reproductive medicine

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Communication

Improvement in Sperm Recovery Rate and Total Motile Sperm Count Using α-Chymotrypsin in Highly Viscous Semen Sample Without Adversely Affecting Assisted Reproductive Technology Outcomes

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Abstract

Objectives: To investigate the impact of α-chymotrypsin treatment on sperm recovery rate and total motile sperm count (TMC) in highly viscous semen for intrauterine insemination (IUI) and in vitro fertilization (IVF), particularly in cases of severely low sperm count. **Methods**: High viscosity was defined by the inability to form a thread exceeding 2 cm from a semen drop after 30 min of incubation at 37 °C with repeated pipetting. Semen samples were treated with 5 mg of α-chymotrypsin for 5–10 min at 37 °C and washed using a 90% gradient solution. A total of 35 patients were included, with comparisons made to the same patients' prior untreated samples using paired *t*-tests. Severely low sperm count was classified as TMC below 10 million. **Results**: Treatment with α-chymotrypsin significantly improved TMC (22.2 million vs. 11.6 million, $p = 0.0004$) and motile sperm recovery rate (38.9% vs. 16.2%, $p = 0.00002$). In cases of severely low sperm count, α-chymotrypsin treatment resulted in a marked increase in recovery rate (43.0% vs. 10.0%, $p = 0.02$) and TMC (5.89 million vs. 1.21 million, $p = 0.004$). Fertilization using treated samples achieved an 87.8% success rate, with a 56.4% usable blastocyst rate, comparable to standard IVF outcomes ($n = 9$, average age = 34.9 years). **Conclusions**: α-chymotrypsin treatment significantly enhances sperm recovery and TMC in highly viscous semen, demonstrating particular efficacy in patients with severely low sperm counts without affecting fertilization or blastocyst rate in IVF.

Keywords: sperm recovery rate; total motile sperm count; α-chymotrypsin; seminal hyperviscosity; semen analysis

Academic Editor: Jan Tesarik

Received: 28 March 2025
Revised: 18 June 2025
Accepted: 1 July 2025
Published: 2 July 2025

Citation: Ayyar, A.; Khalil, M.; Wong, M.; Chung, R.; Coyne, K.; Findley, J.; Weinerman, R.; Flyckt, R.; Sofaly, K.P.; Kim, S.T. Improvement in Sperm Recovery Rate and Total Motile Sperm Count Using α-Chymotrypsin in Highly Viscous Semen Sample Without Adversely Affecting Assisted Reproductive Technology Outcomes. *Reprod. Med.* **2025**, *6*, 17. https://doi.org/10.3390/reprodmed6030017

1. Introduction

Approximately 15% of couples struggle with infertility, of which male infertility contributes 30–50% [1]. Concurrent evaluation of both the female and male partners should

be completed. This encompasses a full evaluation of the male, including a thorough a histological, physical, and semen analysis [2]. A semen analysis includes an assessment of the semen volume, concentration, motility, and morphology. Abnormalities in these parameters (most importantly, total motile sperm count) decreases a couple's chance of spontaneous conception [3].

Semen ejaculate is composed of the human sperm and the seminal plasma, which is produced by the male reproductive accessory organs [4]. After ejaculation, the semen undergoes coagulation. While in this coagulative state, components of the human semen impair the motility of the sperm [5]. After a few minutes, the semen then undergoes liquification under the influence of prostatic proteases. The prostate-specific agents allow for initiation of sperm motility [6]. Semen hyperviscosity (SHV) occurs when there is an impairment in the balance of coagulation and liquification and occurs in 12–29% of ejaculates. Potential contributing factors for SHV include infection, oxidative stress, inflammation, and leukocytospermia [7].

While intrauterine insemination (IUI) and in vitro fertilization (IVF) are used to overcome male infertility, SHV poses challenges to these treatments. Andrology laboratories process ejaculated sperm in order to prepare the specimen; however, IUI and IVF are dependent on the recovery of motile sperm. Our lab has shown that a sperm sample with a total motile count (TMC) $\geq$ 5 million is a significant predictive factor for pregnancy, further emphasizing the importance of high total motile count [8]. Preparations of sperm samples with SHV have absent or poor sperm recovery [9]. Furthermore, SHV is linked to lower fertilization rates after conventional IVF (not intracytoplasmic sperm injection) [10].

