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IMPACTS OF COMMON SEMEN HANDLING METHODS ON SPERM FUNCTION

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Abstract

Introduction: To become fertilization competent, sperm must undergo a maturational process known as capacitation. This process involves plasma membrane changes that occur in response to stimuli within the female tract. Methods currently used in semen analysis, and/or sperm preparation for ART, could affect sperm membranes. Here, capacitation was evaluated using localization of the ganglioside G_{M1} (Cap-Score™). In particular, we compared methods of liquefaction and washing used to reduce viscosity and determined their effects on capacitation.

Methods: Three common methods to reduce viscosity were evaluated. Ejaculates were: 1) Incubated for 0.25, 1.25 or 2 hrs; 2) diluted 1:1 with Modified Human Tubal Fluid (mHTF; Irvine Scientific; Santa Anna, CA) and then passed through a wide orifice transfer pipette (WOTP) or a Pasteur pipette (PP); or 3) Enzymatically digested with chymotrypsin (chymo; 5mg/ejaculate). Early pilot studies had revealed that passage through a hypodermic needle negatively affected motility and membrane integrity and was not studied further. After liquefaction and treatment to reduce viscosity , samples were washed and incubated under capacitating (CAP) and non-capacitating (NC) conditions. Cap-Score values were obtained via fluorescence microscopy.

Results: Liquefaction time, dilution and pipetting did not alter Cap-Score. Control (incubation only), WOTP and PP treated samples had Cap-Scores of 41±4, 40±5, and 41±6 (n=5; CAP). Decreased response to capacitating stimuli was observed when samples were liquefied using chymo (*P*=0.03). Control samples had Cap-Scores of 40±6 (n=5; CAP) whereas samples enzymatically liquefied had Cap-Scores of 31±4 (n=5; CAP). Because chymo is a protease that can cleave membrane proteins and potentially change membrane curvature and surface lipid expression, we checked if the Cap-Score reduction resulted from an alteration in labeling. Samples not exposed to capacitation stimuli were compared and no difference in the percentage of labeled cells was observed. Control and enzymatically liquefied samples had Cap-Scores of 22±4 and 21±5 (n=5; NC). These data support the view that treating semen with chymo can inhibit the ability of sperm to respond to capacitation stimuli.

Conclusions: Liquefaction times of up to 2 hours and mechanical liquefaction using WOTP and/or PP did not influence capacitation. In contrast, the use of enzymes such as chymo reduced the ability of sperm to capacitate, as measured by Cap-Score. These results demonstrate the importance of knowing how semen processing methods impact sperm function.

Introduction

A variety of sample handling and liquefaction techniques are used in ART clinics. Semen analysis parameters such as concentration and motility have been used to determine whether these techniques affect seminal quality, but there has been less focus on sperm function. In this study, the Cap-Score was used to determine if capacitation is affected by liquefaction techniques.

