# TIMING OF SPERM CAPACITATION VARIES AMONG MEN AND IS CONSISTENT WITHIN MEN

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## Abstract

**Introduction**: If inseminated too late, sperm may not complete capacitation before egg quality declines, preventing fertilization. Cap–Score™ reflects the percent of sperm that can capacitate, as determined by $G_{M1}$ distribution patterns. Here we compare differences in capacitation at two time points within semen samples, both within and among individuals. We used ability to undergo acrosome exocytosis (AE) to confirm capacitation status.

**Methods**: Semen from fertile men was liquefied, washed, and incubated for 3 hrs under capacitating (Cap) conditions, then fixed and analyzed immediately (Day0); after being incubated 3 hrs under Cap conditions then maintained 22–24 hrs in fix (Day1–fix); or after 22–24 hrs incubation under Cap conditions prior to fixation (Day1). In all cases, a light fixative previously shown to allow membrane lipid movements was used. Two subsets of the Day1 and Day0 samples (n=10, and n=10-11) were treated with calcium ionophore A23187 or progesterone and compared to samples incubated under Cap conditions without ionophore or progesterone, to confirm capacitation status with two different stimuli for AE. We did not assess AE in Day1–fix cells because of the presence of the fixative and its potential effect on proteins involved in membrane fusion.

**Results**: Day1–fix and Day1 Cap–Scores were greater than Day0 ($p<0.001$; n=25), whereas Day1–fix and Day1 Cap–Scores were equivalent ($p=0.43$; n=25). When 123 samples from 52 men were analyzed, 94% (49/52) of the individuals showed an increase from Day0 to Day1–fix. An increase of more than 1SD (7.7; calculated previously from a fertile cohort) was observed in 42% (22/52) of the men. To test whether the difference from Day0 to Day1–fix was consistent within an individual, 52 samples from 11 fertile men (≥4 samples per donor) were classified into either "early" (difference < 1SD) or "late"(difference ≥ 1 SD) groups. The average capacitation group concordance within a donor was 81%. Median absolute deviation (MAD) was used to assess the tightness of clustering within individuals. The average (2.21) and median (1.98) MAD confirmed consistency within individuals. As expected, significant decreases in Cap–Score were observed following treatment with either ionophore or progesterone in both Day0 and Day1 samples.

**Conclusion**: These data show that the timing of capacitation differed among men and was consistent within them. Knowing the response time of an individual's sperm to capacitation stimuli could be used to personalize and optimize insemination time in different ART procedures.

## Introduction

Sperm must mature functionally in the process of capacitation to become able to fertilize. Capacitation depends on membrane lipid changes, and can be assessed by redistribution of the ganglioside $G_{M1}$, the basis of the Cap-Score™ sperm function test. Here we set out to use Cap-Score™ to assess whether there were differences in the timing of capacitation among and within men.

## Results

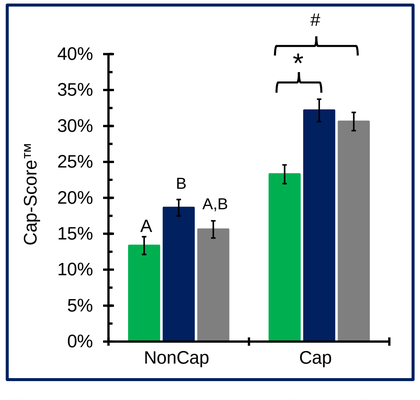


**Figure 1.** ***Changes in Cap-Score™ from Day0 to Day1 reflect membrane changes that are associated with capacitation.*** Samples from 25 fertile donors were incubated under non-capacitating (NonCap) and capacitating (Cap) conditions and then analyzed for Cap-Score after 3 hrs of incubation (Day0; green bars), after overnight incubation in a light fixative (Day1-fix; blue bars) and after overnight incubation in physiologic media followed by fixation (Day1; gray bars). The y-axis shows the average Cap-Score ± the standard error and the x-axis shows the different incubation treatments. Differences among NonCap samples are illustrated by different letters ($p<0.05$) and show an increase in spontaneous capacitation like events from Day0 to Day1-fix, which was not different than Day1. Samples were compared within a treatment using Fisher's LSD. There was a significant difference between Day0 and Day1-fix Cap samples (*$p<0.001$) and between Day0 and Day1 (#$p<0.001$). However, there was no difference between Day1-fix and Day1 Cap samples ($p=0.43$). These observations confirmed that the changes in membrane lipids detected by the Cap-Score did occur in the presence of the light fixative, as described previously in murine sperm (Selvaraj et al. 2006), and showed that those changes occurred in the same percentage of cells as if they had just been incubated in the Cap medium.