A proposed treatment for SHV is the use of α-chymotrypsin during treatment. α-chymotrypsin is a proteolytic enzyme that breaks down proteins and polypeptides [9]. Morales et al. showed the activity of α-chymotrypsin during the acrosome reaction at the time of fertilization [11]. α-chymotrypsin is used by andrologists to assist in liquification in viscous semen samples [12].

The aim of the present study was to investigates the impact of α-chymotrypsin treatment on sperm recovery rate and TMC in highly viscous semen for sperm preparation by paired analysis, particularly in cases of severely low sperm count.

2. Materials and Methods

Patient semen samples with plans for sperm preparation were collected fresh at our fertility center. Each sample was allowed to liquefy for 30 min at 37 °C. The samples were examined macroscopically in order to characterize the viscosity of each sample. This project defined high viscosity as the absence of a thread over 2 cm long from a semen drop after a 30 min incubation period at 37 °C with repeated pipetting [13]. A total of 35 specimens that were categorized as high viscosity through the macroscopic examination were initially analyzed microscopically by an experienced andrologist for motility and concentration of sperm cells using a microcell disposable counting chamber. Each specimen was then divided in half by volume to create an experimental group and a control group for each sample. The experimental portion of the sample was treated with 5 mg of α-chymotrypsin (QwikCheck^{TM} Liquefaction Kit, Medical Electronic Systems, Encino, CA, USA) for 5 min at 37 °C. The α-chymotrypsin treated sample and the untreated control sample were each placed over a 90% gradient solution (Irvine Scientific, Santa Ana, CA, USA) and centrifuged for 15 min at 400× *g*. After centrifugation, each group's pellet was resuspended in 2–4 mL of sperm wash media (Irvine Scientific, Santa Ana, CA, USA) and concentrated via centrifugation for 5 min at 400× *g* to the final washed sample. The final washed sample was microscopically analyzed again for motility and concentration by an experienced andrologist using the microcell disposable counting chamber. The total

motile concentration (TMC) was calculated from the initial microscopic examination and the post-wash microscopic examination for both groups. From this information, a recovery rate was calculated for both the experimental and control group.

Sperm recovery rate and TMC were calculated using the following equations:

$$\text{Sperm Recovery Rate } (\%) = \frac{\textit{Total number of motile sperms before processing}}{\textit{Total number of motile sperms after processing}} \times 100$$

$$\text{TMC} = \text{sample volume (mL)} \times \text{Sperm concentration } \left(\frac{\textit{sperm}}{\text{mL}}\right) \times \text{Motility fraction } \left(\frac{\textit{\% motile sperm}}{100}\right)$$

The recovery rate and TMC of the same patients from previous sperm preparation not treated with α-chymotrypsin were compared. As a subset analysis, a severely low sperm count was considered if TMC was less than 10 million.

The fertilization rate of intracytoplasmic sperm injection (ICSI) and the usable blastocyst rate in IVF cycles were calculated using the following equations:

$$\text{Fertilization Rate } (\%) = \frac{\textit{Total number of normally fertilized oocytes}}{\textit{Total number of ICSI oocytes}} \times 100$$

$$\text{Usable Blastocyst Rate } (\%) = \frac{\textit{Total number of transferrable blastocysts}}{\textit{Total number of normally fertilized oocytes}} \times 100$$

Comparative statistics were performed using a paired *t*-test.

3. Results

3.1. Paired Analysis for Total Motile Sperm Count (TMC) and Recovery Rate in α-Chymotrypsin Treatment

A total of 35 liquefied semen samples were equally divided into an experimental group (treatment with α-chymotrypsin) and a control group followed by sperm preparation by the gradient method. Then, TMC and sperm recovery rate were measured for both groups. TMC was significantly increased in the α-chymotrypsin treatment group (22.2 million vs. 11.6 million, $p = 0.0004$) and the average recovery rate of motile sperm was significantly higher in the α-chymotrypsin-treated group (38.9% vs. 16.2%, $p = 0.00002$), as seen in Figure 1.

