

Abstract

Introduction and objective: The diagnosis of male infertility is based predominantly on the results of standard semen analysis. However, standard semen analysis provides little information on sperm functional competence. Localization of the ganglioside G_{M1} (Cap-Score™) identifies sub-populations of sperm capable of undergoing the functional maturation process known as capacitation and tracks strongly with fertility (Paniza et al., ASRM 2014). Here Cap-Score results were compared to standard semen analysis parameters obtained from men questioning their fertility.

Methods: Semen samples from consenting patients were liquefied, washed and incubated under both non-capacitating and capacitating conditions. Semen analysis was performed according to WHO guidelines. Cap-Score values were obtained via fluorescence microscopy. Statistical analyses were done using Microsoft Excel (2013) and XLSTAT (2015).

Results: Samples from 122 men referred to an infertility specialist were analyzed and had Cap-Scores ranging from 13 to 52% (mean from a population with known fertility was 36.5 ± 7.8%; n=61). An analysis of variance was done to compare Cap-Scores and sperm morphology. Samples were classified as having 0, 1, 2, 3, or ≥4% normal forms (scores ≥4% are considered normal, WHO) and mean Cap-Scores were compared among the groups. No relationship between Cap-Score and morphology was observed (P=0.28). Next, sperm concentration (range 3x10⁶ to 210x10⁶/mL) was compared to Cap-Score using the Pearson product-moment correlation coefficient and no connection was found (r=0.04, P=0.67). Lastly, Cap-Score was compared to total % motility (range 15 to 80%). Linear regression suggested a modest relationship between motility and Cap-Score (r²=0.05, p=0.02). This analysis shows that motility accounts for only 5% of the variability in Cap-Score, leaving 95% of the variability unrelated to motility. Since motility data were collected in an ordinal fashion, rather than as a continuous measure, we also analyzed the data in bins of 5% as they were collected. Bins having less than three observations were removed and an ANOVA was performed. No difference in Cap-Score was detected across the 6 bins (p=0.14). Multiple donors who were classified as normal by WHO criteria had Cap-Scores more than 2 SD below the normal mean, supporting the view that even normal appearing sperm can have functional abnormalities.

Conclusion: Traditional semen analysis identifies only 50% of male infertility cases. These studies show that there is no substantive relationship between Cap-Score and standard semen analysis parameters. Since capacitation is necessary for fertilization, the addition of Cap-Score to traditional semen evaluations could both identify cases of idiopathic infertility and help clinicians counsel couples towards the most appropriate treatment. Funded by Androvia LifeSciences.

Introduction

There are over 73 million infertile couples globally, with >40% of infertility having a male factor. Standard semen analysis, assessing sperm count, motility, and morphology, diagnoses approximately half of all male infertility. The other half have defects in sperm function and are only diagnosed by repeated failed cycles of IUI. Historically, several assays designed to test sperm function were shown to correlate with one of the semen analysis parameters, preventing them from adding value to diagnostic efforts. Here, we evaluated whether a new test for sperm function/fertilizing ability, the Cap-Score, correlated with any of the standard semen analysis parameters or added distinct, complementary information.

