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Seminal hyperviscosity is not associated with semenogelin degradation or sperm deoxyribonucleic acid damage: a prospective study of infertile couples

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Objective: To investigate the association between seminal hyperviscosity, the extent of semenogelin degradation, and sperm DNA integrity (DNA fragmentation index [DFI] and high DNA stainability [HDS]) in semen from infertile couples.
Design: Prospective study.
Setting: University-affiliated fertility center.
Patient(s): Twenty-four consecutive infertile couples with moderate or high seminal viscosity (hyperviscosity group) and 25 consecutive infertile couples with normal semen viscosity (control group) undergoing standard IVF.
Intervention(s): Semen volume and seminal hyperviscosity, sperm concentration, motility, and morphology, level of semenogelin degradation (by immunoblotting), and sperm chromatin damage (by sperm chromatin structure assay and expressed as %DFI and %HDS) were evaluated.
Main Outcome Measures(s): Sperm %DFI and %HDS in the hyperviscosity group and the control group and the relationship between the extent of semenogelin degradation and seminal viscosity.
Result(s): Semen volume in couples with moderate and high seminal viscosity was significantly lower as compared with the control group. In addition, total motility and normal morphology were significantly lower in the couples with high seminal viscosity as compared with the control group; however, there were no significant differences in sperm %DFI and %HDS between the hyperviscosity group and the control group. In addition, there was no relationship between the extent of semenogelin degradation and seminal viscosity.
Conclusion(s): Our data suggest that seminal hyperviscosity (a posttesticular factor) is not an important cause of sperm DNA damage. Moreover, seminal hyperviscosity is not related to the degree of semenogelin degradation. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Semen hyperviscosity, sperm DNA damage, semenoglin, infertile men

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Of the couples unable to conceive, less than half of all cases of infertility are a result of female conditions, whereas male infertility is present in more than 50% of infertile couples alone or in combination with female factors (1). The pathophysiology of male infertility is still poorly understood, and there is no particular test to accurately determine the underlying cause of sperm fertilization ability. Sperm DNA fragmentation is a new adjunct to the conventional semen analysis, and this test may provide additional diagnostic and prognostic information (2). In several studies, sperm nuclear DNA fragmentation has been associated with lower pregnancy rates (natural and assisted) and increased rates of abortion after IVF or intracytoplasmic sperm injection (2, 3).

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At ejaculation, human sperm are suspended in the seminal plasma, a heterogeneous mixture originating from the testicular and epididymal fluids, as well as secretions of seminal vesicles, prostate, and bulbourethral glands (4). Shortly after ejaculation, semen coagulates and becomes a semisolid mass. This coagulum is formed through binding of semenogelin secreted from the secretory epithelium of seminal vesicle at ejaculation with zinc ions from the prostate (5, 6). Semenogelin I (52 kDa) and the less-abundant semenogelin II (71 and 76 kDa) are the major components of human semen coagulum and represent 20–40% of seminal plasma proteins (7). Semenogelin prevents sperm capacitation (8, 9) and is responsible for sperm immobilization in the seminal coagulum (6). Within a few minutes at room temperature the semen usually begins to liquefy by the action of prostatic proteases, and improper degradation of semenogelin prevents the initiation of sperm motility. Semenogelin is degraded into several peptides of specific molecular masses but none of these peptides is a predominant product (6, 7).

During semen analysis, normal-viscosity semen makes small discrete drops when dripped from a pipette; however, semen specimens with high viscosity show homogeneous stickiness, and their consistency stays unchanged with time. High viscosity can be recognized by the elastic properties of the sample, which adheres strongly to itself when attempts are made to pipette it (10). Seminal hyperviscosity occurs in 12%–29% of ejaculates, but its etiology has not been investigated carefully (11, 12). We have shown in our previous studies that seminal hyperviscosity is associated with lower sperm motility and normal morphology, poor outcome of controlled ovarian stimulation and IUI, as well as lower implantation and clinical pregnancy rates in couples undergoing IVF cycles (13, 14).