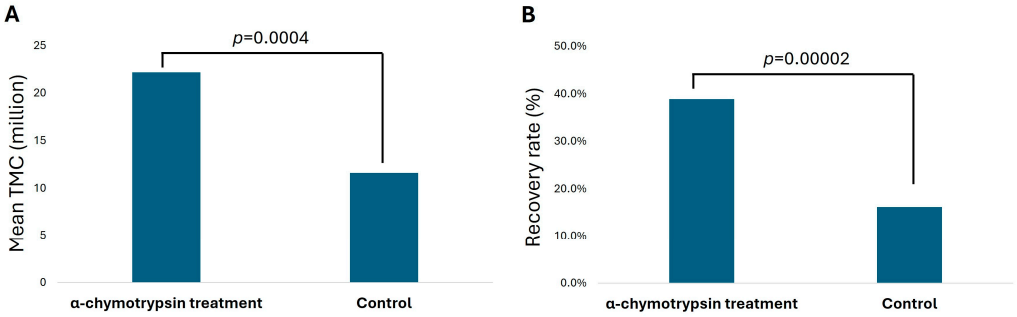

Figure 1. Comparison of total motile sperm count (**A**) and average recovery rate (**B**) of motile sperm between in α-chymotrypsin treatment and control group after sperm preparation.

3.2. TMC and Average Recovery Rate in Patients with Severely Low Sperm Count

In the sub-analysis of patients ($n = 8$) with severely low sperm count, which was defined as TMC of less than 10 million, there was a significant improvement in TMC (5.89 million vs. 1.21 million, $p = 0.004$) and the rate of recovery (43.0% vs. 10.0%, $p = 0.02$) in the α-chymotrypsin-treated group, as seen in Figure 2.

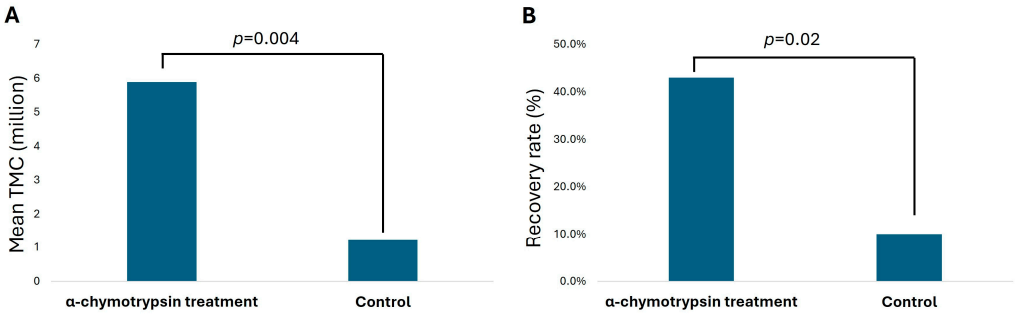

Figure 2. Comparison of total motile sperm count (**A**) and average recovery rate (**B**) of motile sperm between α-chymotrypsin treatment and the control group within patients with severely low sperm count after sperm preparation.

3.3. Fertilization and Blastocyst Rate in IVF Cycle of α-Chymotrypsin Treated Semen

To evaluate whether sperm function was affected by α-chymotrypsin treatment, outcomes from nine IVF cycles were analyzed (mean patient age: 34.9 years). The fertilization rate with α-chymotrypsin-treated semen was 87.8% (101/115), with a usable blastocyst rate of 56.4% (57/101), comparable to the current average IVF success rates. The average fertilization rate and blastocyst rate in the control group (no α-chymotrypsin treatment) were 85.5% (124/145) and 53.2% (66/124) in same female age group. This was not significantly different (p = 0.715 for fertilization rate; p = 0.6869 for blastocyst rate), as seen in Figure 3.

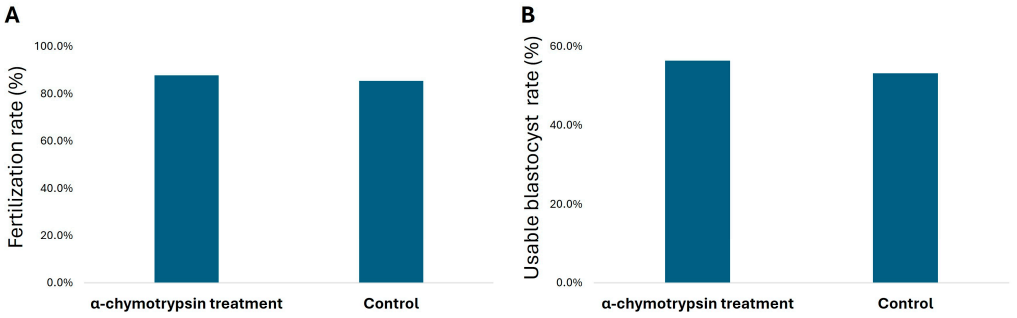

Figure 3. Fertilization rate (**A**) and usable blastocyst rate (**B**) in IVF cycle of α-chymotrypsin-treated sperm.