Results

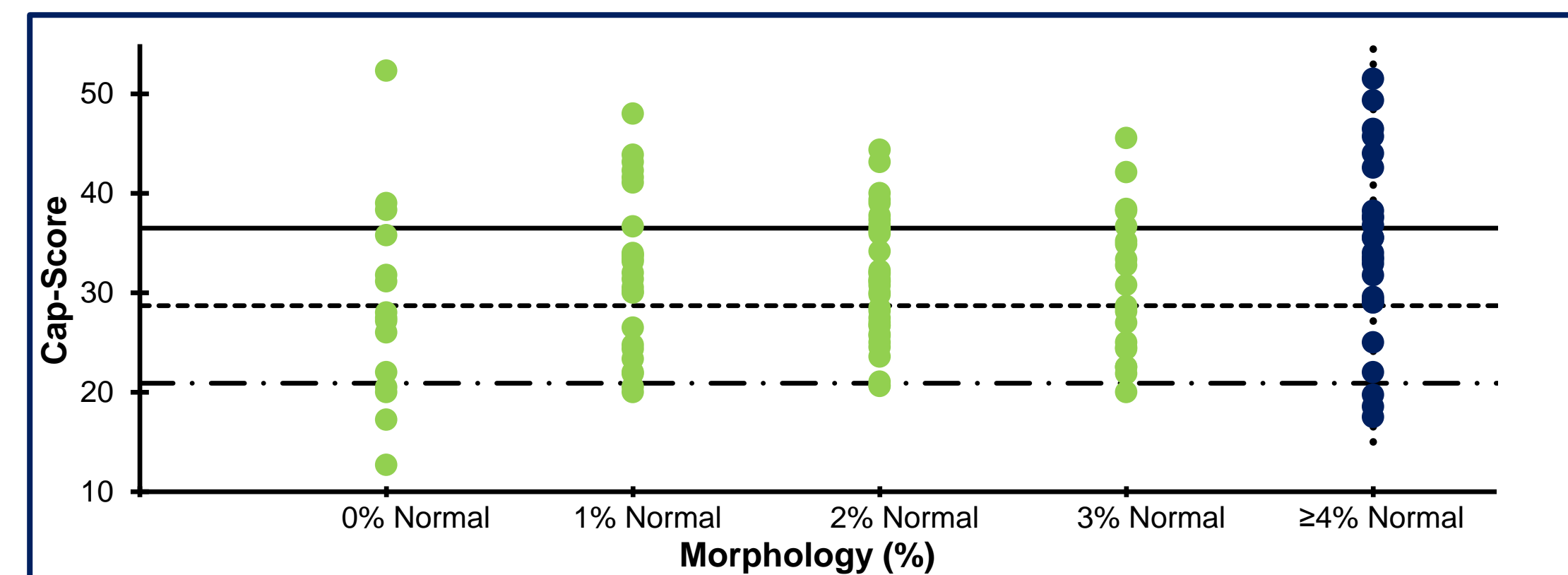


Fig. 1. Morphology plotted against Cap-Score. Semen samples were obtained from 122 men seeking fertility assessment. The percent of sperm showing normal forms (blue) within each ejaculate is shown along the x-axis. 79% (96/122) of the population (green) had abnormal morphology (<4% normal forms; cut-off shown by vertical dotted line). The Cap-Score for each sample is shown on the y-axis. Horizontal lines illustrate the mean and one and two SD below the mean for a population of fertile men. ANOVA revealed no relationship between morphology and Cap-Score (p=0.28).

