CAPACITATION TIMING VARIES AMONG MEN, BUT IS CONSISTENT AMONG EJACULATES WITHIN INDIVIDUALS

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Abstract

Objective: If inseminated too late, sperm may not obtain fertilizing ability before egg quality declines, preventing pregnancy. For fertilization to succeed, sperm must first capacitate, a process dependent upon sterol efflux and altered dynamics of the ganglioside G_{M1}. Cap-Score™ reflects the percent of capacitation competent cells as determined by G_{M1} distribution patterns and was used to assess capacitation timing.
Design: The percentage of capacitated sperm was compared among and within fertile men, controlling for presence of capacitation stimuli and timing of incubation and fixation.
Materials and Methods: Semen was collected from fertile men (pregnant partner or recent father), as approved by WIRB (20152233). Samples were liquefied, washed, and aliquots incubated under non-capacitating (NC) and capacitating (CAP) conditions for 3hrs. The sperm were fixed and analyzed immediately (Day 0) or after overnight incubation in fix (Day 1). In another trial, NC and CAP samples were incubated for 3 hrs and 24 hrs prior to fixation. Cap-Score was compared using T-Tests (Microsoft Excel 2013).
Results: An increase in Cap-Score was observed in CAP samples from Day 0 to Day 1 (p=0.0001; n=53). To investigate if this was physiological, 25 samples were evaluated in a second trial. Cap-Score was greater for Day 1 CAP than Day 0 CAP (p=0.00007), but was the same for samples incubated overnight in fix or media (p=0.44). These data are consistent with prior work, demonstrating that physiologic membrane lipid changes associated with capacitation can occur in certain fixatives. Variation in capacitation timing among ejaculates was evaluated by scoring 124 samples from 53 men on Day 0 and Day 1. An increase in Cap-Score was observed in 82% (102/124) of CAP samples, with 44% (54/124) increasing more than 1 SD (7.7; defined using data from fertile men). These data support capacitation timing differences among ejaculates. The reproducibility of capacitation timing was assessed by classifying 52 ejaculates from 11 men as either early or late capacitators (Day 1 - Day 0>7.7). The average concordance was 81%, supporting the view that capacitation timing is consistent within men.
Conclusions: Capacitation timing differs among men, but is consistent among ejaculates within individuals. This information could be used to optimize insemination timing in procedures such as IUI, IVF, and natural conception leading to personalized management of infertility.
Support: Androvia LifeSciences

Introduction

The timing of capacitation is an important factor that can affect various ART procedures. Here, differences in capacitation timing were reproducibly observed for multiple samples from individual men. With this information, it is possible to use capacitation timing as a way to personalize ART procedures such as IUI and IVF.

Results

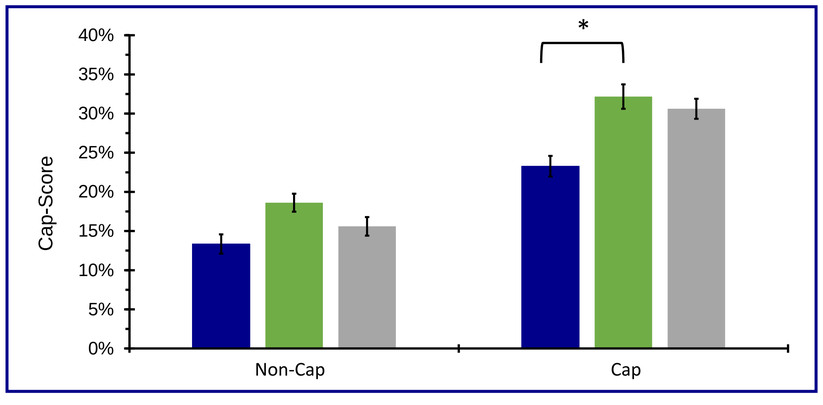

Figure 1: ***Changes in Cap-Score from Day 0 to Day 1 are due to membrane changes involved in capacitation.*** Samples from 25 fertile donors were analyzed on Day 0 (blue bars), after overnight incubation in a light fixative (Day 1; green bars) and after 24 hours of incubation in either non-capacitating (Non-Cap) or capacitating (CAP) media (24 hrs; gray bars). The Y axis shows the average Cap-Score and the X axis shows the different incubation/fixation treatments. No treatment differences were seen in the Non-Cap samples. There was a significant difference between Day 0 and Day 1 CAP samples (*p=0.00007). However, there was no difference between Day 1 CAP samples and CAP samples after 24 hrs of incubation in capacitating medium (p=0.44). This indicates that the Cap-Score change observed between Day 0 and Day 1 is physiological and not due to incubation in light fixative.

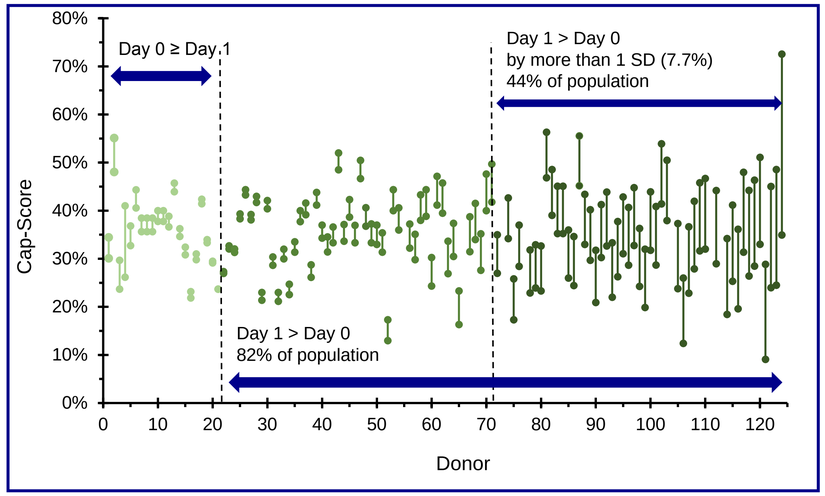

Figure 2: ***Change in Cap-Score from Day 0 to Day 1 revealed a difference in timing of capacitation among individuals.*** Capacitation was assessed for 124 samples at Day 0 and Day 1. The X axis shows the sample number and the Y axis shows the Cap-Score, with the dumbbell plot connecting the values for Day 0 to Day 1 per sample. Out of 124 samples from 53 fertile donors, 82% had an increase in Cap-Score from Day 0 to Day 1 (102/124). 44% (54/124) of the population had an increase in Cap-Score greater than 1 standard deviation (7.7%).