4. Discussion

Sperm motility is essential for fertility and successful conception. Semen viscosity impacts sperm motility, potentially hindering its ability to penetrate the cervix. This can ultimately decrease the chances of fertilization and conception. High viscosity in semen can also reduce the recovery of motile sperm during sperm preparation for IUI or IVF. We have shown significantly lower pregnancy rates in IUI cycles with <5 million of TMC at insemination [8]. Other studies also suggest that pregnancy rates per IUI cycle were 2.3% when TMC is below 10 million, compared to 8.4% for counts between 10 and 30 million [14]. Others proposed a TMC threshold of 1 million as a predictor of IUI success, with higher counts correlating with improved outcomes [15]. In IUI cycles with suboptimal sperm count in viscous semen, a lower TMC is expected after sperm preparation, likely leading to poor pregnancy rates due to insufficient TMC. To enhance motile sperm recovery in such cases, semen viscosity should be minimized prior to sperm preparation.

In this study, we assessed the effectiveness of α-chymotrypsin treatment in improving sperm recovery rate and TMC in highly viscous semen samples. Our findings demonstrate a significant enhancement in both parameters compared to the control group, particularly in patients with severely low sperm counts. The treatment group showed a marked increase in sperm recovery rate (38.9% vs. 16.2%, p = 0.00002) and TMC (22.2 million vs.

11.6 million, $p = 0.0004$), indicating that enzymatic breakdown of semen viscosity facilitates the retrieval of motile sperm. This trend was consistent in the subset of patients with a TMC of less than 10 million, where both recovery rate (43.0% vs. 10.0%, $p = 0.02$) and TMC (5.89 million vs. 1.21 million, $p = 0.004$) were significantly improved. These findings suggest that α-chymotrypsin is particularly beneficial for men with severe oligospermia, where other traditional sperm processing methods may be insufficient for IUI.

Recent studies on highly viscous semen samples treated with mild α-chymotrypsin found no significant difference in fertilization rates compared to untreated samples. Additionally, pregnancy rates remained unchanged, indicating that α-chymotrypsin can effectively reduce semen viscosity without adversely affecting ART outcomes [12]. Our findings also confirm that α-chymotrypsin treatment does not compromise sperm function, as evidenced by favorable in vitro fertilization (IVF) outcomes. The fertilization rate (87.8%) and usable blastocyst formation rate (56.4%) were consistent with reported IVF success rates (Figure 3).

While our study highlights a promising approach to optimizing sperm recovery in highly viscous semen, some limitations should be acknowledged. The sample size remains relatively small, and further studies with larger cohorts are warranted to confirm these findings. Additionally, while no adverse effects on fertilization or embryo development were observed, long-term outcomes of offspring conceived with α-chymotrypsin-treated sperm remain unexplored.

Overall, our results suggest that α-chymotrypsin treatment significantly improves sperm recovery and TMC in highly viscous semen without compromising ART outcomes. This enzymatic approach may provide a valuable alternative for managing oligozoospermia with severe semen viscosity, potentially improving fertility treatment outcomes. Future randomized controlled trials with larger sample sizes are needed to optimize α-chymotrypsin treatment protocols and evaluate its wider clinical applications.

Author Contributions: Conceptualization, S.T.K.; methodology, S.T.K.; validation, S.T.K.; formal analysis, A.A. and S.T.K.; investigation, K.P.S. and S.T.K.; resources, S.T.K.; data curation, A.A., M.W., R.C. and S.T.K.; writing—original draft preparation, M.K., A.A. and K.P.S.; writing—review and editing, A.A., K.P.S., M.W., R.C., R.F., K.C., J.F., R.W. and S.T.K.; visualization, M.K., A.A. and S.T.K.; supervision, S.T.K.; project administration, S.T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of University Hospitals (IRB#: STUDY20201371) in November 2024.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data supporting reported results can be requested via email to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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