In men seeking fertility assistance, defects in sperm capacitation/fertilizing ability are common in all age groups, unlike traditional semen analysis parameters

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Abstract

Objective: Social and economic factors are causing couples to delay parenthood. This trend raises several concerns related to reproductive success. It is generally accepted that maternal age is inversely related with fertility and pregnancy outcome. However, the influence of paternal age is still contentious. While traditional Semen Analysis (SA) is the standard for evaluating male fertility, it often fails to predict reproductive outcome; many men pass SA but have defects in sperm function/fertilizing ability. Sperm must capacitate prior to fertilization. Localization patterns of the ganglioside G_{M1} (Cap-Score™) identify sperm capable of capacitation and prospectively predict pregnancy. The objective of this study was to determine how Cap-Score changes with paternal age.

Materials and Methods: Cap-Score and SA measures (Volume, Concentration, Motility) were collected from men seeking fertility assistance at reproductive endocrinology offices. The population was separated into the following paternal age groups 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, & 50+, with the respective age groups having the corresponding number of observations for the SA assessment 31, 293, 1024, 922, 402, 154, & 66 and Cap-Score analysis 31, 296, 1040, 934, 410, 156, & 67. Kruskal-Wallis Tests with multiple comparisons were done to evaluate the associations between SA, Cap-Score and age.

Results: No association was detected between age and sperm concentration (p=0.921). While motility (p=0.006) and volume (p<0.0001) declined significantly with age, their means were both within normal WHO ranges in the 50+ group (41.9±2.5% and 2.54±0.22 mL). Neither the overall Kruskal-Wallis Test (p=0.466) nor the multiple comparisons detected Cap-Score differences among the age groups. Unfortunately, there were only 67 observations in men above 50 preventing us from making any meaningful analysis compared to the younger age groups.

Conclusions: The influence of paternal age on semen quality and male fertility is still under investigation. The literature supports a decrease in *in vivo* fertility as men age. However, *in vitro* this doesn't appear to be the case. This discrepancy could be a result of confounding factors, such as maternal age or environmental factors, that can be more easily removed *in vitro*. The data presented here suggest that confounding factors may indeed have a large impact. Traditional SA measures are poorly related to male fertility and declined with age. In contrast, capacitation ability has been shown by multiple groups to be the best predictor of male fertility and defects were equally prevalent in younger men as in old.

Impact Statement: Younger men presenting with fertility problems are more likely to have higher SA metrics than older men, but are equally likely to have defects in sperm function, making these tests more sensitive at identifying male infertility across age groups.

Guzick, et al. 2001 N Engl J Med. 345:1388-1393
Cardona, et al. 2017 Mol Repro and Devel. 84(5), 423-435
Moody, et al. 2017. Mol Repro and Devel. 84 (5), 408-422
Schinfeld, et al. 2018. Mol Repro and Devel. 85 (8-9), 654-664
Sharara, et al. 2020. RBMO. 41(1), 69-79

Introduction

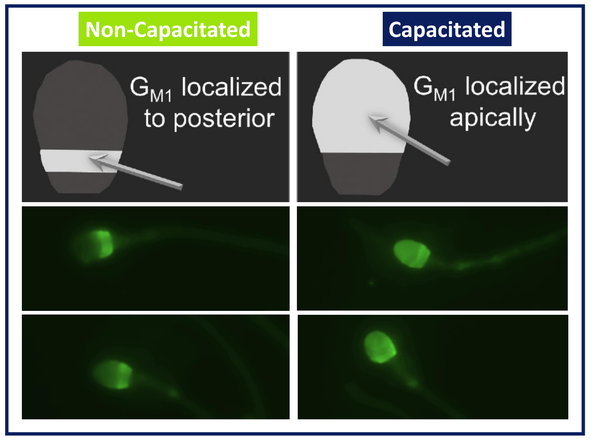


Figure 1. ***Cap-Score™.*** Cap-Score™ is defined as the percentage of capacitated sperm within an ejaculate and is determined by distribution patterns of the ganglioside G_{M1} (Moody et al 2017). The images in the left column show respectively, a diagram and fluorescent microscopy images typical of sperm that have not been exposed to, or have not responded to, stimuli for capacitation. The right column shows G_{M1} distributions that are typical for cells that have responded to stimuli for capacitation.