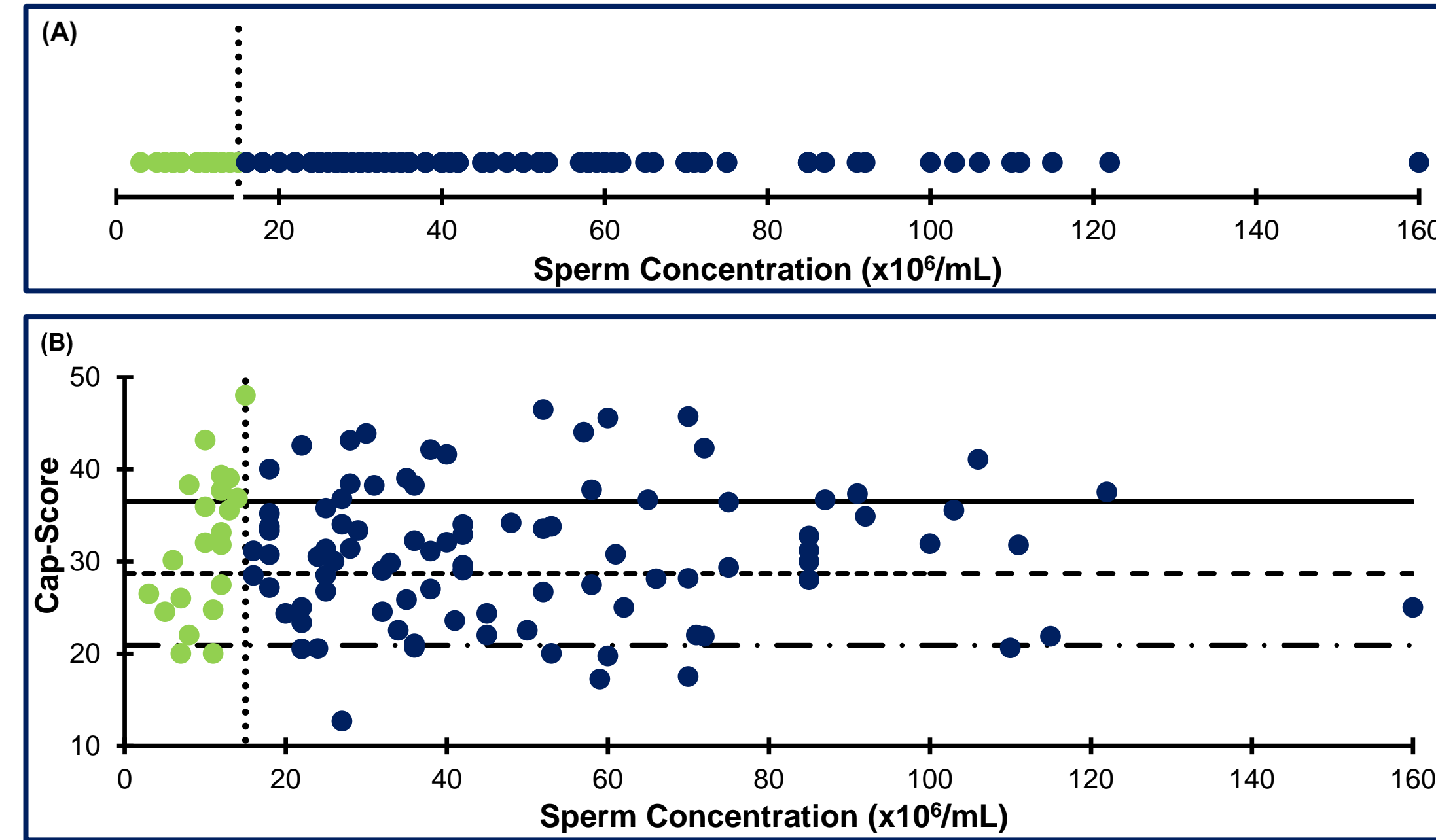


Fig. 2. (A) Traditional view of concentration. (B) Concentration plotted against Cap-Score. No significant correlation was found between sperm concentration (x-axis) and Cap-Score (y-axis; n=122; r=0.04; p=0.67). Horizontal lines denote the mean and one and two SD below the mean Cap-Score for a population of fertile men. Using WHO criteria, 17% (21/122) of men (green) demonstrated oligozoospermia ($\leq 15 \times 10^6$ /mL; cut-off shown by vertical dotted line). 39% (47/122) of men exhibited Cap-Scores more than 1SD below the mean, and of these, 83% (39/47) had normal sperm concentrations.

