CONSISTENT DIFFERENCES AMONG MEN IN CAPACITATION TIMING COULD PERSONALIZE IUI/ART

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Abstract

Introduction: To become fertilization competent, sperm must first capacitate. This process depends on changes in membrane lipids, including removal of sterols and redistribution of the ganglioside G_{M1}. Here, we examine the timing of capacitation among and within different individuals. Reliable differences in capacitation timing could be used to personalize IUI/ART protocols.

Methods: Semen samples from consenting men were liquefied, washed, and aliquots incubated under non-capacitating (NC) or capacitating (CAP) conditions. Samples from fertile men (pregnant partner or recent father) and samples from men seeking fertility exams were incubated for 3hrs, fixed and analyzed immediately (Day0) or after overnight incubation in fix (Day1). In another trial, NC and CAP samples were incubated for 3 hrs or 24 hrs prior to fixation. Capacitation was assessed using localization of G_{M1} (Cap-Score™).

Results: A change in Cap-Score between Day0 to Day1 was observed for multiple samples. To determine whether this change was physiological or an artifact of being in fix overnight, semen samples from 19 fertile men were analyzed at Day0, Day1 and after 24hrs of incubation in medium and then fixed. Day1 CAP was significantly greater than Day0 CAP (p=0.013), but Cap-Scores for samples incubated overnight in fix or in capacitating media were the same (p=0.967). Consistent with prior literature, these data show that membrane changes involved in capacitation still occur in certain fixatives. 117 samples from 61 fertile men were evaluated at Day0 and Day1. An increase in Cap-Score was observed in 82% (96/117) of CAP samples, with 42% (49/117) increasing more than 1 SD (7.8%). In 19 men seeking fertility treatment, 32% (6/19) increased more than 7.8%. These data suggest that sperm achieve capacitation at different times in different ejaculates. To see if this was reproducible for an individual, 52 samples from 11 fertile men were classified as either early or late capacitators (Day1-Day0>7.8). The average concordance of change within donors was 84%, showing that capacitation timing was highly consistent within men.

Conclusions: These data show that capacitation timing differed consistently among men. Determining capacitation rates could eventually lead to personalized management of infertility, including optimal times for IUI relative to ovulation, and capacitation times prior to co-incubation of sperm and oocytes for IVF. Funded by 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

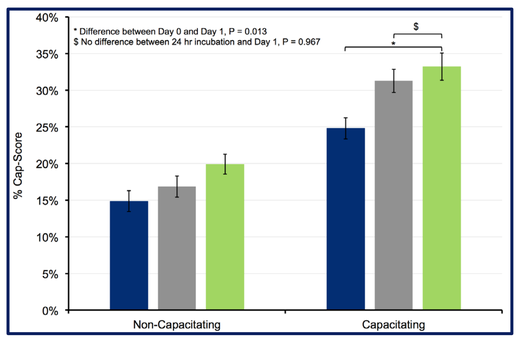


Figure 1: Changes in Cap-Score from Day0 to Day1 reflect membrane changes associated with capacitation. Samples from 19 fertile donors were analyzed on Day0 (blue bars), after 24 hours of incubation in non-capacitating or capacitating media (24 hrs; gray bars), and after overnight incubation in a light fixative (Day1; green bars). The Y axis represents the average Cap-Score and the X axis represents the capacitation treatment (NC and CAP). There were no differences among the NC treatments. Differences among the CAP treatments were detected by ANOVA (P< 0.0001). A multiple comparisons analysis showed that CAP Day1 was greater than CAP Day0 (P= 0.013); however, there was no difference between CAP Day1 and CAP 24 hrs (P= 0.967). These data show that the change in Cap-Score between Day0 and Day1 for the Cap treatment was physiologic.

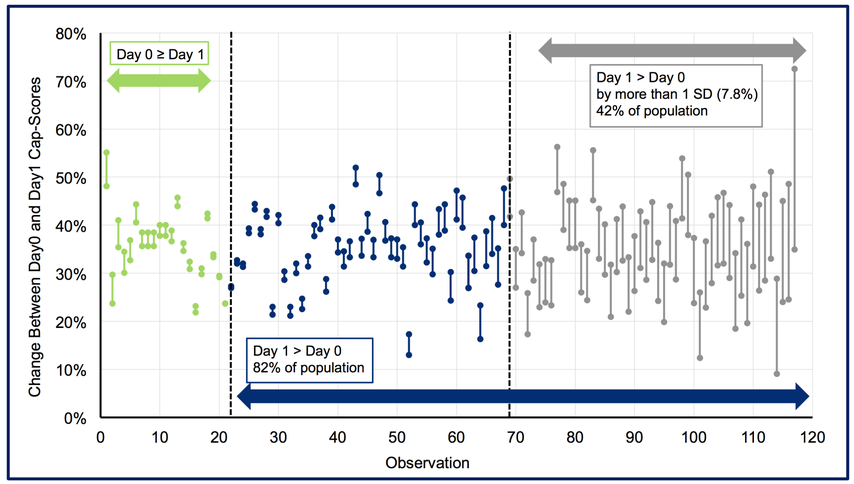
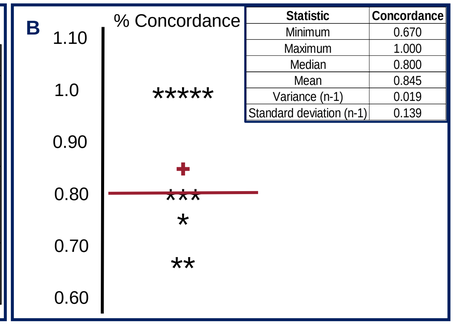


