onto an HPLC column (Vydac C4, The Separation Group, Hesperia, Calif). The eluate containing purified Sg II was lyophilized and stored at −80°C until use. Protein concentration was measured by the bicinchoninic acid assay (Smith et al, 1985) using BSA as a standard. All measurements were performed in duplicate, and the mean was taken. The minimum detectable concentration was 15 ng/mL, and intra- and interassay coefficients of variation were 6.3% and less than 10%, respectively. A typical standard curve for HPLC-purified native Sg II and the mean ± standard deviation of the coefficient of variation (%) (n = 5) are shown in Figure 1. We used the linear segment of the standard curve that ranged from 31 to 250 ng/mL for our measurements. The dilutions of seminal plasma samples were selected from 3 values [1) 1.6×10^5, 2) 3.2×10^5, or 3) 6.4×10^5] in order that the measured Sg level was within the range.

Statistical Analysis

Statistical analysis was conducted using the Mann-Whitney *U* test for unpaired values and Spearman rank correlation coefficients for paired values; a *P*-value $<.05$ was considered significant. Simple linear regression analysis was evaluated between the log-transformed Sg concentrations, sperm motility, and total protein concentrations. A log transformation was used to obtain a normal distribution of Sg concentrations.

Results

Immunoreactivity of the mAb F11

According to the Western blot analysis, the mAb F11 recognized the full-length rSg I (52 kd), rSg I (−repeat), and rSPMI but did not recognize rSg I (−SPMI). The antibody also recognized the 14- and 18-kDa polypeptides among Sg fragments in liquefied semen as well as the full-length Sg I (52 kDa) and Sg II (71 and 75 kDa) in seminal plasma when fragmentation was prevented with 8 M urea before liquefaction (Figure 2). Immunohistochemical staining with this antibody showed reactivity in glandular epithelial cells of the seminal vesicles (Figure 3A through C). No staining was observed in the prostate, epididymis, or testis (data not shown). Figure 3E shows typical frequency changes as a function of the mAb F11 immobilized on the QCM electrode with the addition of peptide fragments EP1–3 of SPMI (Figure 3D) in PBS. After the EP3 peptide fragment was injected at 10 μg/mL, the frequency decreased (the mass increased) grad-