Results

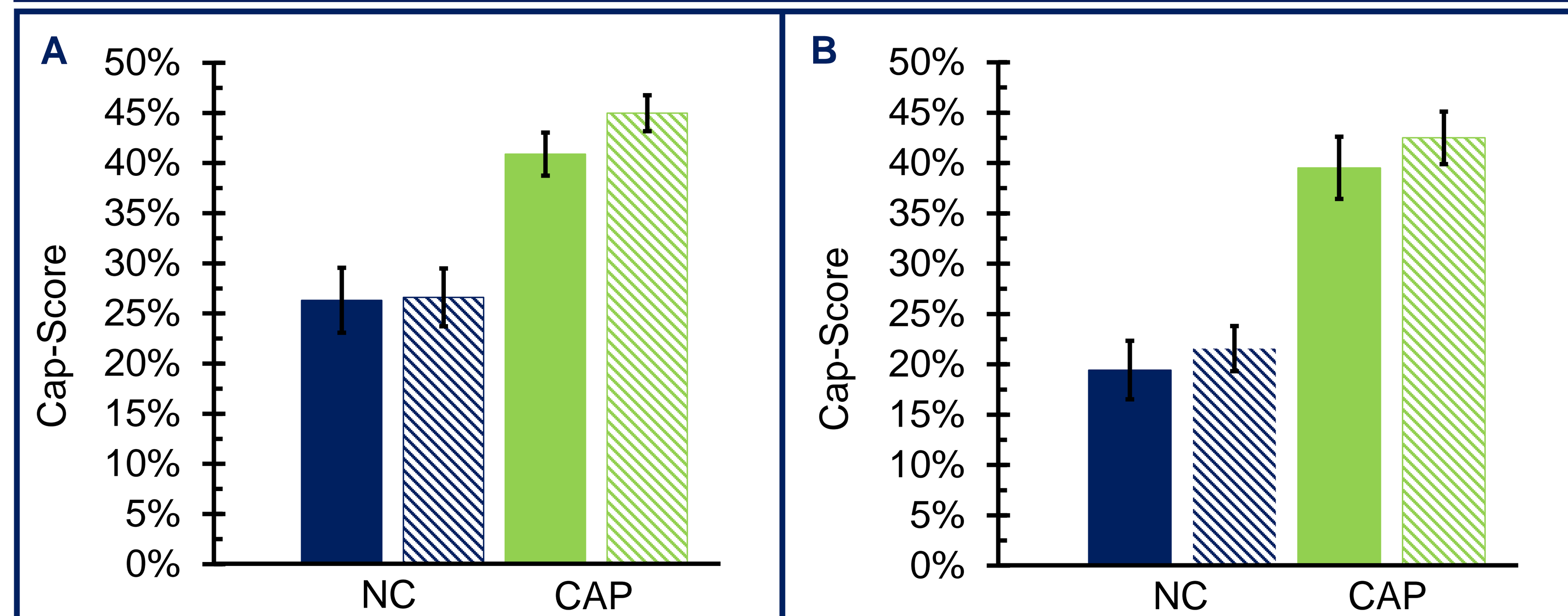

Figure 1: Cap-Scores are similar for 15 minute, 1.25 and 2 hour liquefaction. Nine donor ejaculates were split into two portions; one portion was liquefied for 15 minutes (solids bars) and the other portion was liquefied for either 1.25 (**Panel A**; dashed bars) or 2 hours (**Panel B**; dashed bars). After sperm washing, both fractions were incubated for 3 hours at 37°C under NC (blue bars) and CAP (green bars) conditions, and remained in a light fixative for 24 hours. The average Cap-Score and the standard error of the mean (SEM) is shown on the Y axis, and the capacitation treatment is shown on the X axis. The average Cap-Scores in panel A are 26%±3% (NC 15 min), 27%±3% (NC 1.25 hrs), 41%±2% (Cap 15 min) and 45%±2% (Cap 1.25 hrs). The average Cap-Scores in panel B are 19%±3% (NC 15 min), 22%±2% (NC 2 hrs), 40%±3% (Cap 15 min) and 43%±3% (Cap 2 hrs). There is no difference in Cap-Score between the liquefaction times for either capacitating treatment.

