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Supplementary Materials for

An automated smartphone-based diagnostic assay for point-of-care semen analysis

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The PDF file includes:

Table S3. Raw data of cryopreserved samples collected for measuring sperm concentration.
Table S4. Raw data collected from clinical specimens at MGH fertility clinic tested by trained users.
Table S5. Repeatability measures for the concentration parameter.
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Table S7. Diagnostic results for samples tested by trained users.
Table S8. Comparison of the performance of three different phones with the smartphone-based semen analyzer.
Table S9. Raw data collected from clinical specimens at MGH fertility clinic tested by untrained users.
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Table S11. CASA triplicate measurements at MGH fertility clinic for 112 samples.
Table S12. Measurement of CASA accuracy against manual analysis.
Legend for movie S1

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/9/382/eaai7863/DC1)

Movie S1 (.mp4 format). Video of a semen sample recorded with the smartphone-based semen analyzer.

Materials and Methods

Weight scale design: We designed a miniaturized weight scale that was wirelessly connected to the smartphone-based platform to measure total sperm count and total motile sperm count in addition to sperm concentration and motility (fig. S7). A micro load cell (CZL639HD_3139, Phidgets), was used as the force sensing module along with an amplifier (HX711, Chenbo). The single point load cell was mounted onto a 3D printed base. A small 3D printed platform was attached to the other end of the load cell. The amplifier was interfaced with a low power microcontroller (ATMEGA328, Kookye). The microcontroller was programmed to read the amplified data and calibrated using samples with known weights. This device was designed to function wirelessly via Bluetooth. The serial connection setup was achieved by interfacing the Bluetooth module (HC-05, JBtek) with the microcontroller. Our customized software application on the smartphone was able to communicate with the device to collect data and calibrate the system when necessary. The 3D printed weight scale was ~76 × 60 × 15 mm in size and weighed ~37 g. The weight scale was powered by two 3 V batteries (CR1620).

Weight scale experiments: We tested the functionality of the portable weight scale using semen samples with clinically relevant sample weights up to 25 g (fig. S8A). The weight scale module was designed to be able to measure samples with a maximum weight of 120 g. We observed a perfect Pearson's correlation coefficient 'r' of 1 (n=27) between the portable scale and a standard laboratory-based grade weight scale (EP64, Explorer Pro).

We proceeded to test the capability of our portable scale to estimate total sperm count and total motile sperm count of semen samples (fig. S8B, C). We tested a total of 14 patient semen samples in our preliminary experiments. The samples' weight was converted to volume by multiplying it by a factor of 1.01 as recommended by the WHO 2010 semen analysis guidelines. The total sperm count and total motile sperm count of the samples were calculated based on the sample volume, concentration, and motility. These results were compared with the results obtained using CASA and a weight scale used at the MGH fertility clinic. A Pearson's coefficient analysis showed a good correlation between the results obtained by the smartphone-based platform and CASA (fig. S8B, C, n=14).

CASA repeatability: In an effort to evaluate the repeatability of the CASA used in our study, we performed replicate measurements for 112 semen samples and calculated its repeatability measurement (table S6). The repeatability of measurements obtained by CASA was defined as the percentage of variation in measured magnitudes of concentration or motility for each individual sample.

It is given by the following formula:

$$\%\,\text{repeatablity} = \left(\frac{\text{mean of measured magnitudes}}{\text{mean of measured magnitudes} + \text{standard error of mean}}\right) * 100$$

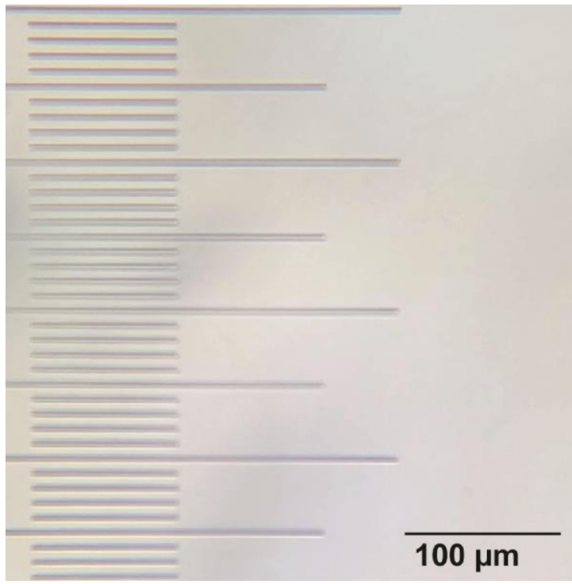


Fig. S1. Image of a micrometer scale taken with the smartphone-based semen analyzer. The spacing between the divisions of the stage micrometer (36121, Edmund optics) is 10 µm. The total image area in pixels is 886 ×886. One micron is represented by 2.4 pixels in this image.