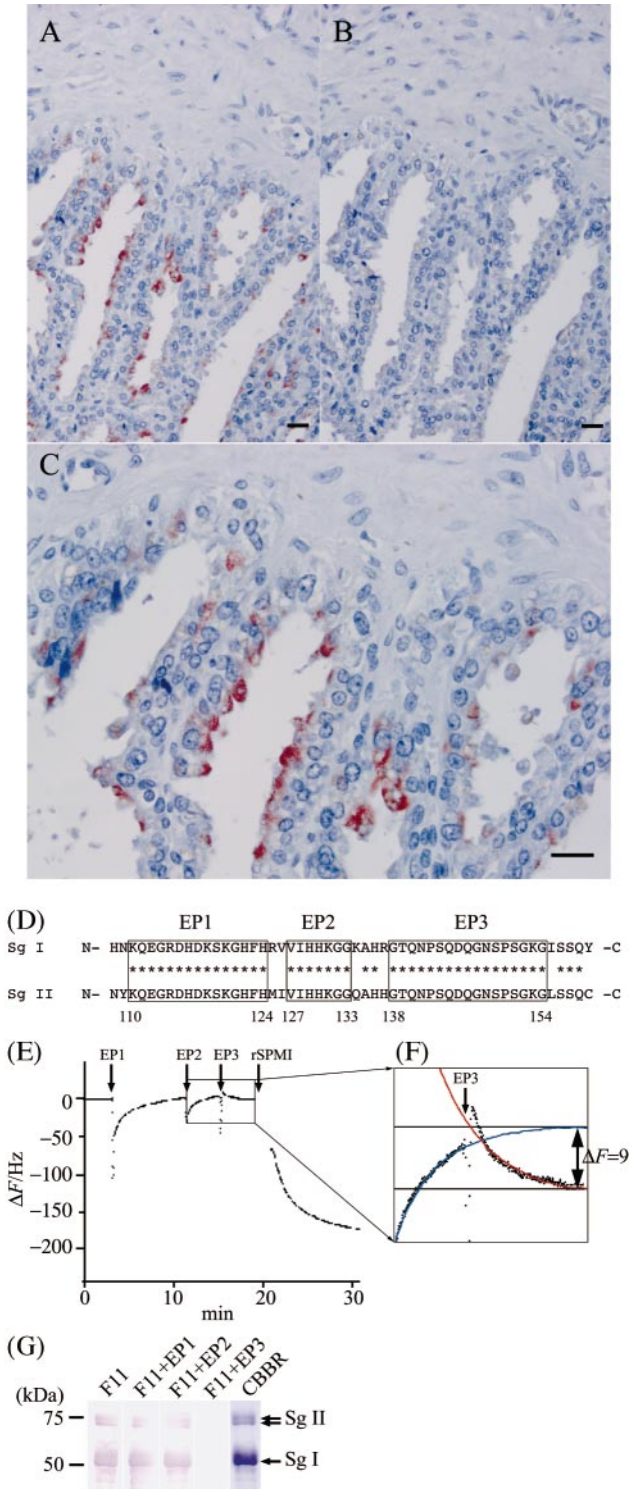

Figure 3. Localization of seminal plasma motility inhibitor/semenogelin (SPMI/Sg) in seminal vesicles by immunohistochemical staining using anti-Sg F11 monoclonal antibody (mAb) **(A, C)**. The negative control is a nonspecific mouse immunoglobulin that has replaced the Sg antibody **(B)**. Bar = 20 μm. **(D–F)** Determination of the epitope of anti-Sg F11 mAb by synthesized peptides and QCM. Amino acid sequenes of SPMI fragments region in Sg I and Sg II **(D)**. For epitope determination, EP1, EP2, and EP3 peptides ere used. Typical time courses of frequency changes of the anti-sg F11 mAb immobilized ACM, responding to the addition of EP1, EP2, and EP3 peptides **(E)**. When the EP3 peptide was added, the QCM frequency decreased. The blue line and the red line show the predicted regression curve after adding EP2 and EP3 **(F)**. Western blot analysis with preabsorbed mAfF11 with EP1–3. The mAbF11 perabsorbed by excess EP3 peptides shows no immunological staining to purified Sg proteins **(G)**.

Table 1. *Spearman correlation coefficients (ρ) and P-values for correlations between Sg concentration and semen quality in healthy male volunteers**

	Sg	
	ρ	*P*
Total protein	.754	<.0001
Age	.009	.9316
Volume	.171	.8890
Sperm concentration	.058	.5629
% progressive sperm cells	−.178	.0772

* Sg indicates semenogelin.

ually for approximately 5 minutes at $-\Delta F = 9$ Hz as a result of the binding of the peptide fragment EP3 onto the mAb F11 (Figure 3E and F). The addition of rSPMI also showed the binding to the mAb F11 (Figure 3E). For the Western blot analysis with preabsorbed mAb F11 with EP1–3, the incubations with mAb F11 preabsorbed with excess EP3 peptides showed no immunological staining to purified Sg I and II (Figure 3G) and the fragments of Sg in seminal plasma (data not shown).

Correlation of Sg Levels With Sperm Parameters

The average Sg concentration in liquefied seminal plasma was 19 ± 13 mg/mL (range, 4–68 mg/mL, n = 100). The SPMI/Sg concentrations were correlated with the total protein concentrations but not with any other semen parameters (Table 1). However, after log transformation, the SPMI/Sg concentrations were correlated positively with the total protein concentrations ($r = 0.793$, $P < .0001$) and negatively with the percentages of sperm with forward progression ($r = -0.229$, $P = .0220$) (Figure 4). No significant difference in the mean of SPMI/Sg concentration was found between the oligozoospermic group (sperm concentration $\leq 20 \times 10^6$/mL) and the group with the normal sperm concentration (sperm concentration $> 20 \times 10^6$/mL) or between the asthenozoospermic group (sperm motility ≤50%) and the group with normal sperm progression (sperm motility >50%) (Table 2).

