CAPACITATION DEFECTS ARE COMMON IN MEN QUESTIONING THEIR FERTILITY AND ARE INDEPENDENT OF STANDARD SEMEN ANALYSIS PARAMETERS

Alexander J. Travis[1,3], Cristina Cardona[1], Alana J. Simpson[1], Melissa A. Moody[1], Eric K. Seaman[2], and G. Charles Ostermeier[1],
[1]Androvia LifeSciences, Mountainside, NJ; [2]Urology Group of New Jersey, Millburn, NJ; [3]Cornell University, Ithaca, NY

Abstract

Objective: Semen analysis fails to diagnose defects in capacitation. Sperm must capacitate to be able to fertilize. Localization of the ganglioside G_{M1} (Cap-Score™) identifies cells capable of capacitating, providing a bioassay for sperm fertilizing ability (Paniza et al., ASRM 2014). However, those data were obtained solely from men seeking fertility treatment. The objectives of this study were to compare the Cap-Scores of men seeking fertility work ups versus men with known fertility, and to evaluate if Cap-Score provided novel functional data or merely tracked with standard semen analysis parameters.
Design: Cohort comparison between presumed fertile (Cohort 1, pregnant or recent father) and potential subfertile/infertile men (cohort 2, men questioning fertility). Relationships between Cap-Score and traditional semen measures were also explored.
Material and Methods: All studies approved by WIRB (20152233). Semen samples were liquefied, washed, and incubated under non-capacitating and capacitating conditions. Sperm were fixed overnight and Cap-Score determined via fluorescence microscopy. Semen quality measures were evaluated according to WHO. T-Test, ANOVA and correlation analyses were done using MS Excel (2013) and XLSTAT (2015).
Results: Cap-Score values were normally distributed in Cohort 1, with 13.2% having Cap-Scores more than one SD below the mean (35.3±7.7%). Significantly more men in Cohort 2 had Cap-Scores greater than one SD below the normal mean (33.6%; p=0.001). For cohort 2, no relationship was observed between Cap-Score and morphology (p=0.28), motility (p=0.14) or concentration (p=0.67). 93.4% (114/122) of men in cohort 2 exhibited normal percent motility, yet 30.7% (35/114) of them had Cap-Scores > 1 SD below the mean for cohort 1. Similarly, 101 of 122 men (82.7%) exhibited normal concentration with 32.6% (33/101) having Cap-Scores > 1 SD below the mean for cohort 1.
Conclusions: These results show that capacitation defects are common in men having difficulty conceiving and that Cap-Score provides functional data complementing traditional semen analysis. Because capacitation is required for fertilization, the Cap-Score can provide an important functional complement to standard semen analysis and may help in choosing the most appropriate fertility treatment.

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. Here we use Cap-Score to compare men with known fertility versus men seeking fertility assessment. We also evaluate whether Cap-Score correlates with any of the standard semen analysis parameters or adds distinct, complementary information.