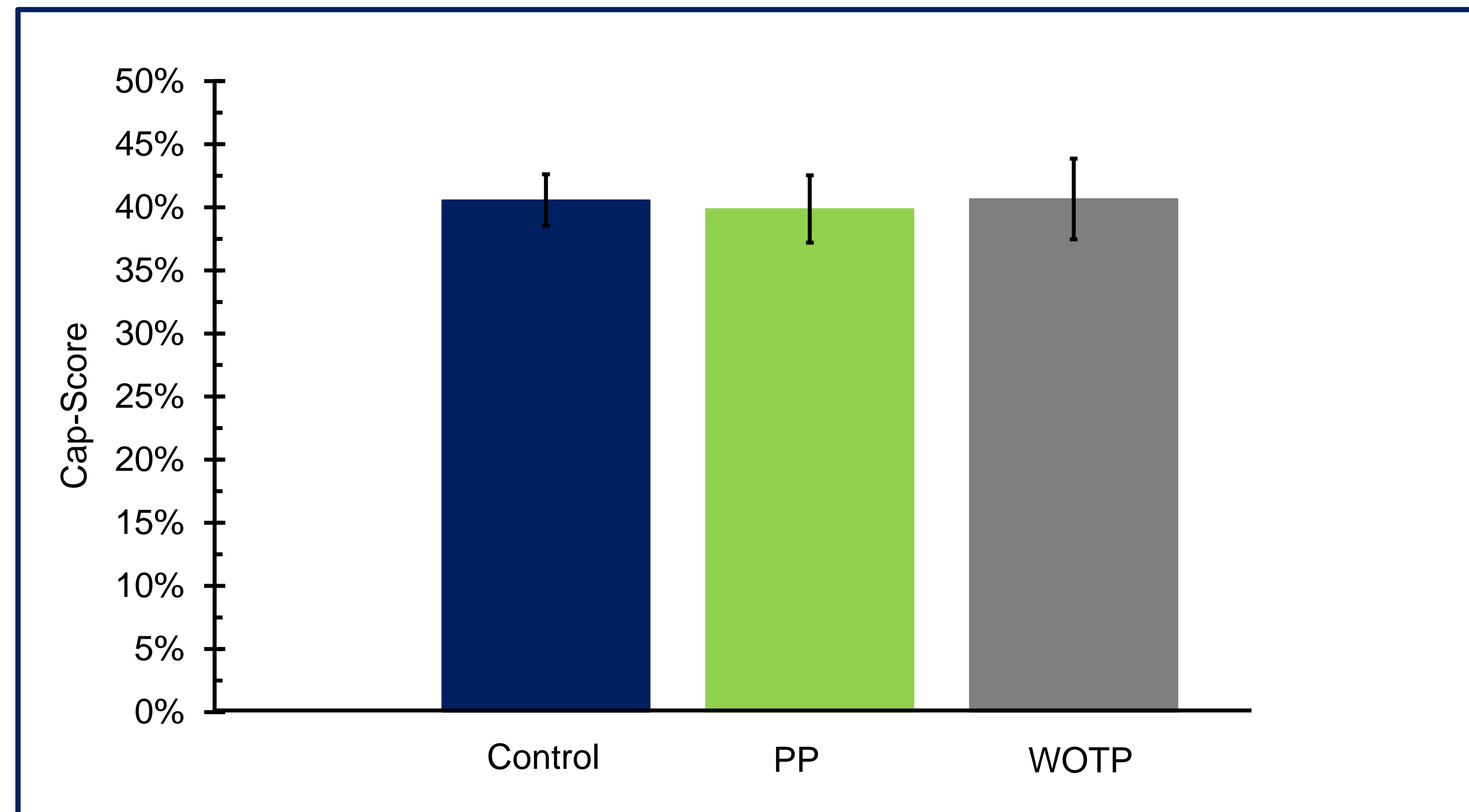

Figure 2: Pasteur pipettes and wide orifice transfer pipettes do not affect capacitation when used for mechanical liquefaction. Ejaculates of five individuals were split into three portions: Mechanical liquefaction was not done (Control; blue bar); 1:1 dilution with mHTF medium and mechanically liquefied with Pasteur pipettes (PP; green bar); and 1:1 dilution with mHTF medium and mechanically liquefied with wide orifice transfer pipettes (WOTP; gray bar). All three portions were incubated for 3 hours at 37°C under CAP conditions and remained in a light fixative for 24 hours. The average Cap-Score and SEM is located on the Y axis and the liquefaction treatments are located on the X axis. There is no difference in the average Cap-Scores of 41%±2% (control), 40%±2% (PP) and 41%±3% (WOTP).