Discussion

We generated an mAb recognizing the consensus amino acid sequence in the SPMI fragment of Sg I and II. In Western immunoblotting, the antibody reacted with the 52-

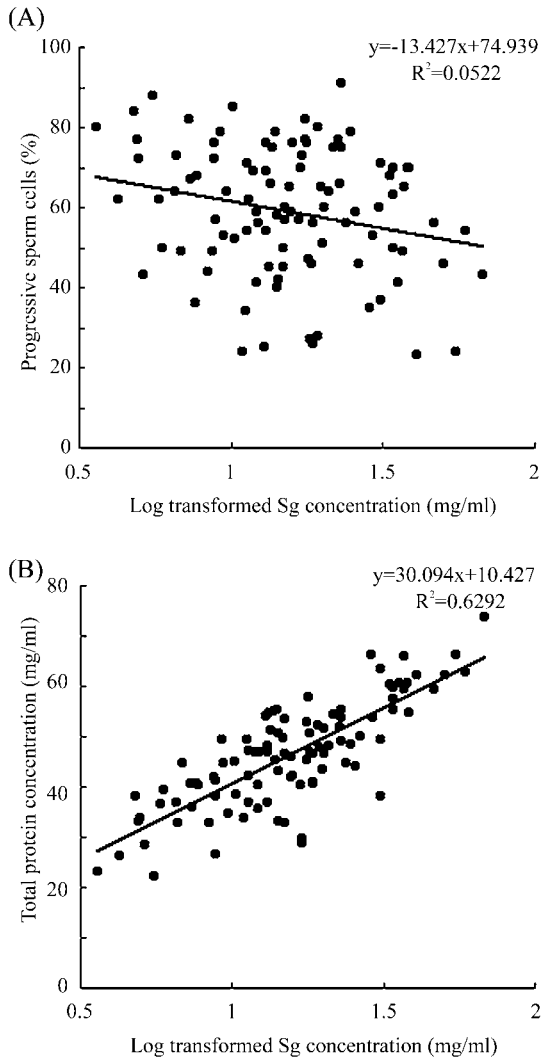

Figure 4. Seminal plasma motility inhibitor/semenogelin (SPMI/Sg) levels in relation to sperm motility **(A)** or to total protein concentration of seminal plasma **(B)** in healthy male volunteers (n = 100).

kDa band for the Sg I band, with the 71- and 76-kDa bands for Sg II in seminal plasma before liquefaction, and with 2 low-molecular-mass bands of 14 and 18 kDa in seminal plasma after liquefaction. The antibody also recognized rSg I, rSg I (−repeat), and rSPMI but did not react with rSg I (−SPMI). The higher-molecular-weight forms in the recombinant preparations were contaminated proteins, and the higher-molecular-weight immunoreactive form in rSg I may be due to the accumulation of rSg I. The specificity of the antibody was confirmed using QCM. This is a new method for epitope mapping; however, there have been many studies for detecting molecular interactions without any labeling (eg, protein–DNA interaction) (Okahata et al, 1998). The antibody recognized a peptide with the sequence GTQNPSQDQGNSPSGKG (aa 138–154), which exists in the carboxyl position of the SPMI fragment (aa 108–159) in both Sg I and II. This result was confirmed by Western blotting with the preabsorbed antibody. The antibody also interacted with whole rSPMI. The decrease of frequency of the QCM electrode after adding rSPMI was larger than the decrease caused by adding the peptide E3 because the molecular mass of rSPMI is larger than that of E3. Immunohistochemical staining with this antibody showed reactivity in glandular epithelial cells of the seminal vesicles, while it showed no reactivity in the normal region of the prostate, epididymis, and testis. These immunohistochemical localizations were similar to staining with other anti-Sg mAbs reported previously (Evans and Herr, 1986; Bjartell et al, 1996; Koistinen et al, 2002).