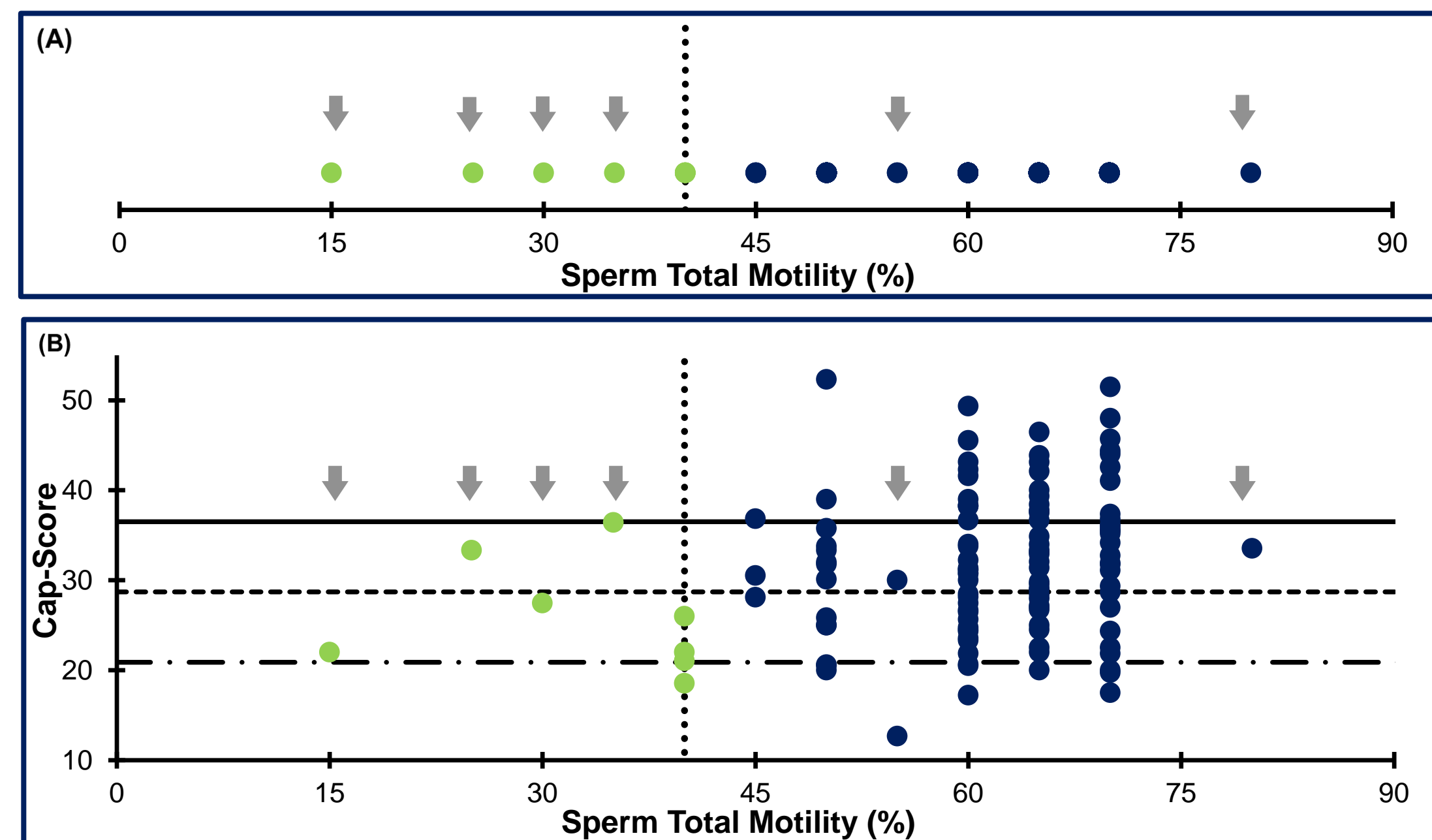


Fig. 3. (A) Traditional view of motility. (B) Motility plotted against Cap-Score. Total motility was collected in an ordinal fashion and data are presented in bins, or increments of 5% (x-axis). Those bins having less than 3 observations were removed and are indicated by gray arrows in panels A and B. The corresponding Cap-Score for each observation is shown on the y-axis. Horizontal lines represent the mean and one and two SD below the mean Cap-Score for a population of fertile men. No difference in Cap-Score was detected across the 6 bins (n=115; ANOVA; p=0.14). 7% (8/122) of men (green) were asthenozoospermic by WHO criteria ($\leq 40\%$ total motility; cut-off shown by vertical dotted line). 36% (41/114) of sperm having normal motility exhibited Cap-Scores more than 1SD below the mean.