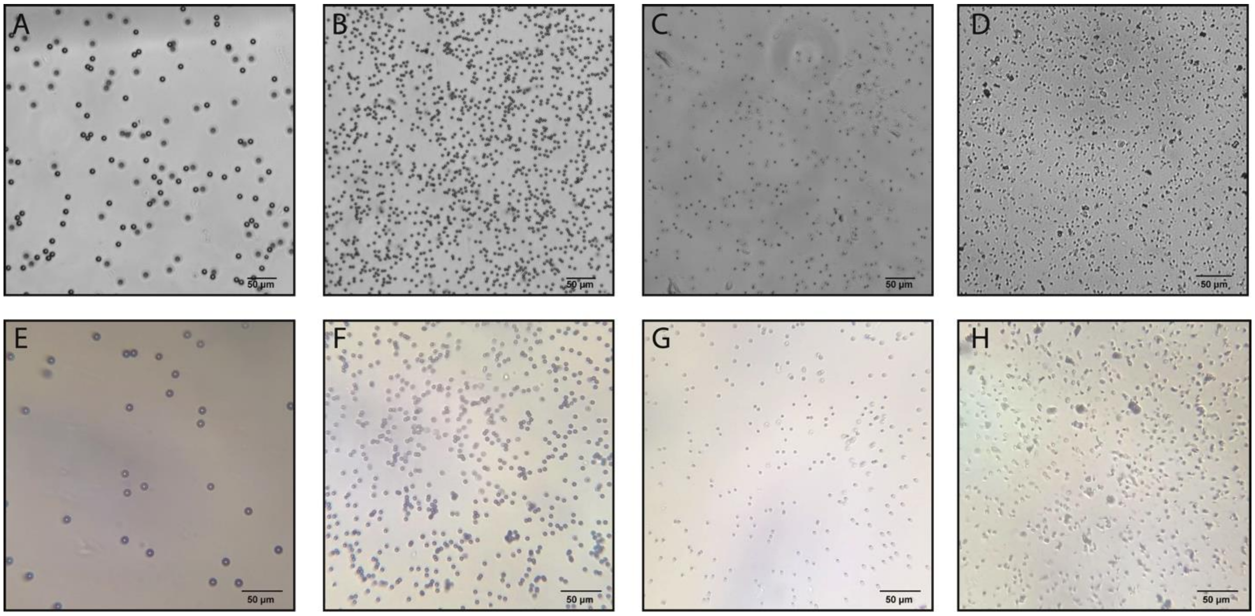


Fig. S2. Images of microbeads and sperm taken with a laboratory-based microscope and the smartphone-based semen analyzer. Monochrome images of (A) 10 µm, (B) 5 µm, (C) 3 µm polystyrene beads, and (D) sperm taken with a desktop microscope (Observer D1, Zeiss) with a 10× objective. Color images of (E) 10 µm, (F) 5 µm, (G) 3 µm polystyrene beads, and (H) sperm taken with the smartphone-based semen analyzer.