The mAb F11 was found suitable for the quantification of Sg in seminal plasma after liquefaction, because fragments of 14 and 18 kDa can be found that react with this antibody in seminal plasma after liquefaction. Sg is the physiological substrate of PSA, but the SPMI fragment region appears to be resistant to its protease activity (Robert et al, 1997). The detection antibody in this ELISA system, anti-Sg pAb, was demonstrated to be a suitable antibody for the detection of Sg fragments as a marker for human semen identification in forensic samples because of its high sensitivity and broad detection range of Sg fragments (Sato et al, 2001). Therefore, this ELISA system is suitable for the quantification of Sg as the precursor protein of SPMI in seminal plasma after liquefaction. The estimated Sg I concentration in seminal plasma immediately after ejaculation was 7–11 mg/mL (Robert and Gagnon, 1996). The present study showed that the mean Sg concentration, 19 ± 13 mg/mL, was the sum of Sg I and II. Analysis of the gene structure of Sg indicates that an equal number of molecules of both Sg I and II are expressed, so that the total Sg present was estimated to be twice that of Sg I, 14–22 mg/mL. This estimated value was within the range of the results from the present study. Another quantification of Sg in seminal plasma us-

Table 2. *SPMI/Sg concentrations (mg/mL) in different sperm concentrations or sperm motility groups in healthy male volunteers**

Group	Mean (SD)	Median (range)
Sperm concentration ≥20 × 10⁶ (n = 84)	18.6 (12.0)	15.7 (4.2–67.9)
Sperm concentration <20 × 10⁶ (n = 16)	20.2 (15.7)	14.6 (3.6–55.3)
Sperm motility ≥50% (n = 69)	17.5 (10.8)	15.0 (3.6–59.2)
Sperm motility <50% (n = 31)	21.8 (15.5)	14.9 (5.2–67.9)

* SPMI indicates seminal plasma motility inhibitor; Sg, semenogelin.

ing an immunofluorometric assay showed that the concentration of Sg was 153 ± 61 μM (20–350 μM) (Koistinen et al, 2002). If the calculation was performed as the sum of Sg I and II, the concentration of Sg is estimated at 9.6 ± 3.8 mg/mL (1.3–2.2 mg/mL). The use of different methods and standards as well as dilutions of seminal plasma may cause such a difference.

In this study, we showed that the SPMI activity of Sg in liquefied human seminal plasma was estimated as the quantity of the SPMI fragment of Sg and that the log-transformed SPMI/Sg levels were negatively correlated with the sperm motility ($r = -0.23$, $P = .0213$). As previously reported, both Sg and fragmented Sg inhibit sperm motility; however, this effect on intact spermatozoa is less than on demembranated-reactivated spermatozoa (Iwamoto and Gagnon, 1988a). Previously, levels of SPMI activity in liquefied human seminal plasma measured by a biological assay using demembranated spermatozoa were not correlated with sperm motility (Iwamoto, 1999). The assay system was sensitive for detecting SPMI activity but not under physiological conditions. An immunofluorometric assay using an mAb, which was developed recently by another group, did not show a correlation between Sg levels and sperm motility (Koistinen et al, 2002). The antibody they used may be specific to the Sg region except for the SPMI fragment, but they did not show the epitope of the antibody. Moreover, these 2 previous studies were concerned with infertile patients. The use of healthy male volunteers was one reason we detected the correlation between SPMI/Sg levels and sperm motility.

There was no significant difference in the mean of the SPMI/Sg concentration between the asthenozoospermic group (≤50%) and the group with normal sperm progression (>50%) (Table 2). The results from this study suggest that the cause-and-effect relationship between SPMI/Sg and asthenozoospermia depends on the condition of the spermatozoa; therefore, the receptor and signaling mechanism of SPMI must be elucidated before further discussion about the relationship between the levels of SPMI/Sg and asthenozoospermia can occur.

Acknowledgments

The authors are grateful to the staff of the male infertility clinic at St Marianna University School of Medicine for collection of tissue and semen specimens. We also thank Dr E. de Lamirande for a critical review of the manuscript.

References

Bjartell A, Malm J, Moller C, Gunnarsson M, Lundwell A, Lilja H. Distribution and tissue expression of semenogelin I and II in man as demonstrated by in situ hybridization and immunocytochemistry. *J Androl.* 1996;17:17–26.

Chaistitvanich N, Boonsaeng V. Molecular structure of human seminal coagulum: the role of disulfide bonds. *Andrologia.* 1983;15:446–451.

Evans RJ, Herr JC. Immunohistochemical localization of the MHS-5 antigen in principal cells of human seminal vesicle epithelium. *Anat Rec.* 1986;214:372–377, 390–371.

