SPERM FROM FERTILE MEN AND THOSE SEEKING FERTILITY EXAMS DIFFER IN THEIR ABILITY TO CAPACITATE

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Abstract

Introduction: Routine semen analysis correctly identifies male infertility only half the time. Localization patterns of G_{M1} in human sperm are highly associated with capacitation and their ability to fertilize. Here, the laboratory developed Cap-Score™ Sperm Function Test was used to define a standard capacitation profile for men of proven fertility, which was then compared to a group of men seeking male fertility workups.
Methods: Two cohorts were defined: 1) normal fertile men (pregnant partner or having a child within 3 years), and 2) potential subfertile/infertile men seeking semen analysis at the Urology Group of New Jersey. Subsequent to obtaining consent, collection and liquefaction, sperm were washed, incubated, fixed and then evaluated via fluorescence microscopy to determine Cap-Score.
Results: The Cap-Score mean (μ=40) and SD (σ=7.1) from 34 fertile men were used to estimate the number of fertile men needed to establish a robust fertile capacitation profile. For a power analysis, an acceptable range about the mean was set at 3%, and a two-tailed t-test at the p<0.01 level with a probability of detecting a difference this large of 90% was applied. Results suggested that a valid standard can be established with ≥85 individuals. A preliminary fertile standard was created using 173 observations from 61 unique donors. Cap-Scores were averaged by donor and converted to z-scores ((X-μ)/σ; X=observation, μ=36.5; σ=7.8). This transformed the μ to 0 and the σ to 1, with converted values representing the distance from the μ (mean) in units of σ (S.D.). The normal fertile standard was tested against Cap-Scores from 122 men seeking fertility exams. This cohort scored significantly below the fertile population (p=1.0E-4), with 9.8 and 28.7% having z-scores ≤-2 and between -1 and -2. Only 61.5% scored near or above the mean. These data suggest that in comparison to fertile men, many men seeking fertility exams have defects in capacitation.
Conclusions: Classic analyses provide little information on the ability of a semen sample to fertilize. Capacitation is required for fertilization and can be assessed using G_{M1} localization. A robust capacitation profile can be defined and employed for identifying abnormalities. Remarkably, 38.5% of men questioning their fertility had z-scores ≤-1 versus an expected result of 9.8%. Combining the Cap-Score™ Sperm Function Test with traditional analyses should prove valuable in diagnosing male infertility.

Introduction

Half of infertile men have defects in sperm function that current diagnostic tests fail to identify. Sperm functional maturation is known as capacitation and is required for fertilization. Localization of the ganglioside G_{M1} (Cap-Score) identifies sub-populations of sperm capable of capacitating. Men producing high numbers of such sperm are more likely to conceive by natural conception and/or IUI (Paniza, et al., 2014). Here we use the laboratory-developed Cap-Score Sperm Function Test, based on G_{M1} localization, to compare men with known fertility with a cohort of men seeking fertility assessment.

Results

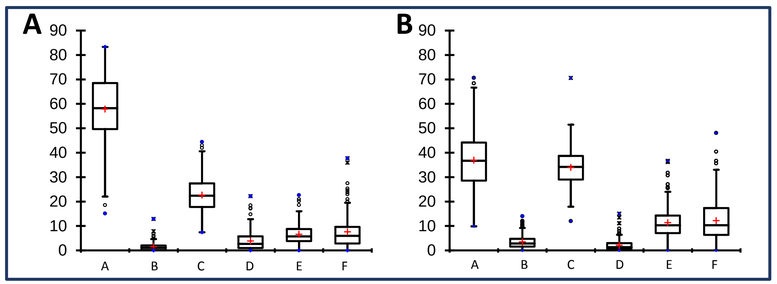
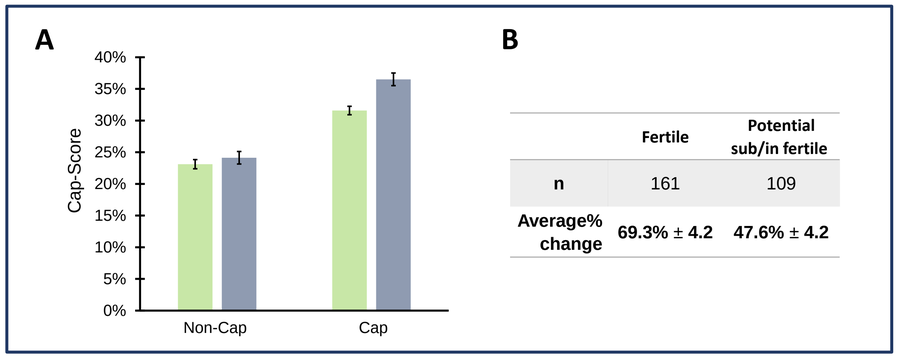


Figure 1. ***G_{M1} localization patterns in human sperm after incubation with capacitation stimuli.*** A) Sperm incubated under non-capacitating conditions, n=162 (fertile men). B) Sperm incubated under capacitating conditions, n=173 (fertile men). Percent of cells demonstrating each specific localization pattern is shown on the y-axis (different patterns are given letter designations). The first and third quartiles are at the ends of the box, the median is indicated with a horizontal line in the interior of the box, the mean is indicated by the red cross. The ends of the whiskers were calculated as (+/-1.5*Inner Quartile Range). Minimum/Maximum values are shown as blue diamonds. Outliers are shown as circles and asterisk.