Figure 2: Changes in Cap-Score from Day0 to Day1 reveal a difference in timing of capacitation among individuals. Capacitation was assessed for 117 samples from 61 fertile donors on Day0 and Day1. The sample number is shown on the X axis and the Cap-Score on the Y axis. The two points per sample number represent the change from Day0 to Day1. 82% of the population showed an increase in Cap-Score from Day0 to Day1 (96/117). 42% (49/117) of the population showed an increase in Cap-Score greater than 1 standard deviation (7.8%). In 19 men who sought fertility treatment (not shown), 32% showed an increase greater than one standard deviation (6/19). These data reveal that there are differences in timing of capacitation among individuals.

A

Donor	Sample	Day 1 - Day 0	Early vs Late Capacitation	% Concordance
1	1	11%	Late	
1	2	11%	Late	
1	3	12%	Late	
1	4	14%	Late	
1	5	8%	Early	
1	6	7%	Early	**67%**
2	1	-4%	Early	
2	2	1%	Early	
2	3	-3%	Early	
2	4	8%	Early	
2	5	11%	Late	**80%**
3	1	13%	Late	
3	2	12%	Late	
3	3	10%	Late	
3	4	10%	Late	**100%**

B



Statistic	Concordance
Minimum	0.670
Maximum	1.000
Median	0.800
Mean	0.845
Variance (n-1)	0.019
Standard deviation (n-1)	0.139

Figure 3: Capacitation timing is consistent within men. Cap-Scores were obtained for 52 samples from 11 fertile donors on Day0 and Day1 (at least 4 observations per donor). The samples were categorized as either early capacitation (Day1 – Day0 < 1 SD: 7.8%), or late capacitation (Day1 - Day0 > 1 SD: 7.8%). Concordance of samples within donors was calculated. **Panel A** shows examples of how % concordance was calculated for 3 donors. The first donor represents 67% late concordance (4/6 samples), the second donor represents 80% early concordance (4/5 samples), and the third donor represents 100% late concordance (4/4 samples). In **Panel B,** the scatter plot shows the % concordance per donor (black asterisks) along the Y axis. The average (84%; red plus sign) and the median (80%; red line) concordance show that capacitation timing is highly consistent within men.

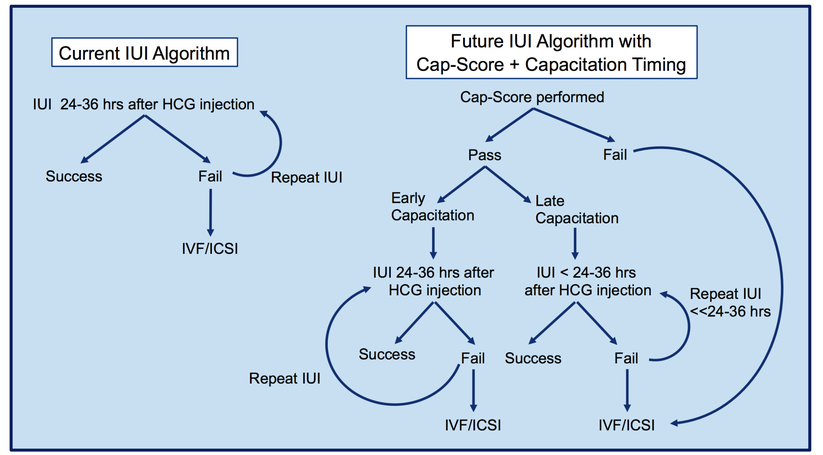


Figure 4: Personalizing IUI with capacitation timing. The use of the Cap-Score along with timing of capacitation could lead to personalized ART treatment for individuals. Typically, the time for insemination to be performed in IUIs is ~24-36 hours after HCG injection. However, If it takes sperm 24 hours 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 individuals.
- Capacitation timing is highly consistent within men.
- When performed under varying incubation and/or fixation conditions, a comparative use of the Cap-Score might 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.

Funding