Herr JC, Summers TA, McGee RS, Sutherland WM, Sigman M, Evans RJ. Characterization of a monoclonal antibody to a conserved epitope on human seminal vesicle–specific peptides: a novel probe/marker system for semen identification. *Biol Reprod.* 1986;35:773–784.

Herr JC, Woodward MP. An enzyme-linked immunosorbent assay (ELISA) for human semen identification based on a biotinylated monoclonal antibody to a seminal vesicle–specific antigen. *J Forensic Sci.* 1987;32:346–356.

Iwamoto T. Physiological action and role of seminal plasma motility sperm inhibitor. *Nippon Funin Gakkai Zasshi.* 1999;44:103–108.

Iwamoto T, Gagnon C. A human seminal plasma protein blocks the motility of human spermatozoa. *J Urol.* 1988a;140:1045–1048.

Iwamoto T, Gagnon C. Purification and characterization of a sperm motility inhibitor in human seminal plasma. *J Androl.* 1988b;9:377–383.

Keil W, Bachus J, Troger HD. Evaluation of MHS-5 in detecting seminal fluid in vaginal swabs. *Int J Legal Med.* 1996;108:186–190.

Koistinen H, Soini T, Leinonen J, et al. Monoclonal antibodies, immunofluorometric assay, and detection of human semenogelin in male reproductive tract: no association with in vitro fertilizing capacity of sperm. *Biol Reprod.* 2002;66:624–628.

Lilja H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J Clin Invest.* 1985;76:1899–1903.

Lilja H, Abrahamsson PA, Lundwall A. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. *J Biol Chem.* 1989;264:1894–1900.

Lilja H, Laurell CB. Liquefaction of coagulated human semen. *Scand J Clin Lab Invest.* 1984;44:447–452.

Lilja H, Laurell CB. The predominant protein in human seminal coagulate. *Scand J Clin Lab Invest.* 1985;45:635–641.

Lilja H, Oldbring J, Rannevik G, Laurell CB. Seminal vesicle–secreted proteins and their reactions during gelation and liquefaction of human semen. *J Clin Invest.* 1987;80:281–285.

McGee RS, Herr JC. Human seminal vesicle–specific antigen during semen liquefaction. *Biol Reprod.* 1987;37:431–439.

McGee RS, Herr JC. Human seminal vesicle-specific antigen is a substrate for prostate-specific antigen (or P-30). *Biol Reprod.* 1988;39:499–510.

Miyano S, Yoshida K, Yoshiike M, Miyamoto C, Furuichi Y, Iwamoto T. A large deletion of the repeat site in semenogelin I is not involved in male infertility. *Int J Mol Med.* 2003;11:435–440.

Murakami J, Yoshiike M, Satoh M, Furuichi Y, Iwamoto T. Characterization of recombinant precursor proteins of the human seminal plasma sperm motility inhibitor synthesized in insect cells. *Int J Mol Med.* 1998;2:693–700.

Okahata Y, Masunaga Y, Matsuno H, Furusawa H. Quantitative detection of a DNA ligase reaction on a quartz-crystal microbalance. *Nucleic Acids Symp Ser.* 1999;147–148.

Okahata Y, Niikura K, Sugiura Y, Sawada M, Morii T. Kinetic studies of sequence-specific binding of GCN4-bZIP peptides to DNA strands immobilized on a 27-MHz quartz-crystal microbalance. *Biochemistry.* 1998;37:5666–5672.

Robert M, Gagnon C. Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: identity with semenogelin. *Biol Reprod.* 1996;55:813–821.

Robert M, Gibbs BF, Jacobson E, Gagnon C. Characterization of prostate-specific antigen proteolytic activity on its major physiological sub-

strate, the sperm motility inhibitor precursor/semenogelin I. *Biochemistry.* 1997;36:3811–3819.

Sato I, Yoshiike M, Yamasaki T, Yoshida K, Takano S, Mukai T, Iwamoto T. A dot-blot-immunoassay for semen identification using a polyclonal antibody against semenogelin, a powerful seminal marker. *Forensic Sci Int.* 2001;122:27–34.

Smith P, Krohn R, Hermanson G, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem.* 1985;150:76–85.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interactions*. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.