Likert scale (1=very easy, 5=difficult). A total of 27 men uploaded both sets of results (i.e., completed study) and received a $20 e-gift card. The distribution of semen parameters was as expected for a population-based cohort of men aged 24-40 (semen volume: median=3.0, interquartile range (IQR): 2.5-4.2; sperm concentration: median=60.0, IQR: 26.5-90.0; total sperm count: median=157.5, IQR: 71.3-330.0). 6 men (17%) reported abnormal concentration or volume results (< 15 M/mL or < 1.5 mL) further emphasizing utility of early testing. Table 1 shows mean values for selected semen parameters reported using Trak according to selected lifestyle, anthropometric, socioeconomic, and medical history variables. These data provide early evidence that the semen results are consistent with findings from the literature for several health and lifestyle variables and that a geographically diverse group of men from the general population will participate in semen studies.

Conclusion: The Trak System provides a convenient, reliable, easy-to-use, and cost-effective means to measure semen parameters for epidemiological research by allowing men to test at home.

Table 1. Differences in mean semen parameters by selected characteristics, PRESTO / Trak pilot study.

	Semen volume (ml) ß (95% CI)	Sperm conc (M/ml) ß (95% CI)	Total sperm count (M) ß (95% CI)
Age at baseline, years	-0.41 (-3.90, 3.09)	0.83 (-3.32, 74.98)	-1.77 (-17.5, 13.99)
Abstinence time, days	0.50 (0.05, 0.94)	6.55 (-48.5, 17.94)	46.7 (-11.2, 104.53)
Less than college education vs. college education	1.95 (0.66, 3.23)	4.66 (-62.5, 71.8)	165.8 (-105.7, 437.4)
White Non-Hispanic vs. Other Race/Ethnicity	0.74 (-0.28, 1.77)	-8.07 (-43.7, 27.5)	15.8 (-144.6, 176.3)
BMI 25-29 vs. BMI <25 kg/m^2	0.29 (-0.86, 1.43)	-3.02 (-43.8, 37.8)	7.3 (-142.2, 156.8)
BMI ≥30 vs. BMI <25 kg/m^2	0.53 (-0.72, 1.79)	-32.6 (-103.2, 38.0)	-36.2 (-320.7, 248.3)
Current smoker vs. non smoker	-0.06 (-1.48, 1.36)	46.5 (-26.5, 120.4)	135.4 (-106.4, 377.2)
Vigorous physical activity, hours/week	-0.07 (-0.26, 0.12)	-1.46 (-9.60, 6.67)	-1.5 (-28.7, 25.6)
Perceived Stress Scale (PSS-10) score	-0.02 (-0.11, 0.06)	5.23 (-0.46, 10.9)	16.7 (-2.44, 35.8)
Major Depression Inventory (MDI) score	-0.10 (-0.18, -0.01)	-3.03 (-7.78, 1.71)	-15.9 (-32.7, 0.91)
Job hours worked per week	0.06 (-0.01, 0.13)	0.18 (-2.87, 3.24)	5.0 (-5.6, 15.6)
Laptop use on lap, hours/week	-0.19 (-0.33, -0.04)	-5.63 (-11.97, 0.71)	-28.2 (-52.3, 4.03)
Seat heaters (yes vs. no)	0.24 (-0.81, 1.30)	20.0 (-18.3, 58.3)	79.6 (-77.1, 236.3)
Sauna use in previous 6 months (yes vs. no)	-0.87 (-2.15, 0.41)	13.558 (-63.5, 90.40)	-91.31 (-348.16, 165.54)

ß= difference in mean semen parameter for a one-unit increase in characteristic. Parameters demonstrated sufficient normality.
36 men contributed 63 semen samples to this analysis. GEE models take into account clustering of samples within man.

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LOCALIZATION PATTERNS OF THE GANGLIOSIDE GM1 IDENTIFY SPERM CAPABLE OF UNDERGOING CAPACITATION AND ACROSOME EXOCYTOSIS

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Introduction: Sperm must first capacitate and then undergo acrosome exocytosis (AE) to fertilize. Localization patterns of GM1 change in response to capacitation stimuli (Cap-Score™) and track strongly with human clinical fertility. This indirect evidence supported the notion that individual human sperm showing "capacitated" GM1 patterns represented capacitated sperm. Here, we examined directly if "capacitated" GM1 patterns represent cells that are capacitated and can undergo AE.

Methods: 10 semen samples were collected from fertile men (pregnant partner or recent father). Samples were liquefied, washed, and aliquots incubated under non-capacitating (NC) and capacitating (CAP) conditions. For 7 of these samples, a CAP+calcium ionophore A23187 treatment was used to induce AE. Cells were then attached to slides and dual labeled with PNA, a lectin that binds specific carbohydrate residues associated with the acrosome, and Cholera Toxin B to determine GM1 localization and capacitation status.

Results: Under NC conditions, a specific localization pattern of GM1 ("NC") predominated (68.3±3%; n=10). Most of the cells with the NC GM1 pattern did not label with PNA (58.2±6.6%), reflecting an intact plasma membrane and acrosome. The percentage of sperm having the "CAP" pattern of GM1 localization increased from 16.3±2.5% (n=10) under NC conditions, to 28.0±2.6% (n=10) after 3 hours under CAP conditions ($p<0.01$). The majority (63.1±4.4%) of cells with the CAP pattern labeled with PNA over the acrosome. Only 19.3±2.8% had no label, and 17.6±4.0% had equatorial label. These data are consistent with knowledge of plasma membrane dynamics during capacitation, in which point-fusions occur with the underlying outer acrosomal membrane, exposing acrosomal matrix contents. Samples exposed to CAP+A23187 showed a decrease in Cap-Score when compared to CAP samples (CAP: 28.0±2.6%, n=10; CAP+Ionophore: 19.4±1.4%, n=7; $p=0.02$). This finding is consistent with sperm undergoing acrosome exocytosis originating from the sub-population that would otherwise have had a CAP pattern of GM1 localization, and corroborates prior data using mouse sperm.

Conclusion: On a single cell level, sperm having the "CAP" pattern of GM1 localization are those that responded to capacitation stimuli and those that underwent AE. These data substantiate earlier clinical studies on populations of sperm, linking the Cap-Score with sperm function and male fertility.

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STRUCTURAL ORGANIZATION OF THE ACTIN CYTOSKELETON OF THE MOUSE SPERM FLAGELLUM

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The sperm flagellum is essential for sperm motility during their journey through the female reproductive tract. In addition, once near the egg, sperm experience a dramatic change in their motility called hyperactivation that involves a highly asymmetrical movement of the tail and high amplitude bending of the head. The sperm tail can be divided into three regions: midpiece, principal piece, and end piece. In the midpiece, the ODFs are surrounded by a mitochondrial sheath where mitochondria are localized. In the principal piece, the ODFs are surrounded by a fibrous sheath that becomes progressively thinner towards the end piece. To date, the axoneme together with the ODFs and the fibrous sheath are considered the main cytoskeletal components of the sperm tail involved in cell motility. In addition to tubulin, another protein found in the sperm tail is actin. ATP-dependent polymerization of globular monomeric actin (G-actin) to filamentous actin (F-actin) is essential for diverse cellular functions such as cell shape, cell motility, membrane organization, and cytokinesis. F-actin structure depends on the interaction of actin filaments with a variety of interactor proteins such as adducin, ankirin, spectrin

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