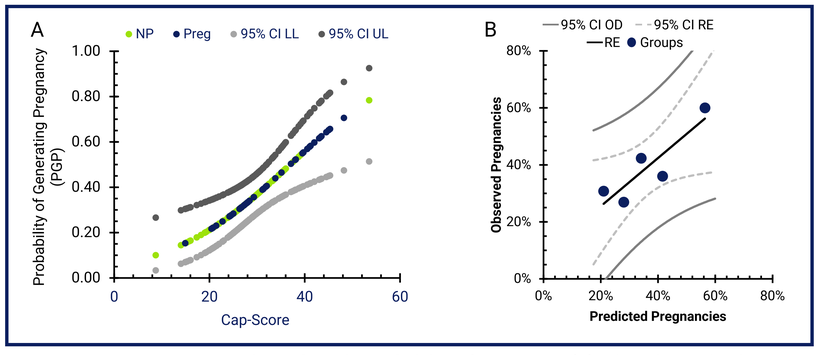


Figure 2. ***Cap-Score accurately predicts the probability of pregnancy.*** **(A)** The relationship between Cap-Score and a man's probability of generating pregnancy (PGP) was defined previously (Schinfeld, et al. 2018). Logistic regression transformed Cap-Score into PGP. The green dots represent non-pregnant (NP) and the blue dots represent pregnant (Preg) couples, respectively. The dark and light gray dots represent the 95% confidence interval (CI). **(B)** A prospective study tested this relationship (Sharara et al, 2020). Cap-Score and its associated PGP were determined for 128 new individuals. The couples were then followed over 3 rounds of IUI. The population was ordered by PGP and then divided into 5 equally sized groups (blue dots). No difference was detected between the predicted and observed pregnancies, substantiating the relationship between capacitation ability and a man's fertility. The black line shows the regression equation (RE). The dotted and solid gray lines show the 95% CI for the RE and observed data (OD), respectively.

Experimental design

Cap-Score and SA measures (Volume, Concentration, Motility) were collected from men seeking fertility assistance at reproductive endocrinology offices. The population was compared to a previously acquired population of men of known fertility and then separated into the following paternal age groups 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, & 50. The population of men questioning their fertility was compared to the fertile population using a Mann-Whitney test. Kruskal-Wallis Tests with multiple comparisons were done to evaluate the associations between age, SA and Cap-Score.

Results

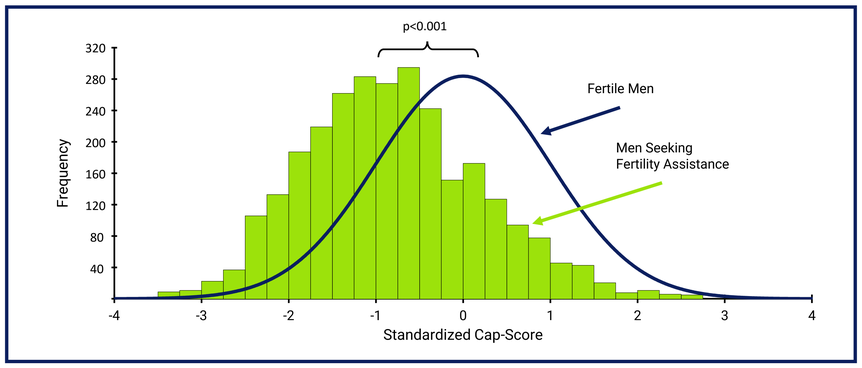
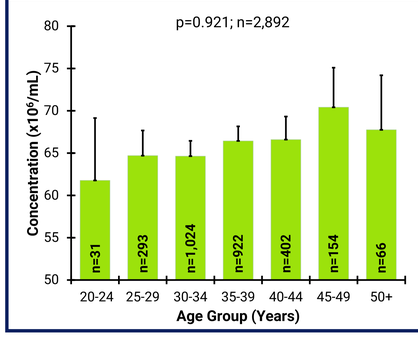


Figure 3. ***Comparison of Cap-Scores between men of know fertility and men seeking fertility assistance.*** Cap-Scores were collected from men seeking fertility assistance at reproductive endocrinology offices (n=2,934; green histogram) and a previously acquired population of known fertile men (n=76; blue bell curve; Cardona et al 2017). Both distributions were standardized so that the Cap-Score mean (35.3) and standard deviation (7.7) of the fertile population was set to zero and one unit respectively. There was a significant reduction in Cap-Scores in men who were seeking fertility assistance (0±1 vs -0.81±1; p<0.001).

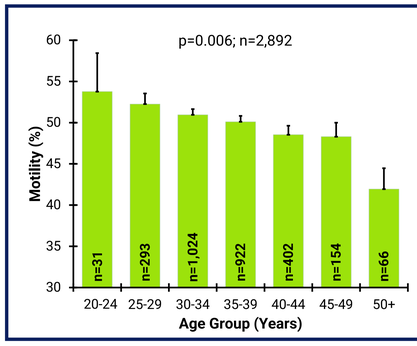


P-Values for multiple comparisons

Age Group	20-24	25-29	30-34	35-39	40-44	45-49	50+
20-24	1						
25-29	0.922	1					
30-34	0.924	0.987	1				
35-39	0.844	0.416	0.239	1			
40-44	0.994	0.794	0.748	0.564	1		
45-49	0.814	0.513	0.459	0.904	0.635	1	
50+	0.797	0.584	0.562	0.874	0.681	0.948	1

Bonferroni corrected significance level: 0.0024

Figure 4. ***The relationship between age and concentration in men seeking fertility assistance.*** The bar chart shows the means and standard errors, while the table shows the p-values for the multiple comparisons between the age groups. No association was detected between age and sperm concentration (p=0.921) and none of the multiple comparisons reached the 0.05 p-value cut-off.