B

	Fertile	Potential sub/in fertile
n	161	109
Average% change	69.3% ± 4.2	47.6% ± 4.2

Figure 2. ***Changes in ganglioside G_{M1} localization patterns (Cap-Score) in fertile and potential sub/in-fertile men.*** A) Bar graphs show average Cap-Scores obtained when sperm from known fertile and potential sub/in- fertile men were incubated under non-capacitating conditions (Non-Cap) and capacitating conditions (Cap). Green bars represents the potential sub/in- fertile population (Non-Cap: n=111; Cap: n=122); Blue bars represents the fertile population (Non-Cap: n= 61 unique donors, total 162 observation; Cap: n=61 unique donors, total 173 observation). Error bars represent standard error of the mean. The potential sub-fertile/infertile men scored below the fertile population (p=1.0E-4). B) Percent change ((Cap – Non-Cap)/Cap) from Non-Cap to Cap was calculated for each individual donor and then averaged. The average % change from Non-Cap to Cap was 69.3% for the fertile and 47.6% for the potential sub/in fertile population.

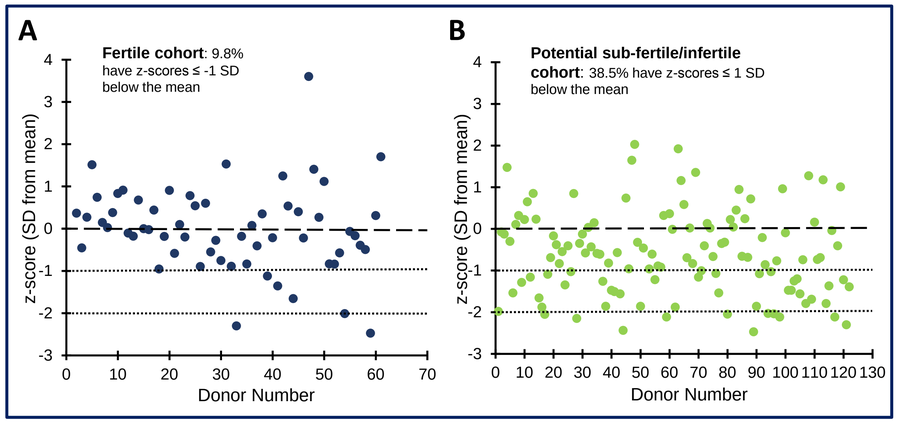


Figure 3. ***Defects in sperm fertilizing ability are quite common in men questioning their fertility.*** A) Scatter plot of Cap-Scores obtained from a fertile population. Blue dots represent average Cap-Scores for 61 unique fertile donors (total 173 observations), plotted as z-scores ((X-μ)/σ; X=observation, μ=36.5; σ=7.8) in comparison to the mean. B) Scatter plot of Cap-Scores obtained from a potential sub-fertile/infertile population. Green dots represent a single donor (n=122). Cap-Scores converted to z scores ((X-μ)/σ; X=observation, μ=36.5; σ=7.8) are on the y-axis and the donor number is on the x-axis. The dashed horizontal line represents the mean and the dotted lines represent 1 and 2 SD below the mean. 38.5% of men questioning their fertility had z-scores lower than -1 versus 9.8% in the fertile cohort.

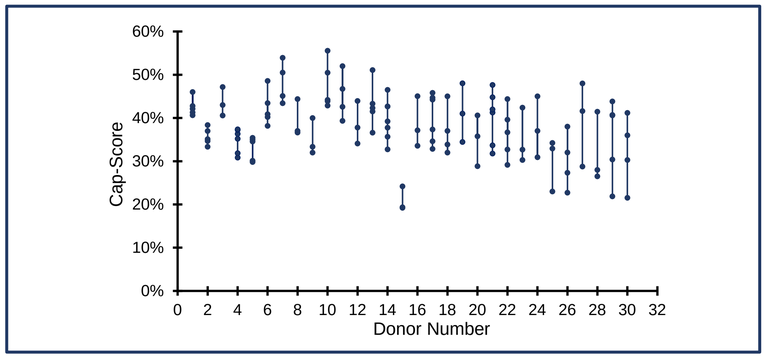


Figure 4. ***Cap-Score reproducibility within individuals.*** Multiple ejaculates were tested from 30 individuals (average 4 ejaculates / donor). The x-axis shows donor number and the y-axis shows Cap-Score. Each point within a single donor number represents a different day of collection. Average coefficient of variation (σ/μ) within donor was 12.6%. Multiple collections varied within 6% points of the average.

Conclusions

- A capacitation profile for a population of fertile men was generated and used as a point of comparison to assess sperm function in a group of men questioning their fertility.
- Significantly more men questioning their fertility had Cap-Scores more than 1 SD below the mean from the fertile population.
- Cap-Score readings are reproducible within individuals, with most collections typically varying within 6% points of their average.
- Cap-Score might explain cases of idiopathic infertility and serve to direct couples to the most appropriate infertility treatment.

Future directions

- Poor nutrition and/or exposure to certain environmental elements can have detrimental impacts on male fertility. Research is needed to determine if Cap-Score could be used as a biomarker to monitor the effects of changing lifestyles, diets and/or surroundings.

Funding