

Semenogelin-Derived Peptides (RSIY-15 and SSIY-15) Enhance Motility in Asthenospermic Semen: A Potential Therapeutic Approach for Male Factor Infertility

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Background:

Infertility affects 13% of couples in the United States, with male factor infertility contributing to 30.4% of assisted reproductive technology (ART) cycles in 2017–2018 (1). Low sperm motility is a major cause of male infertility, but the mechanisms regulating motility remain poorly understood. Semenogelin-1 and -2, the main proteins in semen, initially form a coagulum that restricts sperm movement but are later cleaved into bioactive peptides capable of modulating motility (-2). Since sperm motility parameters strongly predict IVF and IUI success, identifying factors that influence motility, such as semenogelin-derived peptides, is clinically important. Using peptidomic analysis, we previously identified RSIY-11, a semenogelin-derived peptide that enhances motility by inhibiting neutral endopeptidase (NEP) (3). Subsequent studies revealed additional novel semenogelin-derived peptides that, based on sequence analysis, may also act as NEP inhibitors and influence sperm motility.

Objective: The goal of the present study was to identify if other semenogelin-derived peptides (RSIY-15 and SSIY-15) might also work as NEP inhibitors and enhance sperm motility.

Materials and Methods:

75 semen samples (31 normal motility, 44 low motility or asthenospermic as per WHO 5th edition) were collected from men who were evaluated at Montefiore's Institute for Reproductive Medicine for infertility workup. Two novel semenogelin-derived peptides (RSIY-15: GENGVQKDVSQRSIY and SSIY-15: GENGVQKDVSQSSIY), identified through peptidomic analysis of semen and predicted to act as NEP inhibitors, were synthesized and used in a fluorogenic assay to determine NEP inhibition constants (Ki). Sperm motility

parameters including total motility (TM) and progressive motility (PM) were assessed both visually and using a computer-assisted sperm analysis (CASA) system, which also measured additional kinetic parameters such as average path velocity (VAP), curvilinear velocity (VCL), and straight-line velocity (VSL). These evaluations were performed on patient samples at 0, 30, and 60 minutes following the addition of 75 μM of RSIY-15, SSIY-15, RSIY-11, or phosphate-buffered saline (PBS) alone. K_i values were calculated using Prism, and changes in sperm motility after peptide treatment were assessed using mixed effects linear regression and Wilcoxon Rank-Sum tests, with $p < 0.05$ considered statistically significant.

Results:

Lineweaver-Burk analysis showed RSIY-15 and SSIY-15 act as competitive NEP inhibitors. Dixon plots revealed K_i values of $12.58 \pm 1.67 \mu\text{M}$ for RSIY-15 and $13.69 \pm 2.5 \mu\text{M}$ for SSIY-15, both lower than RSIY-11 ($18.4 \pm 1.6 \mu\text{M}$). Visual assessment demonstrated significant improvements in total motility (TM) and progressive motility (PM) at 60 min ($P < 0.05$). In asthenospermic samples, CASA analysis confirmed these effects: compared to PBS controls, RSIY-15 increased TM and PM by 9.13% and 13.74%, and SSIY-15 by 19.5% and 19.23%. VAP, VSL, and VCL were significantly improved with RSIY-15 ($P < 0.05$), while SSIY-15 enhanced VCL at 60 min ($P < 0.05$).

Conclusion:

RSIY-15 and SSIY-15 are semenogelin-derived peptides identified in human semen and characterized as competitive NEP inhibitors in vitro, both demonstrating greater inhibitory potency than RSIY-11. They increased sperm motility in asthenospermic samples to normal ranges. This study uniquely confirms subjective visual assessment with objective CASA analysis. Improvements in CASA parameters, including VSL and VCL (key indicators of fertilizing potential), support the potential clinical use of these peptides in improving ART outcomes.

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