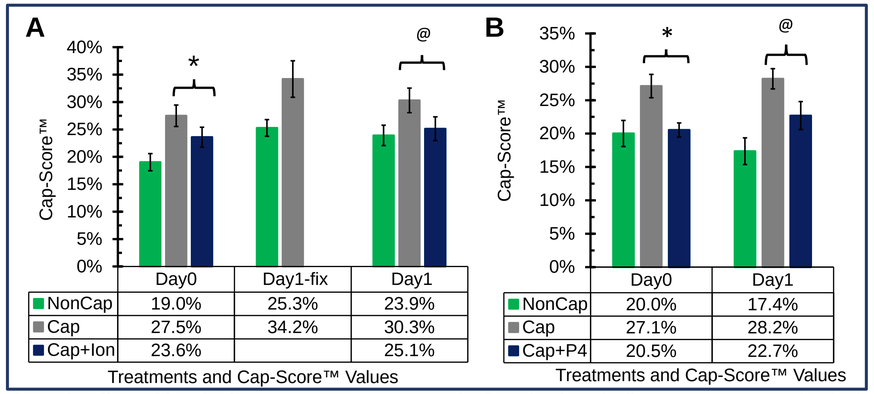


**Figure 2.** ***Effect of incubation on acrosome exocytosis.*** Samples from fertile men were exposed to both non-capacitating (NonCap; green Bars) and capacitating (Cap; Grey Bars) media and then analyzed for Cap-Score after 3 hrs incubation and fixation (Day0), after 3hrs incubation and then maintenance overnight in a light fixative (Day1-fix) and after overnight incubation followed by fixation (Day1). A third treatment was done for the Day0 and Day1 samples and consisted of incubation for the designated time under Cap conditions, followed by the addition of the calcium ionophore, A23187 (**A**; n=10; Cap+Ion, blue bars), or progesterone (**B;** n=11; Cap+P4, blue bars). The y-axis shows the average Cap-Score and standard error, while the x-axis shows the different incubation treatments. Consistent with expectations from our prior experiments, we observed significant decreases in Cap-Score following treatment with A23187 versus sperm incubated under Cap conditions without ionophore in both Day0 (*$p=0.03$) and Day1 (@$p=0.01$) samples. Similar results were obtained with P4 (Day0 Cap vs Day0 Cap+P4, *$p<0.01$; Day1 Cap vs Day1 Cap+P4, @$p=0.02$).

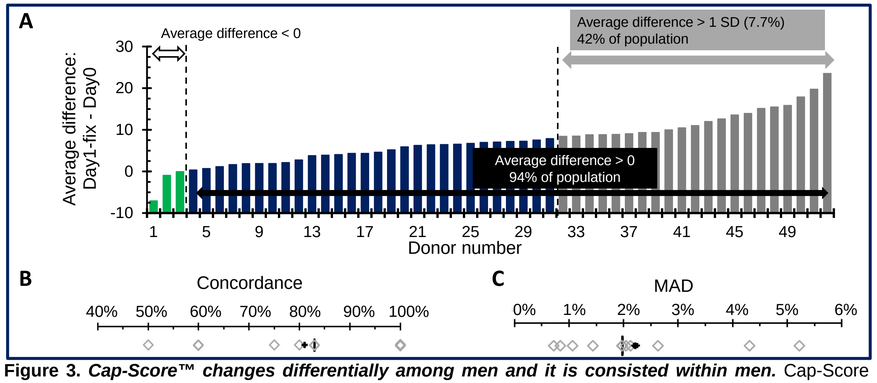


**Figure 3.** ***Cap-Score™ changes differentially among men and it is consisted within men.*** Cap-Score was determined for both Day0 and Day1-fix treatments in 123 samples from 52 fertile donors. **A.** The x-axis shows the donor number and the y-axis show the average Cap-Rate™ of each donor (defined as Day1-fix minus Day 0). Out of 52 donors, 94% had an increase in Cap-Score from Day0 to Day1-fix (49/52). 42% (22/53) of the population had an average Cap-Score increase greater than 1SD (7.7%) defined previously in a population of men with proven fertility (Cardona et al., 2017). These data supported the existence of substantial changes in capacitation over time that differed among men. **B.** The scatter plot shows the distribution of concordance among donors, with each donor being represented by a single diamond. The average (81%) and median (83%) are shown by a black plus sign and line respectively. **C.** The median absolute deviation for Cap-Rate was calculated within a donor (MAD=median ($|x_i$-median(x)|); where $x_i$ is the observed difference for a given collection). The scatter plot shows the distribution of MAD among donors with each donor being represented by a single diamond. The average (2.21) and median (1.98) MAD are shown by a black plus sign and line respectively. These data show consistency of Cap-Rate within men.

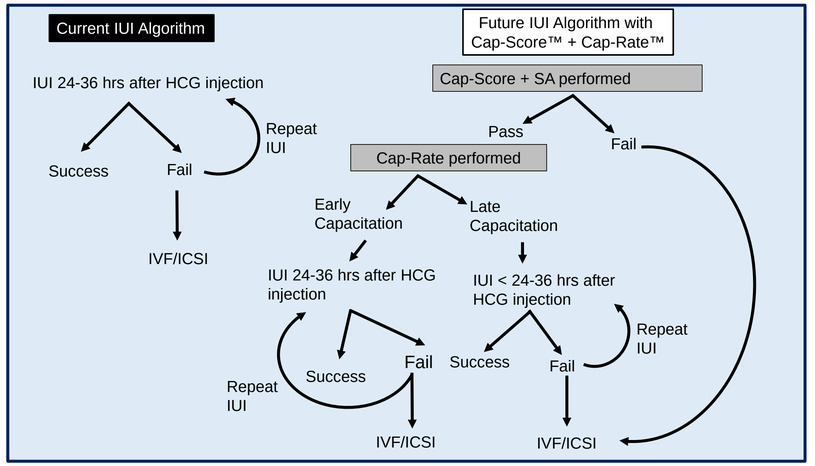


**Figure 4:** ***Current and future algorithms of the IUI process.*** The use of the Cap-Score™ along with timing of capacitation could lead to personalized ART treatment for individuals. Typically, the time for insemination to be performed in IUIs is ~24-36 hours after HCG injection. However, If it 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

- The Cap-Score™ reflects the percentage of sperm with $G_{M1}$ localization patterns associated with a response to capacitating stimuli. Using both A23187 and progesterone as triggers, our findings show that these cells are indeed capacitated and capable of undergoing stimulated acrosome exocytosis.
- Timing of capacitation varies among men.
- Timing of capacitation is highly consistent within men.
- Knowledge of the timing of capacitation, such as obtained by Cap-Rate™, might enable clinicians to personalize infertility treatments for couples by varying the timing of insemination.

Funding