Impacts of TEST (TES and Tris) yolk buffer and cooling on the ability of human sperm to capacitate

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Abstract

OBJECTIVE: Studies across several mammalian species show that G_{M1} localization patterns are indicative of capacitation at the single cell level. The Cap-Score™ Male Fertility Assay reports the proportion of sperm displaying G_{M1} localization patterns consistent with capacitation. Using clinical pregnancy outcomes, Cap-Score was previously shown to prospectively predict a man's fertility, and the relationship between Cap-Score and a man's probability of generating a pregnancy was established. TEST (TES and Tris) yolk buffer (TYB) can prolong the fertilization capacity of sperm. Here, we evaluated whether incubation in TYB overnight at a cool temperature affected human sperm capacitation.

DESIGN: To evaluate the impact of semen extension with TYB and cooling on sperm capacitation, ejaculates were split into control and test samples for a repeated measure design.

METHODS: Studies approved by WIRB (20152233). Semen was collected, liquefied and split into control and test samples. Control samples were processed normally for Cap-Score. Test samples were extended in TYB at 1:1 (n=5), 1:6 (n=7) or 8:5 (n=5; volume ratio of semen:TYB) and cooled overnight in a Styrofoam box with an ice pack. The next day, samples were washed, exposed to non-capacitating (Non-CAP) or capacitating (CAP) conditions for 3 hrs, and then fixed overnight, before Cap-Score determination. Test-samples were compared to controls using paired t-tests.

RESULTS: In all experiments, Cap-Score was greater for control-CAP when compared to control-Non-CAP ($p<0.05$). No differences were observed between the control-CAP and the test-CAP for any dilution (1:1 ratio: 39.7± 0.04 vs 40.0±0.02%; $p=0.87$; 1:6 ratio: 32.0±0.04 vs 34.0±0.03%; $p=0.33$; 8:5 ratio: 36.0±0.02 vs 34.2±0.01%; $p=0.5$).

CONCLUSIONS: A good capacitation response was observed in the controls for all experiments, suggesting proper stimulus by the CAP condition. The ratios of semen:TYB were chosen to mimic typical ejaculate volumes, such that a constant volume of extender could potentially be utilized in an at home semen collection kit that maintains sperm capacitation ability. Addition of a fixed volume of TYB to varying ejaculate volumes would limit the need for user calculation and measurement. Similar Cap-Score values between the control-CAP and test-CAP, no matter the ratio, indicates that ejaculates can be maintained overnight in varying concentrations of TYB with minimal impact on next-day function. At home sample collection could lessen the burden of processing samples at clinics with limited personnel resources. It could also encourage pursuit of workup by men whose main barrier is privacy in producing samples at clinics or bringing them to clinics. It could also broaden the geographical availability of sperm function tests to those living far from clinics, and reduce financial burdens associated with travel and time away from work.

Methods

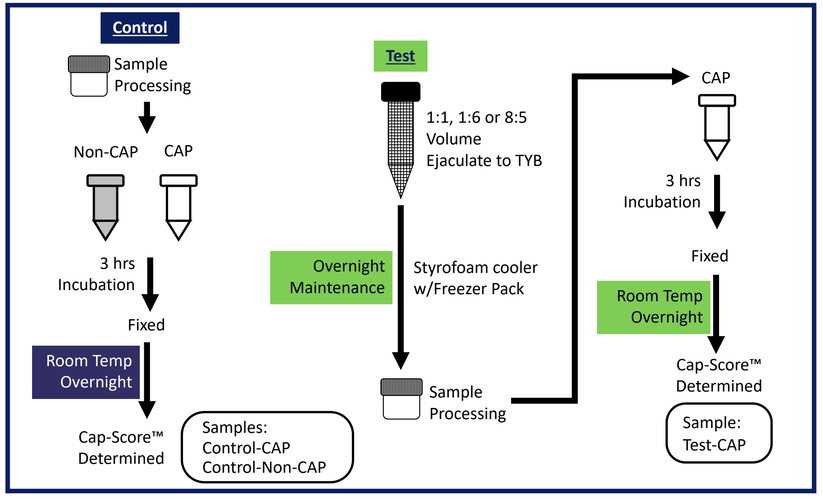
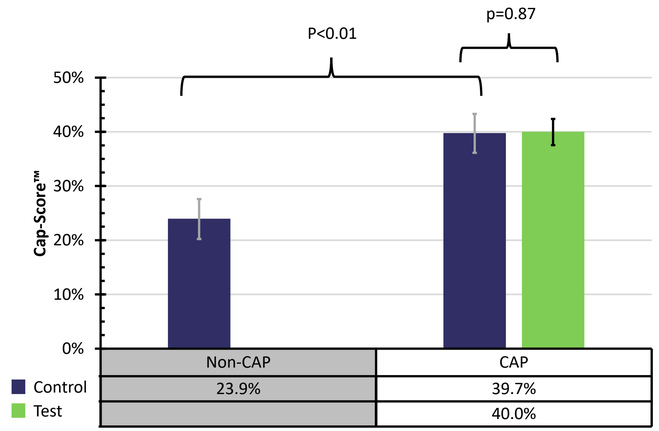


