A Double-blind, Prospective Test Demonstrates an Association Between Capacitation Status and Pregnancy.

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Abstract

OBJECTIVE: Semen Analysis (SA) often fails to predict fertility, apart from extreme cases, highlighting the need for advanced sperm testing. Cap-Score™ is a validated test that uses changes in G_{M1} localization patterns to identify sperm that can and cannot capacitate. Since capacitation is required for fertilization, men must produce sperm with this ability for pregnancy generation. The purpose of this study was to use a double-blind prospective analysis to evaluate how predictive a previously defined Cap-Score reference range (Cardona, et al. 2017) was, of male fertility.

MATERIALS AND METHODS: Cap-Score and SA were performed (n=107), with subsequent clinical Intrauterine Insemination (IUI) outcomes available for 24 at the time of analysis. Outcomes were defined as either completion of, or pregnancy within, 3 cycles of IUI. The chance of pregnancy outcome was predicted as either low (n=9) or normal (n=15), based solely on the previously defined reference range. IUI was done blinded to Cap-Score evaluation. Absolute and cumulative pregnancy rates were compared over 1-3 rounds of IUI using a Kaplan-Meier survival analysis. Semen analysis measures were compared between pregnant (n=8) and not-pregnant (n=16) groups using weighted t-tests, with the weights assigned by the number of IUI rounds.

RESULTS Men having low Cap-Scores showed reduced absolute and cumulative pregnancy outcomes (absolute: predicted low [0%] vs predicted normal [53%; p=0.001]; cumulative predicted low vs. normal: 0 vs 33, 0 vs 58, and 0 vs 58% for cycles 1, 2, and 3 [n=24, 11, and 4 rounds of IUI; p=0.025]). Only Cap-Score (35.4±1.7 vs 31.3±2.2; p=0.04) and motility (81.1±2.2 vs 73.9±3.5; p=0.02), differed between the pregnancy groups. No differences were detected between these groups in semen volume (p=0.47), sperm concentration (p=0.83), total motile sperm (p=0.84), or in male (p=0.07) and female age (p=0.06).

CONCLUSION: Cap-Score was associated with a man's probability of generating a pregnancy, substantiating previous work (Schinfeld, et al. 2018). While motility differed between the pregnancy groups, all men were above the 40% WHO cut-off. All pregnancies occurred within the first two rounds of IUI within the normal Cap-Score group. Quickly identifying men with reduced fertility, rather than after multiple failed IUI attempts, was modeled to improve outcomes and save money (Babigumira, et al 2018). Several studies support the improvement of capacitation through lifestyle changes and(or) surgical intervention. Thus, depending on time, resource and treatment goals, multiple options are available for men with reduced Cap-Scores beyond intracytoplasmic sperm injection.

IMPACT STATEMENT: Accurately identifying male fertility is critical in the treatment of the couple seeking fertility assistance. The sooner patients are on the correct treatment path, the better their experience and expected outcome.

SUPPORT: Androvia LifeSciences provided Cap-Scores.

Moody et al, 2017. Mol Repro and Devel. 84(5):408-422.
Cardona, et al. 2017. Mol Repro and Devel. 84(5):423-435.
Schinfeld, et al. 2018. Mol Repro and Devel. 2018;85 (8-9), 654-664.
Babigumira, et al. 2018. JARG. 35:99-106.

Introduction

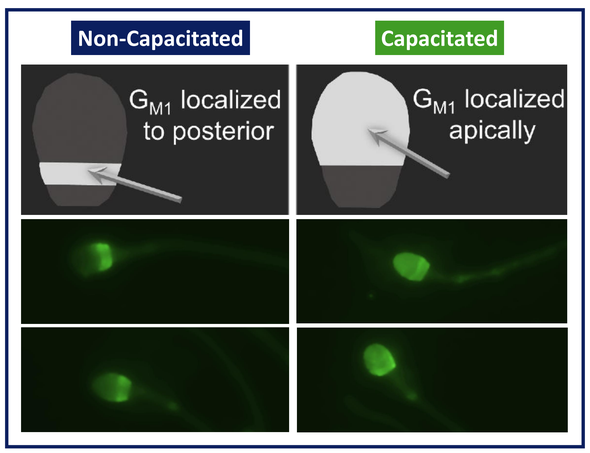

Figure 1. ***Cap-Score™.*** Cap-Score™ is defined as the percentage of capacitated sperm within an ejaculate and is determined by distribution patterns of the ganglioside G_{M1} (Moody et al, 2017). G_{M1} is a key regulator of capacitation and acrosome exocytosis and can be visualized using fluorescence microscopy. 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.

