A Bioassay to Measure Fertilization Competence of Human Spermatozoa

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Abstract

Objective: To test a biomarker-based assay diagnostic of sperm function, specifically the ability to capacitate and fertilize.

Design: We assessed ganglioside G_{M1} localization in sperm as a biomarker to quantify the sub-population that could respond to capacitating stimuli and become fertilization competent. Specific patterns of sperm G_{M1} localization were quantified in basal and capacitating conditions on semen samples of consenting men to predict fertilizing ability.

Materials and Methods: Semen parameters were evaluated according to WHO 2010 criteria. Samples were scored via fluorescence microscopy for G_{M1} localization patterns reflecting capacitation status in at least 200 sperm incubated under both standard and capacitating media. Based on preliminary data and normal timing of human sperm capacitation within 4hrs, we made observations at 1, 2, and 3hrs. Men were categorized as having normal or abnormal capacitation based on pattern frequencies compared to our reference ranges, and then clinical outcomes followed to assess predictive ability.

Results: In all tested men (n=63), average semen parameters were 58.1 ± 20 x10^6/ml, motility of 47.8 ± 8%, and normal morphology of 2.7 ± 1%. We identified 31 men with scores matching the normal reference group, with baseline G_{M1} patterns of 17%-22%-28% in standard and 26%-31%-38% in capacitating media, respectively. We identified 32 men with below reference values of 15%-20%-24% in standard and 20%-25%-29% in capacitating media. Semen parameters were comparable between the two groups. The population with normal range G_{M1} patterns had an IUI pregnancy rate of 45.2% (14/31) of which 8 (25.8%) generated at least one fetal heartbeat. Three additional couples in this group became pregnant on their own. For men with below-reference G_{M1} patterns, the IUI clinical pregnancy rate was only 6.3% (2/32; P=0.03). In this cohort, 13 underwent ICSI and 6 became pregnant (46.2%).

Conclusions: The G_{M1} assay reflected sperm fertilizing ability and could identify men prone to IUI failure irrespective of semen parameters. Assay results may guide selection of optimal ART treatment.

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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. Having the ability to diagnose defects in sperm function would allow clinicians to direct patients toward the most appropriate technology of assisted reproduction. Here we describe a laboratory study designed to assess whether a bioassay—localization of the ganglioside, G_{M1}—could diagnose sperm function. The biological basis of the assay is the process of functional maturation known as capacitation. During capacitation, sperm respond to stimuli within the female tract and acquire the ability to fertilize. Currently, there are no sensitive or simple markers for capacitation that can be used in a clinical setting.

Materials and Methods

Samples from consenting men were assessed for concentration, motility, and morphology according to WHO criteria (WHO, 2010). Specimens were processed to enrich the motile fraction by discontinuous density gradient (Fig. 1). Spermatozoa (2 × 10^6/ml) were incubated in 300 µl control, non-capacitating medium (NON-CAP), or medium with stimuli for capacitation (CAP), prior to localization of G_{M1}. At least 200 sperm were scored for each condition using fluorescence microscopy to determine the relative sperm sub-populations having different G_{M1} localization patterns.

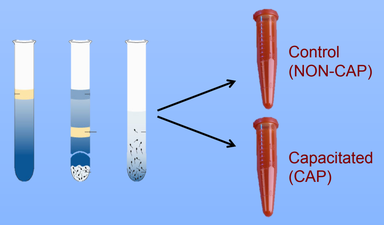

Fig. 1. Motility enrichment by triple layer density gradient followed by incubation in a control or capacitating medium. G_{M1} localization was then determined using fluorescence microscopy.

Table. 1. Comparison of traditional semen analysis parameters revealed no statistically significant differences between men who passed vs failed the assay.

	Concentration (millions/ml) ± S.D.	Motility (%) ± S.D.	Morphology (%) ± S.D.
Pass	62.16 ± 20.79	49.52 ± 6.22	2.73 ± 0.94
Fail	54.20 ± 18.23	46.16 ± 8.49	2.65 ± 0.76