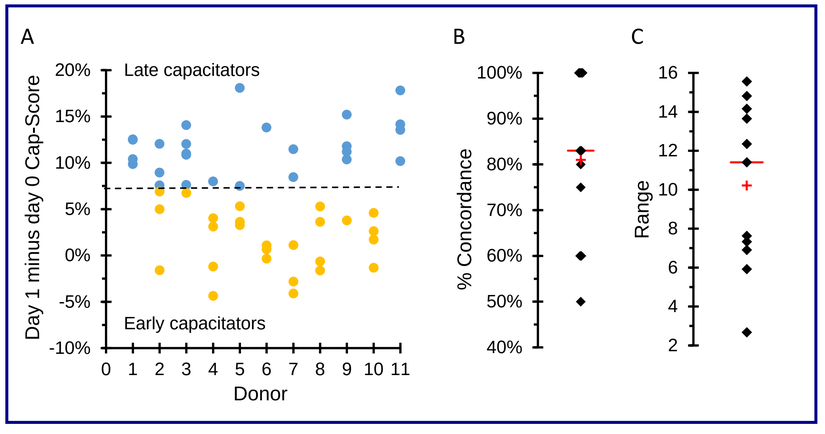

Figure 3: Capacitation timing was consistent within men. Cap-Scores were obtained for 52 samples from 11 fertile donors on Day 0 and Day 1 (at least 4 observations per donor). **A.** The Y axis shows the difference in Cap-Score (Day 1 – Day 0). The dotted line denotes a difference of 7.7% (1 SD) which we used as a cut-off to define early and late capacitators. The X Axis shows the 11 donors. Each observation was categorized as either early capacitation (orange) or late capacitation (blue). Percent concordance was calculated for each of the 11 donors, for example donor number 1 represents 100% late concordance (4/4 samples blue), donor number 6 represents 75% early concordance (3/4 samples orange). **B.** Scatter plot shows the % concordance distribution, with each donor being represented by a single black diamond. The average (81%; red plus sign) and the median (83%; red line) concordance, demonstrate that capacitation timing is highly consistent within individuals. **C.** Scatter plot shows the range of differences between Day 1 and Day 0 within donor, with the average (10.2) and median (11.4) range shown by a red plus sign and red line respectively. Five of the individuals had ranges less than 7.7 (1 SD), again substantiating consistency in capacitation timing.

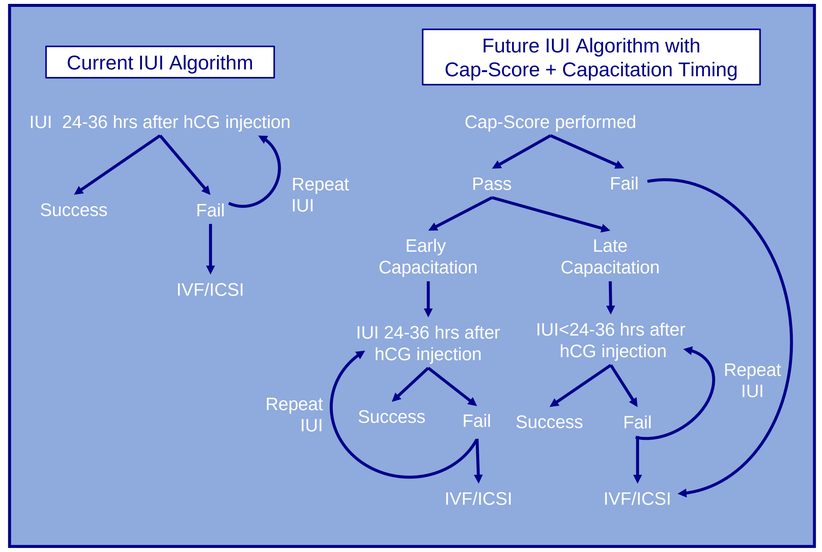

Figure 4: Personalizing IUI with capacitation timing. The use of the Cap-Score along with timing of capacitation could lead to personalized ART treatment for individuals. Typically, the time for insemination to be performed in IUIs is ~24-36 hours after hCG injection. However, If it takes sperm 24 hours to reach full capacitation (i.e. the male exhibits late capacitation), the standard approach might be less likely to succeed because by the time the sperm cells are fully capacitated, the egg may no longer be viable. Future diagnostic algorithms could evaluate both Cap-Score and capacitation timing; with this information, it would be possible to personalize not only the best fertility treatment, but also the timing of that treatment.

Conclusions

- There is a difference in timing of capacitation among men.
- Capacitation timing is highly consistent within individuals.
- When performed on Day 0 and Day 1, a comparative use of the Cap-Score can provide information about the timing of capacitation.
- Differences in capacitation timing might optimize fertilization in a variety of insemination procedures such as IUI, IVF, and natural conception.

Future Directions

- The impact of measures of capacitation timing on success of IUI, IVF, and natural conception need to be established.

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