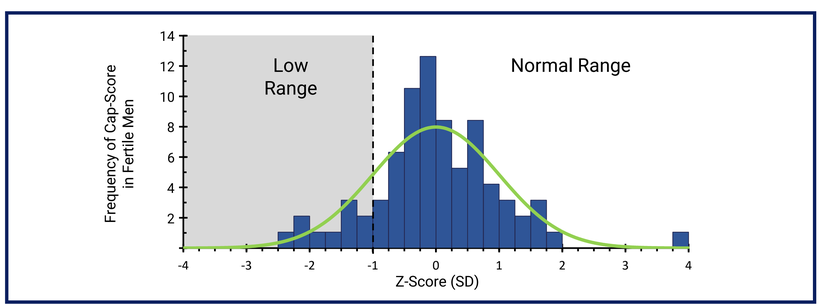

Figure 2. ***Definition of Cap-Score Reference range.*** 187 Cap-Scores, from 76 individuals were obtained. Cap-Scores were averaged by donor and then converted to z-scores, transforming the mean to 0 and each unit to 1 Standard Deviation (SD). The raw data distribution can be viewed in the blue bar chart. A Lilliefor's test determined that the data followed a normal distribution (p = 0.24), which can be seen in the green bell curve. In a normal distribution 68% of values are within one SD of the mean, resulting in approximately 16% of observations being more than one SD below the mean. This was chosen as a cut-off to be conservative, as there is less risk associated with identifying a borderline individual as potentially having low fertility. (Cardona, et al. 2017)

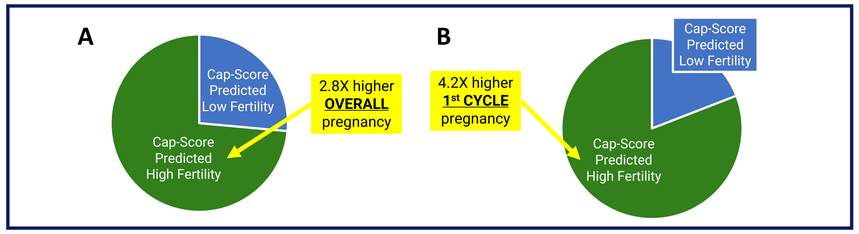

Figure 3. Previously published prospective study results show fertility differences in men with low and normal Cap-Scores. In this prospective study, Cap-Score and semen analysis were performed for 208 couples. Each man's fertility was predicted using the previously defined reference range (**Fig. 2**). Those having Cap-Scores that were more than 1 standard deviation below the mean of the fertile cohort were predicted to have low fertility. Each patient and his partner were followed over three rounds of IUI and pregnancy outcome determined. For this study, female partners were examined, and eligibility was restricted only to those suitable for IUI. At the time of analysis, outcomes were available for 91 couples. (**A**) The proportion of pregnancies following three rounds of IUI, in relation to the number of patients starting treatment, was nearly three times greater for men prospectively predicted of having a normal chance of pregnancy versus those predicted to have a low chance. (**B**) Men predicted of having normal fertility were four times more like to generate a pregnancy in the first round. (Schinfeld, et al. 2018)

Experimental design

Cap-Score and SA were performed (n=107) with clinical Intrauterine Insemination (IUI) outcomes available for 24 at the time of analysis. The chance of pregnancy outcome was predicted as either low (n=9) or normal (n=15), based solely on the previously defined reference range (**Fig. 2**). IUI was done blinded to Cap-Score evaluation. Absolute and cumulative pregnancy rates were compared over 1-3 rounds of IUI using survival analysis (**Tables 1 and 2**). Semen analysis measures were compared between pregnant (n=8) and not-pregnant (n=16) groups (**Table 3**).