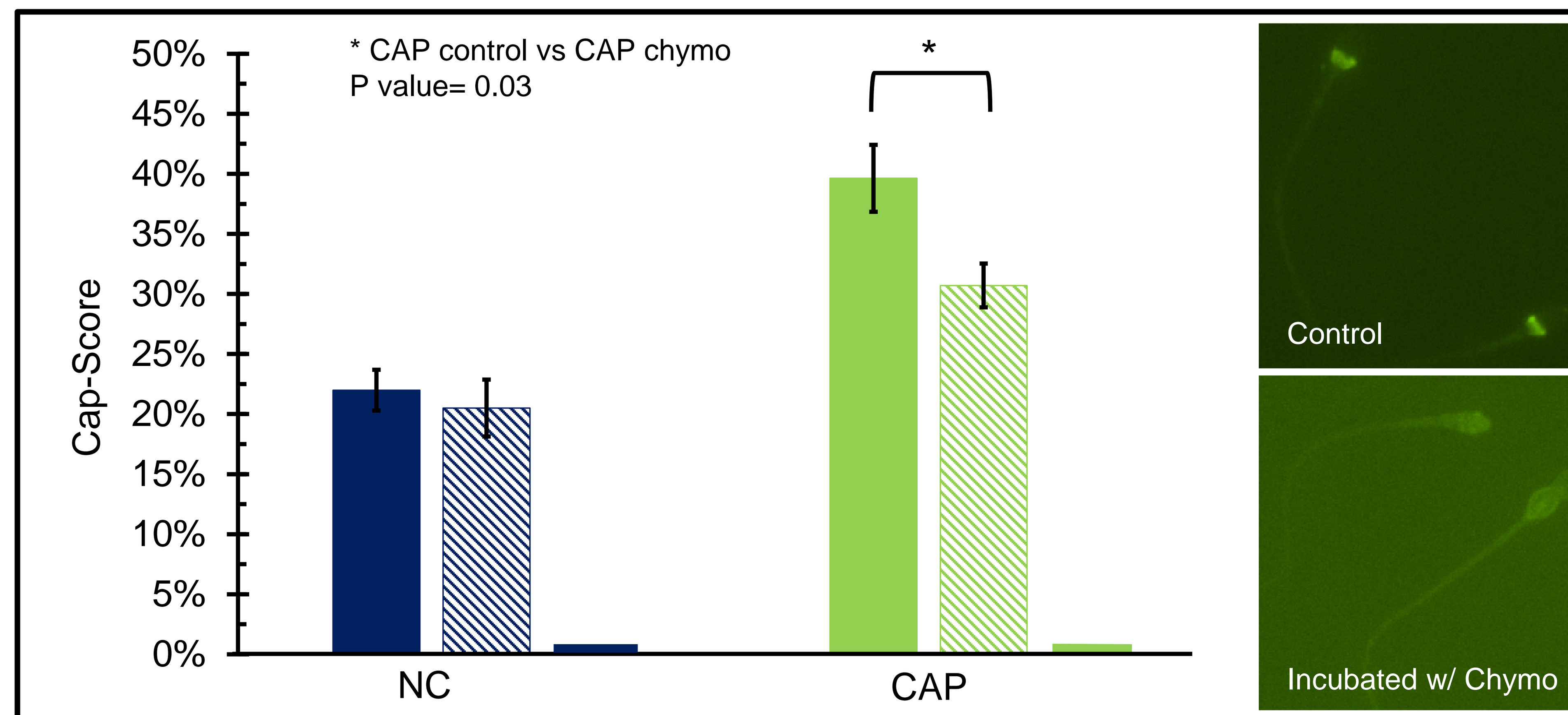

Figure 3: The use of chymotrypsin for liquefaction negatively affects capacitation. Five ejaculates were split into three portions. The first portion was liquefied for 15 minutes (Control; solid bars); the second portion was liquefied with chymo for 15 minutes (5mg/ejaculate; Dashed bars); and the third portion was incubated with chymo (3mg/ml; unscorable). All three portions were incubated for 3 hours at 37°C in either NC or CAP conditions, and remained in a light fixative for 24 hours. The average Cap-Score and SEM is shown on the Y axis, and the incubation conditions are shown on the X axis. There was a significant drop in Cap-Score for CAP samples liquefied with chymo (31%±2%) vs. CAP control samples (40%±3%; P value= 0.03). There was no difference in Cap-Score for the NC treatments. NC and CAP samples incubated with chymo could not be scored due to high levels of membrane damage in the sperm cells, as shown in the image titled "Incubated w/ Chymo" and compared to the image titled "Control."