Results

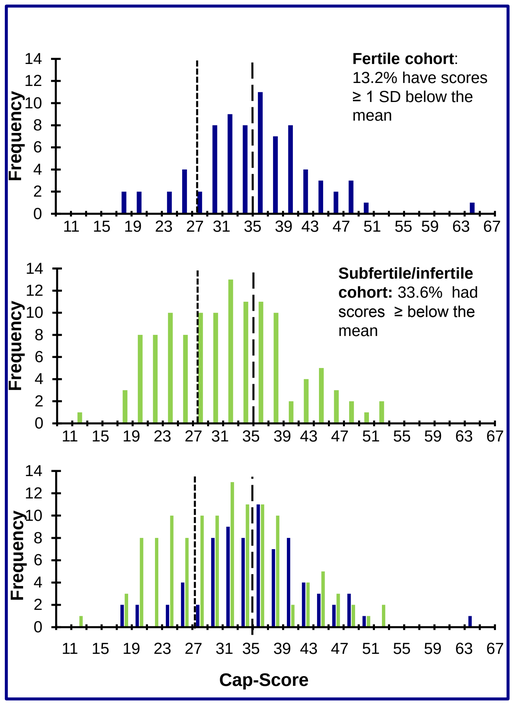

Figure 1. ***Spermatozoa from presumed fertile men showed a robust response to capacitation stimuli when compared to men questioning their fertility.*** X axis shows donor's Cap-Score organized in bins. Y axis shows the number of donors within each bin (frequency). Top histogram, blue bars, shows data obtained from presumed fertile men (pregnant or recent father n=76). Middle histogram, green bars, shows data obtained from a potential sub-fertile/infertile population (n=122, men who were referred for fertility evaluation and not screened for female factor infertility). Bottom, a combination of top and middle histograms. The dashed lines represent the mean of the fertile population (35.3±7.7%) and one SD below the mean. 13.2% of the fertile individuals had Cap-Scores ≥ one SD below the mean. A greater proportion of subfertile/infertile individuals had Cap-Scores ≥ one SD below the mean of fertile population (33.6%; p= 0.001).

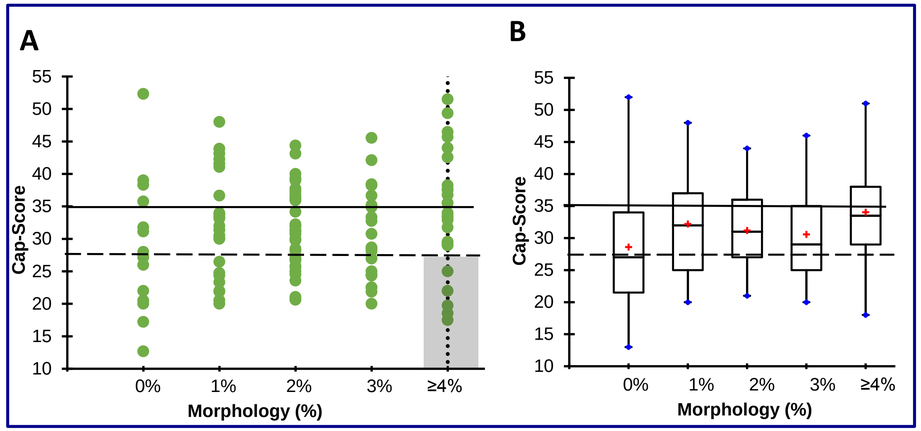

Figure 2. ***There was no correlation between sperm morphology and Cap-Score.*** **(A)** Scatter plot of morphology (% normal forms; X-axis) and Cap-Score (Y-axis) obtained for 122 samples from men questioning their fertility (Cohort 2). 78.7% (96/122) of the population had abnormal morphology (<4% normal forms; cut-off shown by vertical dotted line). 21.3% (26/122) of the population had normal morphology (≥4% normal forms). The solid horizontal line marks the mean and dotted horizontal lines denotes one standard deviation below the mean for a population of presumed fertile men (Cohort 1). 19.2% (5/26, gray shaded area) of men with normal morphology had a Cap-Score more than one SD below the mean of Cohort 1. ANOVA revealed no relationship between morphology and Cap-Score (p=0.28). **(B)** Descriptive statistics of morphology data shown in A: % normal forms is shown on the X-axis and Cap-Score is shown on the Y-axis. 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. Minimum/Maximum values are shown as blue diamonds. Upper and lower fences were calculated using +/-1.5*Inner Quartile Range. Observations smaller than the lower fence and larger than the upper fence were considered outliers. The whiskers extend to the smallest and largest observations that are not considered outliers.