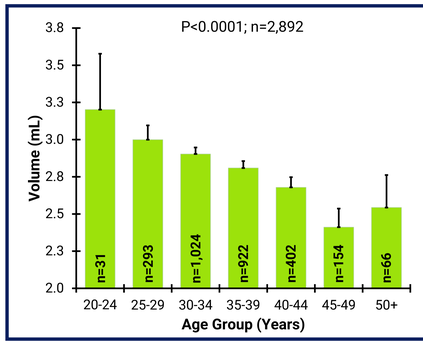


P-Values for multiple comparisons

Age Group	20-24	25-29	30-34	35-39	40-44	45-49	50+
20-24	1						
25-29	0.640	1					
30-34	0.401	0.330	1				
35-39	0.287	0.115	0.364	1			
40-44	0.145	0.017	0.044	0.196	1		
45-49	0.169	0.067	0.173	0.379	0.994	1	
50+	0.010	0.001	0.001	0.004	0.031	0.052	1

Bonferroni corrected significance level: 0.0024

Figure 5. ***The relationship between age and motility in men seeking fertility assistance.*** The bar chart shows the means and standard errors, while the table shows the p-values for the multiple comparisons between the age groups. Those p-values meeting the Bonferroni significance level of 0.0024 are highlighted in yellow. Those that were less than 0.05 are highlighted in gray. While motility declined significantly with age (p=0.006) the mean of the 50+ group was within the normal WHO range (41.9±2.5%).

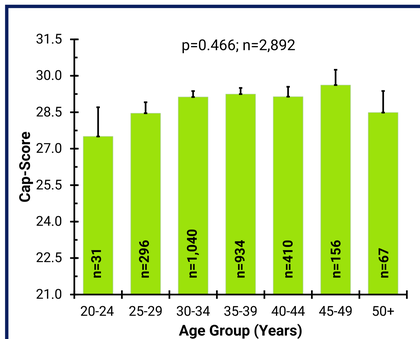


P-Values for multiple comparisons

Age Group	20-24	25-29	30-34	35-39	40-44	45-49	50+
20-24	1						
25-29	0.754	1					
30-34	0.634	0.677	1				
35-39	0.387	0.140	0.116	1			
40-44	0.162	0.009	0.003	0.086	1		
45-49	0.011	<0.0001	<0.0001	<0.0001	0.012	1	
50+	0.034	0.003	0.003	0.017	0.129	0.807	1

Bonferroni corrected significance level: 0.0024

Figure 6. ***The relationship between age and semen volume in men seeking fertility assistance.*** The bar chart shows the means and standard errors, while the table shows the p-values for the multiple comparisons between the age groups. Those p-values meeting the Bonferroni significance level of 0.0024 are highlighted in yellow. Those that were less than 0.05 are highlighted in gray. While volume declined significantly with age (p<0.0001), the mean of the 50+ group was within the normal WHO range (2.54±0.22 mL).



P-Vales for multiple comparisons

Age Group	20-24	25-29	30-34	35-39	40-44	45-49	50+
20-24	1						
25-29	0.370	1					
30-34	0.180	0.255	1				
35-39	0.135	0.120	0.525	1			
40-44	0.206	0.386	0.878	0.526	1		
45-49	0.105	0.131	0.386	0.597	0.376	1	
50+	0.456	0.956	0.513	0.380	0.577	0.283	1

Bonferroni corrected significance level: 0.0024

Figure 7. ***The relationship between age and Cap-Score in men seeking fertility assistance.*** The bar chart shows the Cap-Score means and standard errors, while the table shows the p-values for the multiple comparisons between the age groups. No association was detected between age and Cap-Score (p=0.466) and none of the multiple comparisons reached the 0.05 p-value cut-off.

Conclusions

- Capacitation ability has been shown by multiple groups to be the best predictor of male fertility.
- Traditional SA measures are poorly related to male fertility; over half of infertile men pass SA but have defects in sperm function (Guzick, et al 2001).
- When compared to fertile men, Cap-Scores were reduced in men seeking fertility assistance.
- Motility and volume declined with age in men seeking fertility assistance.
- In contrast, defects in capacitation/sperm fertilizing ability were equally prevalent across age groups in men questioning their fertility.
- These results suggest that young men questioning their fertility are more likely to pass traditional SA, but the prevalence of functional defects is equally common across ages. These data show that tests of sperm function are more sensitive indicators of problems with male fertility across age groups and shouldn't be reserved for older male patients.

Cap-Score results provided by