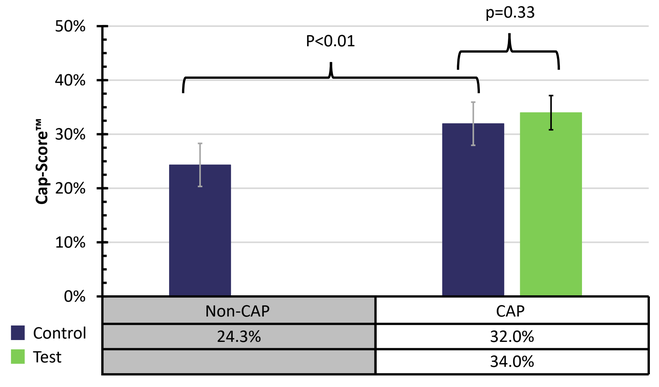
Figure 1. Experimental design. Semen samples were collected and split. Half served as a control (Control) and the other half served as the test (Test). The Control samples were processed as normal for Cap-Score. Briefly, they were incubated under capacitating (CAP) and non-capacitating (Non-CAP) conditions, fixed and then maintained overnight before being evaluated for Cap-Score. The Test samples were diluted 1:1, 1:6 or 8:5 with TEST (TES and Tris) yolk buffer (TYB) and maintained overnight in a cooler with a freezer pack. Following overnight maintenance in the TYB, the test samples were processed. The samples were incubated, fixed, maintained for a second overnight and then Cap-Score was determined the following day.

Results

A Samples Diluted 1:1 w/ TYB and chilled w/ Freezer pack (n=5)



B Samples Diluted 1:6 w/ TYB and chilled w/ Freezer pack (n=7)



C Samples Diluted 8:5 w/ TYB and chilled w/ Freezer pack (n=5)

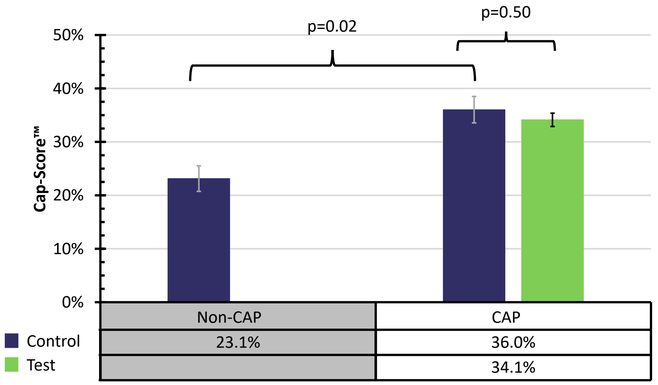


Figure 2. Impacts of different ratios of semen sample:TYB on Cap-Score. Samples were split in two. The first half was processed as usual for Cap-Score and served as a control. The second half was used to test 1:1, 1:6, and 8:5 (semen:TYB) dilutions. These dilutions were chosen to mimic typical ejaculate volumes, such that a constant volume of extender could potentially be utilized in an at home semen collection kit that maintains sperm capacitation ability. Test samples were diluted and then packed into a Styrofoam box with an freezer pack. The following day, samples were processed for Cap-Score (See **Fig. 1**). Since ejaculates were split, paired t-tests were used to compare the control to the test samples. Control Non-CAP samples were lower than the control CAP samples in the 1:1 (panel **A**; $p<0.01$), 1:6 (panel **B**; $p<0.01$) and 8:5 (panel **C**; $p=0.02$) experiments. No differences in Cap-Score were observed between the control and test samples in the 1:1 (panel **A**; $p=0.87$), 1:6 (panel **B**; $p=0.333$) or 8:5 (panel **c**; $p=0.50$) experiments.

Conclusions

- Overnight maintenance of ejaculates in varying concentrations of TYB has minimal impact on capacitation ability.
- These results support the development of an at home collection kit for the Cap-Score Male Fertility Assay.
- At home collection could:
  - Lessen the burden of processing samples at clinics with limited resources.
  - Encourage workups by men whose main barrier is privacy in sample production.
  - Broaden the geographical availability of sperm function tests.

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