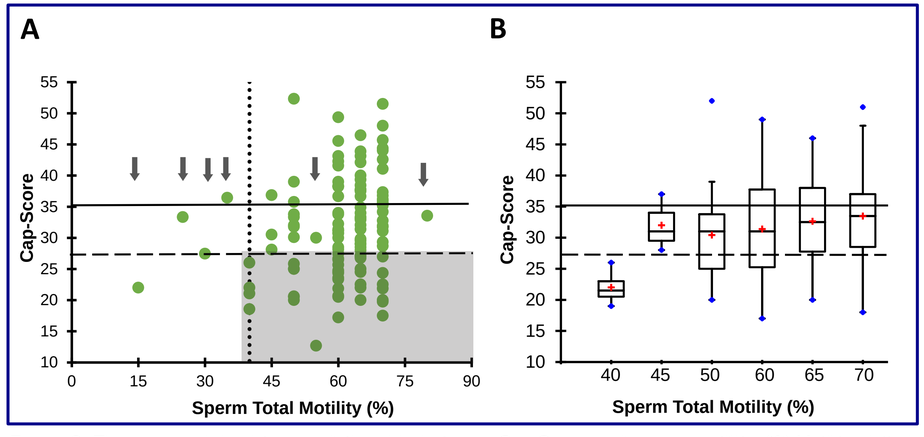

Figure 3. ***There was minimal-to-no relationship between Cap-Score and sperm motility.*** **(A)** Motility plotted against Cap-Score (Cohort 2, n=122)**.** Total % motility was collected in an ordinal fashion and data are presented in bins, or increments of 5% (X-axis). Those bins having less than three observations were removed from analysis and are indicated by gray arrows. The corresponding Cap-Score for each observation is shown on the Y-axis. The solid horizontal line marks the mean and dotted horizontal line denotes one standard deviation below the mean for a population of presumed fertile men (Cohort 1). No difference in Cap-Score was detected across the 6 bins (n=115; ANOVA; p=0.144). 6.6% (8/122) of men were asthenozoospermic by WHO criteria (≤40% total motility; cut-off represented by vertical dotted line). 93.4% (114/122) of men had normal percent motility. 30.7% (35/114) had normal percent motility, but exhibited Cap-Scores ≤1 SD below the mean (gray shaded area). **(B)** Descriptive statistics of the 6 bins (containing more than 3 observations) shown in A. Sperm total percent motility is shown on the X-axis. Cap-Scores are shown on the Y-axis. 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. Minimum/Maximum values are shown as blue diamonds. Upper and lower fences were calculated using +/-1.5*Inner Quartile Range. Observations smaller than the lower fence and larger than the upper fence are consider outliers. The whiskers extended to the smallest and largest observations that are not considered outliers.

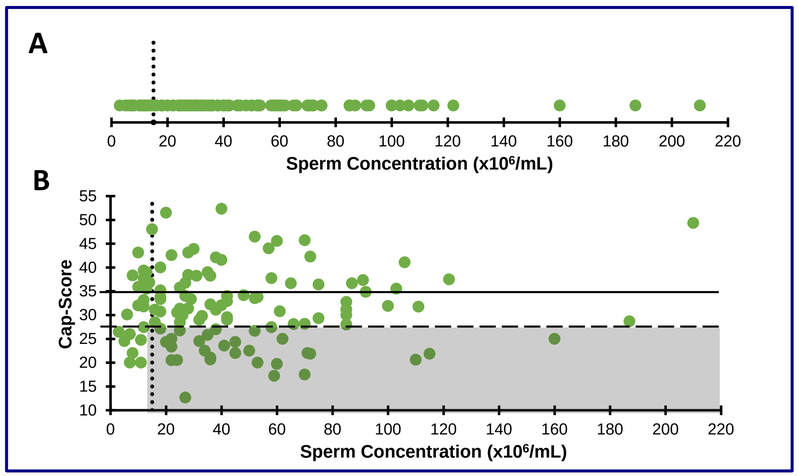

Figure 4. ***There was no relationship between sperm concentration and Cap-Score.*** **(A)** Traditional view of concentration (Cohort 2, n=122)**. (B)** Concentration (X-axis) plotted against Cap-Score (Y-axis) (Cohort 2, n=122). Horizontal lines denote the mean Cap-Score and one standard deviation below the mean for fertile men (Cohort 1). 17.2% (21/122) of men demonstrated oligozoospermia (≤15x10⁶/mL; cut-off shown by vertical dotted line). 82.8% (101/122) of men had normal concentration. 32.7% (33/101) of men with normal concentration had Cap-Scores more than one SD below the mean of the presumed fertile population (gray shaded area). 33.6% (41/122) of men exhibited Cap-Scores ≤1 SD below the mean, and of these, 80.5% (33/41) had normal sperm concentrations.

Conclusions

- Significantly more men questioning their fertility had Cap-Scores more than 1 SD below the mean from the fertile population (33.6% vs 13.2%). This was especially remarkable because no men were excluded because of female factor infertility. The literature suggests that 30-50% of the men in this group should have normal fertility.
- Minimal/no relationship was found between Cap-Score and sperm concentration, morphology or motility.
- In combination with semen analysis, the Cap-Score can be a powerful tool to diagnose male fertility. Its contribution is a unique, quantitative insight into sperm function.

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