Results

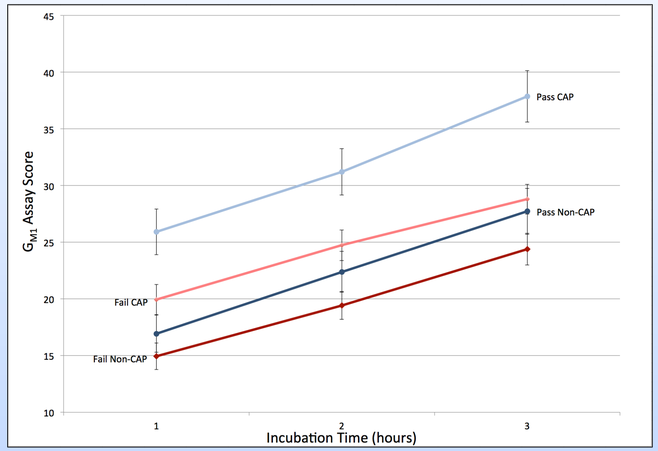

Fig. 2. G_{M1} localization assay scores over time in men who "passed" versus "failed" the assay. Sperm were incubated for 1-3 hours either in basal medium (NON-CAP), or with stimuli for capacitation (CAP). Red lines show the assay scores for men judged as having failed the assay (based on lower rate of change relative to the one hour value, n=32). Blue lines show the assay scores for men judged as having passed the assay (n=31). Error bars show the standard error of the mean. Darker colors reflect NON-CAP conditions and lighter colors reflect CAP conditions.

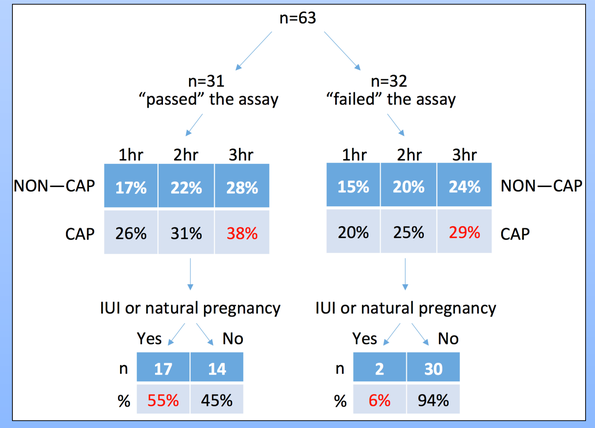

Fig. 3. Success rates of IUI or natural conception was highly correlated with performance in the G_{M1} localization assay. Of the cohort of men who "passed" the assay, 55% had success at IUI or natural conception. In the cohort that "failed" the assay, the IUI clinical pregnancy rate was only 6% (p= 0.03).

Potential Clinical Application

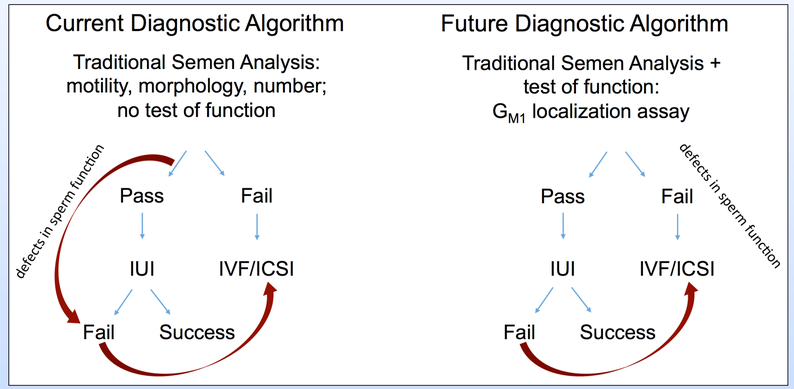

Fig. 4. The G_{M1} localization assay provides a test of sperm function, offering a complement to the descriptive parameters in the traditional semen analysis. Currently, defects in sperm function go undiagnosed by the traditional semen analysis and are only diagnosed by repeated failure at natural conception and IUI, imparting enormous emotional, physical and financial costs to infertile couples. Couples that fail a test of 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.

Conclusions

- Sperm capacitation status was reflected in the pattern of G_{M1} localization; however, the localization patterns need further testing to understand their relationship with male fertility.
- In this pilot study, G_{M1} localization assay scores tended to correlate with IUI results and natural conceptions.
- Samples from men who passed versus failed the assay did not differ significantly in results of traditional semen analysis.

Future Directions

- Reflecting our largely sub-fertile or infertile patient base, more testing is needed to establish normal vs abnormal reference ranges.
- Studies are needed to improve reagent selection, materials specification, instructions for use, incubation parameters, fluorescence imaging, performance data characteristics and guidance for interpretation.
- To become broadly useful, the assay must be refined, adapted for reliability, reproducibility and commercial use, and validated in a broader patient population with controls representing men of normal fertility.