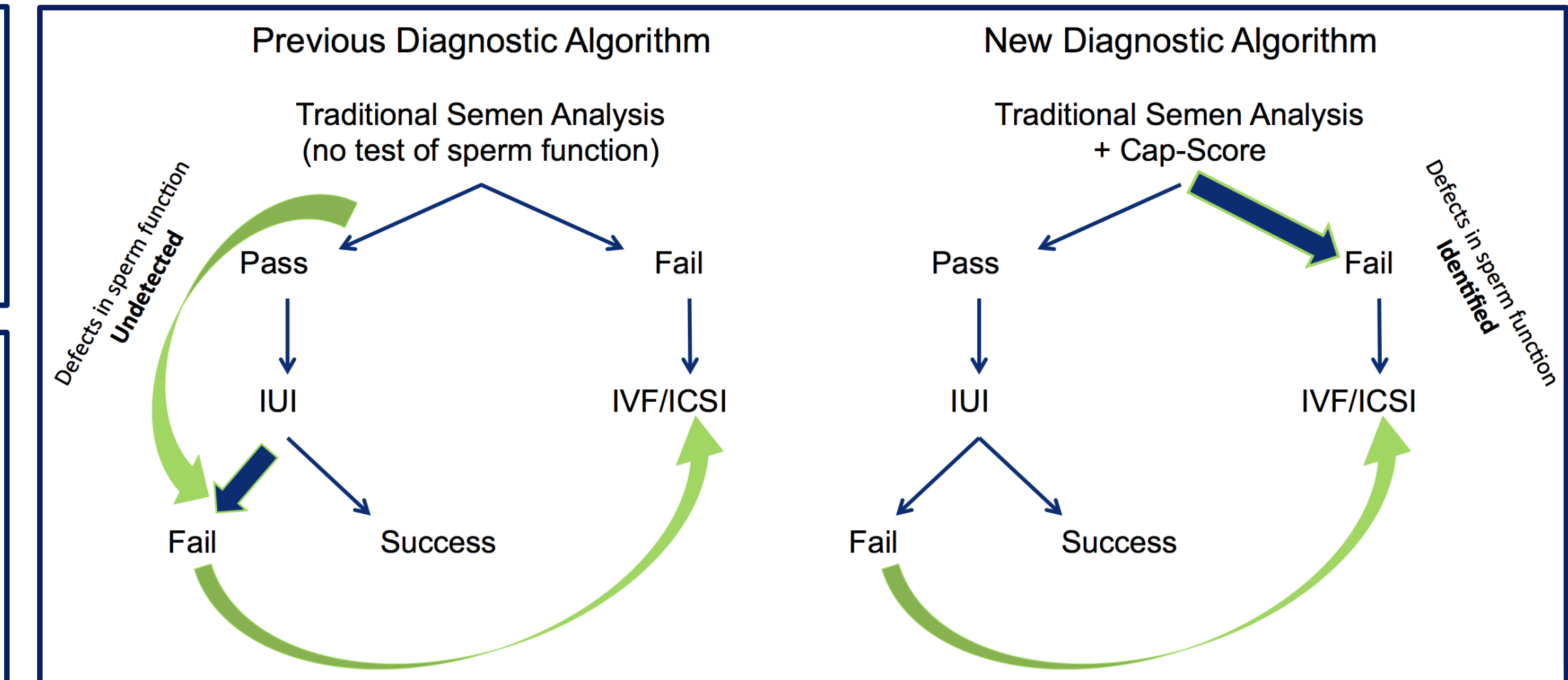


Fig. 4. Comparison of diagnostic/clinical algorithms between historical reliance on semen analysis versus semen analysis performed in conjunction with Cap-Score. The Cap-Score provides a test of sperm function, offering a complement to the descriptive parameters in the traditional semen analysis. Currently, defects in sperm function go undiagnosed by the traditional semen analysis and half of all cases of male infertility are idiopathic, identified only by repeated failure at natural conception and IUI. This diagnostic algorithm imparts enormous emotional, physical and financial costs on infertile couples. With a test of sperm function such as Cap-Score, couples with low sperm function could be spared cycles that are doomed to fail, and immediately directed to a more appropriate form of ART, such as IVF or ICSI.

Conclusion

- Traditional semen analysis parameters, including sperm morphology, concentration and total motility, have no or minimal correlation with the Cap-Score test of sperm function.
- Cap-Score provides functional and complementary information to the major diagnostic tool used to evaluate male fertility.
- Of the 21% (26/122) of men exhibiting normal morphology, 54% (14/26) had Cap-Scores more than 1SD below the mean. 83% (101/122) exhibited normal concentration and of these, 39% (39/101) had Cap-Scores more than 1SD below the mean. 93% (114/122) exhibited normal motility and of these, 36% (41/114) had Cap-Scores more than 1SD below the mean.
- These data show that many men questioning their fertility pass the descriptive parameters of traditional semen analysis, yet have sperm function scores significantly lower than expected when compared to results from a population of fertile men.

Funding