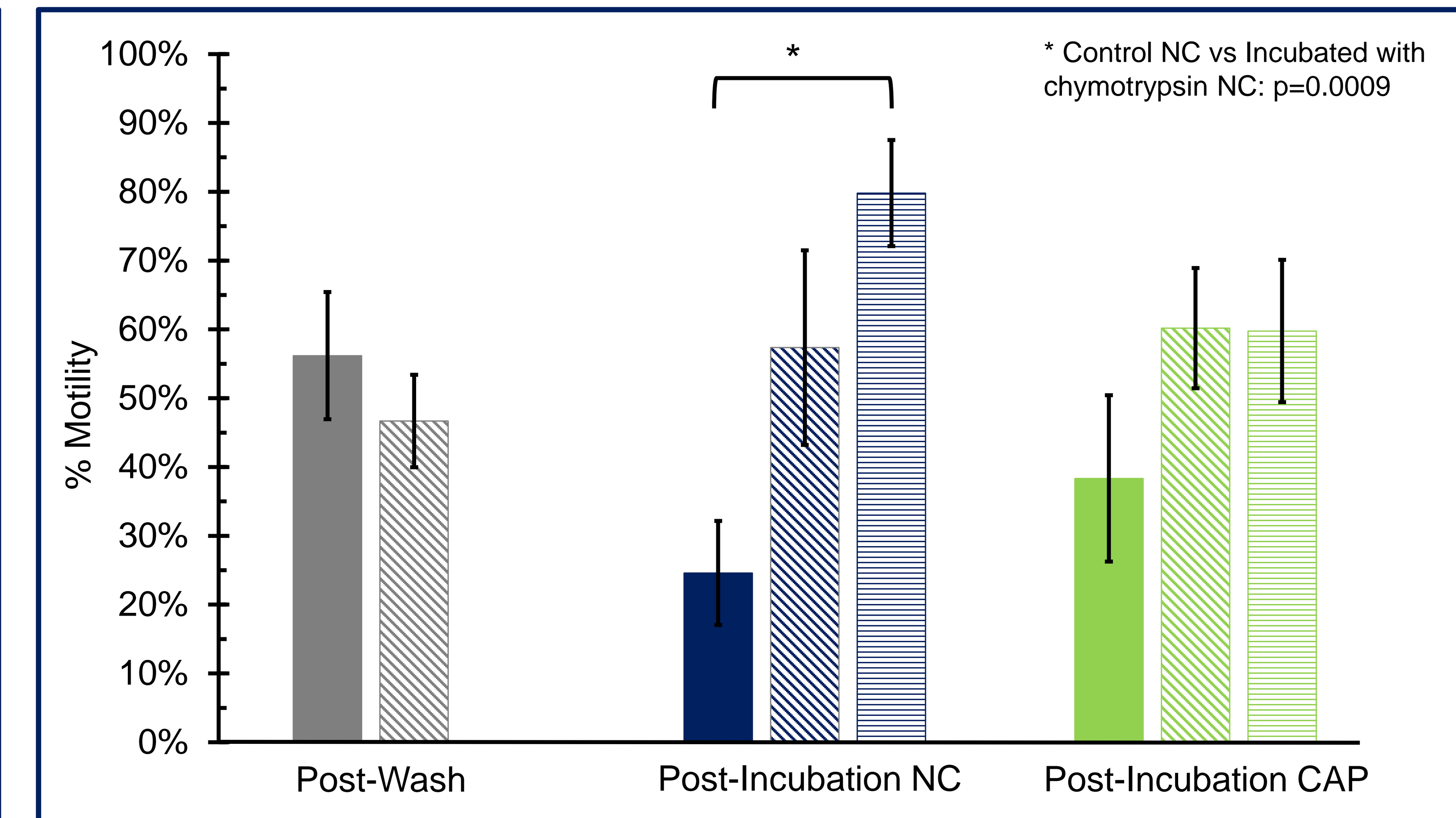

Figure 4: Samples incubated with chymotrypsin have a higher % motility in the NC samples. The data from Figure 3 were analyzed further and post-wash and post-incubation NC and CAP motilities were obtained for samples liquefied for 15 minutes (control; solid bars), samples liquefied with chymo for 15 minutes (dashed bars), and samples incubated with chymo for 3 hours (horizontal lined bars). The Y axis shows the average motility percentages and the SEM, and the X axis shows the incubation conditions. When comparing the average motilities of the control treatments to the chymo treatments in each category, there are no significant differences for the post-wash (gray bars) or post-incubation CAP treatments (green bars). There is a significant increase in motility for samples incubated with chymo compared to the control (Control: 25%±8%, incubated with chymo: 80%±8%) in the post-incubation NC treatment (blue bars; p = 0.0009).

Conclusions

- Liquefaction with chymotrypsin affects capacitation and possibly damages cell membranes.
- Common measures of semen quality do not reflect function.
- Liquefaction up to 2 hours does not affect capacitation.
- Mechanical liquefaction with Pasteur pipettes and wide orifice transfer pipettes does not affect capacitation.

Future Directions

- The Cap-Score can be used to detect changes in capacitation caused by reagents, handling techniques, procedures, or equipment commonly used in ART laboratories.

Funding

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