The aim of the present study was to investigate the association between seminal hyperviscosity, the extent of semenogelin degradation, and sperm DNA integrity (DNA fragmentation index [DFI] and high DNA stainability [HDS]) in semen from infertile men.

MATERIALS AND METHODS

Study Population

A prospective study was conducted in two separate cohorts undergoing IVF at the Toronto Centre for Advanced Reproductive Technology between October 2007 and June 2009. Twenty-four consecutive infertile couples with moderate or high seminal viscosity and 25 consecutive infertile couples with normal semen viscosity (control group) were enrolled in the study. Most of the 49 couples undergoing IVF had female-factor or unexplained infertility (with normal semen parameters), and a minority had mild male factor with mild semen abnormalities (Table 1). The ethics review board at our institution approved the study, and all patients signed an informed consent form. All information remained confidential.

Semen Collection and Analysis

Semen samples were collected on the day of oocyte retrieval and after 48–72 hours of sexual abstinence. Specimens were allowed to liquefy for up to 1 hour after collection, and the volume, appearance, pH, and seminal viscosity were determined. Manual semen analysis was performed according to World Health Organization (WHO) guidelines to determine sperm concentration, motility, and morphology, and values were classified according to WHO recommendations at the time of the study (10). In this study, normal values were as follows: sperm concentration $\geq 20 \times 10^6$/mL, total motility $\geq 50\%$, and normal sperm forms $\geq 30\%$.

As indicated in the WHO guidelines, seminal viscosity was estimated by gently aspirating the sample into a wide-bore, 5-mL sterile pipette and then allowing the semen to drop by gravity. Ejaculates with normal viscosity have a thread length of ≤ 2 cm. If viscosity is abnormal, thread length is more than 2 cm (moderate viscosity) or semen does not drip at all and stays as a whole coagulum (high viscosity). The men were assigned to the hyperviscosity or control group according to the viscosity results of the sample submitted for IVF. To overcome high seminal viscosity, a sterile 5-mL syringe (Becton Dickinson) fitted with a sterile 18G needle was used. The specimen was gently drawn into the syringe and expelled slowly back into the tube. This procedure was repeated few times to ensure the fluidity of the sample. Two aliquots of unprocessed semen (containing $\geq 2 \times 10^6$ spermatozoa) were collected and stored in liquid nitrogen for later assessment of semenogelin degradation and sperm DNA integrity.

TABLE 1

Cause of infertility in patients with seminal hyperviscosity and in controls.

Factor	Hyperviscosity	Control	*P* value
Tubal factor	1 (4)	3 (12)	NS
Unexplained infertility	2 (8.3)	4 (16)	NS
Late maternal age	6 (25)	7 (28)	NS
Endometriosis	2 (8.3)	1 (4)	NS
Poor responder	2 (8.3)	4 (16)	NS
PCOS	3 (13)	1 (4)	NS
Male factor	8 (33)	5 (20)	NS

Note: Values are number (percentage). NS = nonsignificant; PCOS = polycystic ovary syndrome.

Esfandiari. Sperm DNA damage and seminal viscosity. Fertil Steril 2014.

Immunoblotting Experiments

Frozen semen samples were thawed and centrifuged (10,000 × *g*, 5 minutes). Seminal plasma was collected and diluted 20-fold with HEPES-balanced saline. An aliquot was used to test protein concentration (bicinchoninic acid assay, using bovine serum albumin as standard), and another one was supplemented with sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer, boiled for 5 minutes, centrifuged, and electrophoresed (10 μL per well, 31 to 40 μg protein; 34 ± 1 μg protein mean ± SEM) on 12% polyacrylamide gels. Controls and samples with medium and high seminal viscosity were loaded on the gel randomly, the technician not knowing the status of the sample, to prevent any bias in the test. After transfer on nitrocellulose (0.22-μm pore size; Osmonics), proteins were immunoblotted with a polyclonal