Results

Table 1. ***Low Cap-Score (≤ 27.6%)***

IUI #	# Patients	# Pregnant	# Not going to next IUI	Absolute % Pregnant	Proportion Pregnant	Proportion not pregnant	Cum Pregnancy	Cum Nonpreg
1	9	0	6		0	100	0	100
2	3	0	2		0	100	0	100
≥3	1	0	1	0 (0/9)	0	100	0	100

Table 2. ***Normal Cap-Score (> 27.6%)***

IUI #	# Patients	# Pregnant	# Not going to next IUI	Absolute % Pregnant	Proportion Pregnant	Proportion not pregnant	Cum Pregnancy	Cum Nonpreg
1	15	5	2		33	67	33	67
2	8	3	2		38	62	58	42
≥3	3	0	3	53 (8/15)	0	100	58	42

The Cap-Score™ cut off (**Fig. 2**), which was determined using a population of men with known fertility (Cardona et al, 2017), was used to place individuals having undergone IUI into low (**Table 1**) and normal (**Table 2**) groups. Subsequently, information regarding their ability to generate a pregnancy was obtained. A Kaplan-Meier survival analysis was done to prospectively compare the low and normal groups in their abilities to generate pregnancy in relation to the number of IUI attempts (IUI #). This approach clearly demonstrated differences between the low and normal groups in their ability to successfully generate pregnancy (p=0.025). These differences can be observed in the cumulative pregnancies (Cum Pregnancy) columns, which are at least 30 to 60 times greater for those with normal Cap-Scores™.

IUI # - Rounds of IUI attempted; # Patients – number of patients undergoing treatment; # Pregnant – number of patients generating clinical pregnancy; # Not going to next IUI – number of patients discontinuing treatment; Absolute % Pregnant – # pregnant in ≤ 3 IUI / # patients starting treatment; Proportion Pregnant - # Pregnant/# Patients for that IUI #; Proportion not Pregnant – 1-proportion pregnant for that IUI#; Cumulative Pregnacy – 1-Cumulative Nonpreg; Cumulative Nonpreg – Cumulative Nonpreg from previous IUI # * Proportion not Pregnant.

Table 3. ***Semen analysis measures (mean ± Standard Error) for men who were and were not successful in generating pregnancy***

	N	Volume (ml)	Conc (M/ml)	Motility (%)	Total Motile (M)	Male Age (Years)	Female Age (Years)	Cap-Score (%)
Preg	8	2.6 ± 0.5	86.7 ± 12.4	81.1 ± 2.2	188.3 ± 49.6	33.9 ± 1.0	31.9 ± 1.1	35.4 ± 1.7
Not Preg (NP)	16	2.9 ± 0.4	84.1 ± 12.5	73.9 ± 3.5	180.6 ± 30.9	35.7 ± 1.0	33.8 ± 1.0	31.3 ± 2.2
p-value (Preg vs NP)		0.47	0.83	**0.02**	0.84	0.07	0.06	**0.04**

Individuals completed at least one round of IUI; Men were placed in the "Preg" category if their partner conceived in ≤ 3 rounds of IUI. Otherwise, they were placed in the Not Preg (NP) Group; p-value (Preg vs NP) – p-value from a weighted 2-tailed t-test. Weights were determined using the number of IUI rounds. N – Number of observations; Conc – Sperm concentration; Cap-Score™ – proportion of sperm having G_{M1} localization patterns consistent with capacitation (**Fig. 1**). Note that all samples had motilities above 40%, indicating that they were normal according to WHO criteria.

Conclusions

- Cap-Score was associated with a man's chance of generating a pregnancy (**Table 1 and 2**), substantiating previous work (**Fig. 3**; Schinfeld, et al. 2018).
- Although motility differed between the pregnancy groups, all men were above the 40% WHO cut-off (**Table 3**).
- All pregnancies occurred within the first two rounds of IUI within the normal Cap-Score group (**Table 2**).
- Quickly identifying men with reduced fertility, rather than after multiple failed IUI attempts, was previously modeled to improve outcome and save money (Babigumira, et al 2018).
- Several studies support the improvement of capacitation through lifestyle changes and(or) surgical intervention. Thus, depending on time, resource and treatment goals, multiple options are available for men with reduced Cap-Scores beyond intracytoplasmic sperm injection.
- Accurately identifying male fertility is critical in the treatment of the couple seeking fertility assistance. The sooner patients are on the correct treatment path, the better their experience and expected outcome.

Cap-Score results provided by