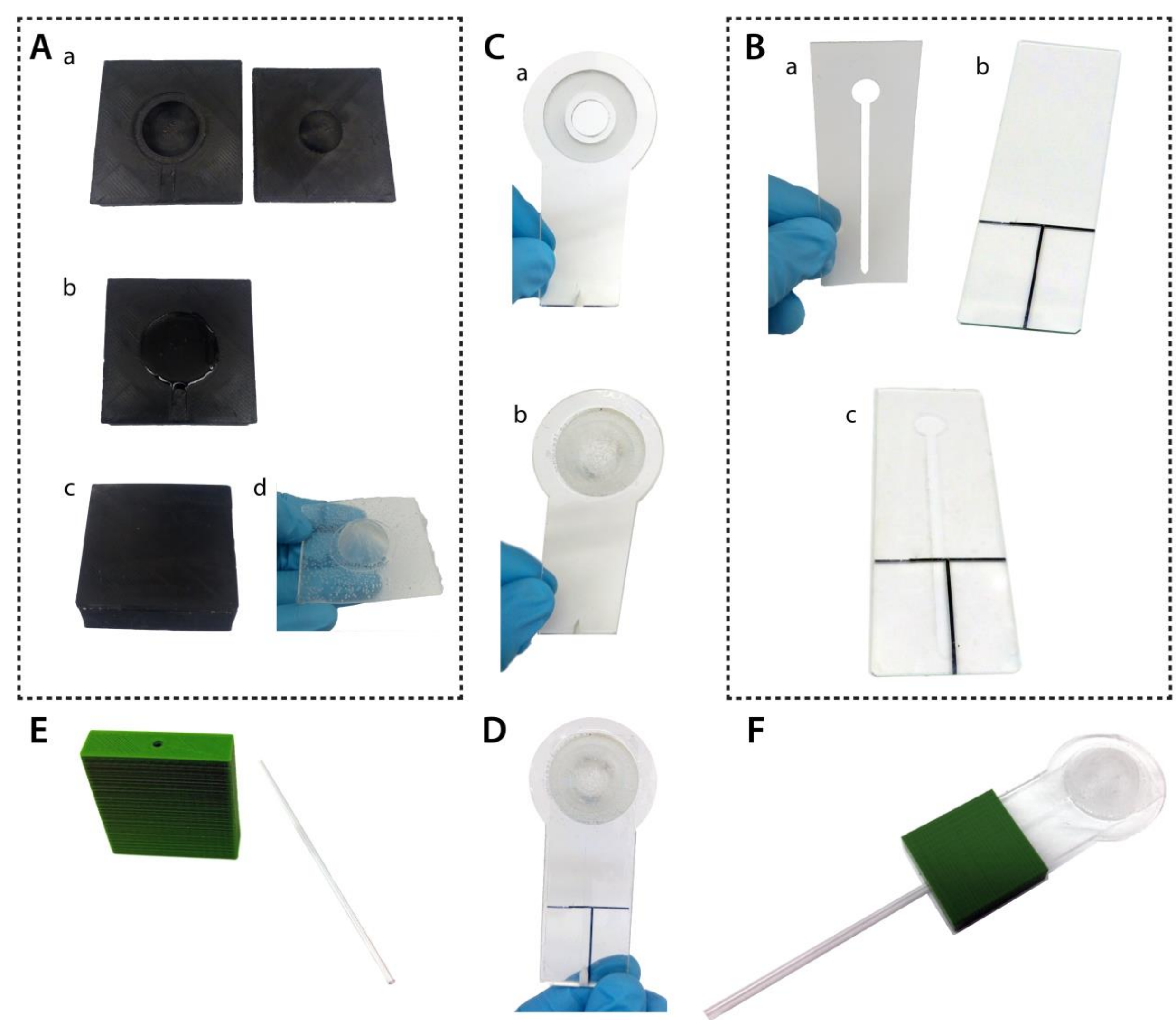


Fig. S3. Process flow of microchip fabrication. (A) Microchip bulb fabrication using PDMS and a 3D printed plastic mold. a) Molds are made by 3D printing ABS to produce a negative and a positive mask. b) The pre-polymer and curing agent are mixed (10:1). c) The positive is placed in the cavity and heated in an oven (60^0 C) for 30 minutes. d) The cured polymer is immediately removed once it has formed. (B) The glass and DSA substrates. a) DSA layer was cut using a laser cutter. b) A regular glass slide was used as one of the substrates. c) The protective thin plastic layer on one side of the DSA was removed, and the DSA was attached to the glass slide. (C) The PMMA substrate and rubber bulb for power-free pumping. a) PMMA was cut and engraved with a laser cutter. The opposite end of the PMMA from the PDMS bulb was engraved to create an inlet for the capillary tube. b) The bulb was attached to the cut PMMA with PDMS. (D) The microchip is completed when (C.b) and (B.c) are attached together. (E) Image of the 3D printed extender and the disposable capillary tip. (F) Image of the actual microchip with the

PDMS bulb. For this design, an extender was 3D printed, a plastic capillary tube was attached to it, the chip inlet was covered with a latex fabric, and a notch was made on the bulb.

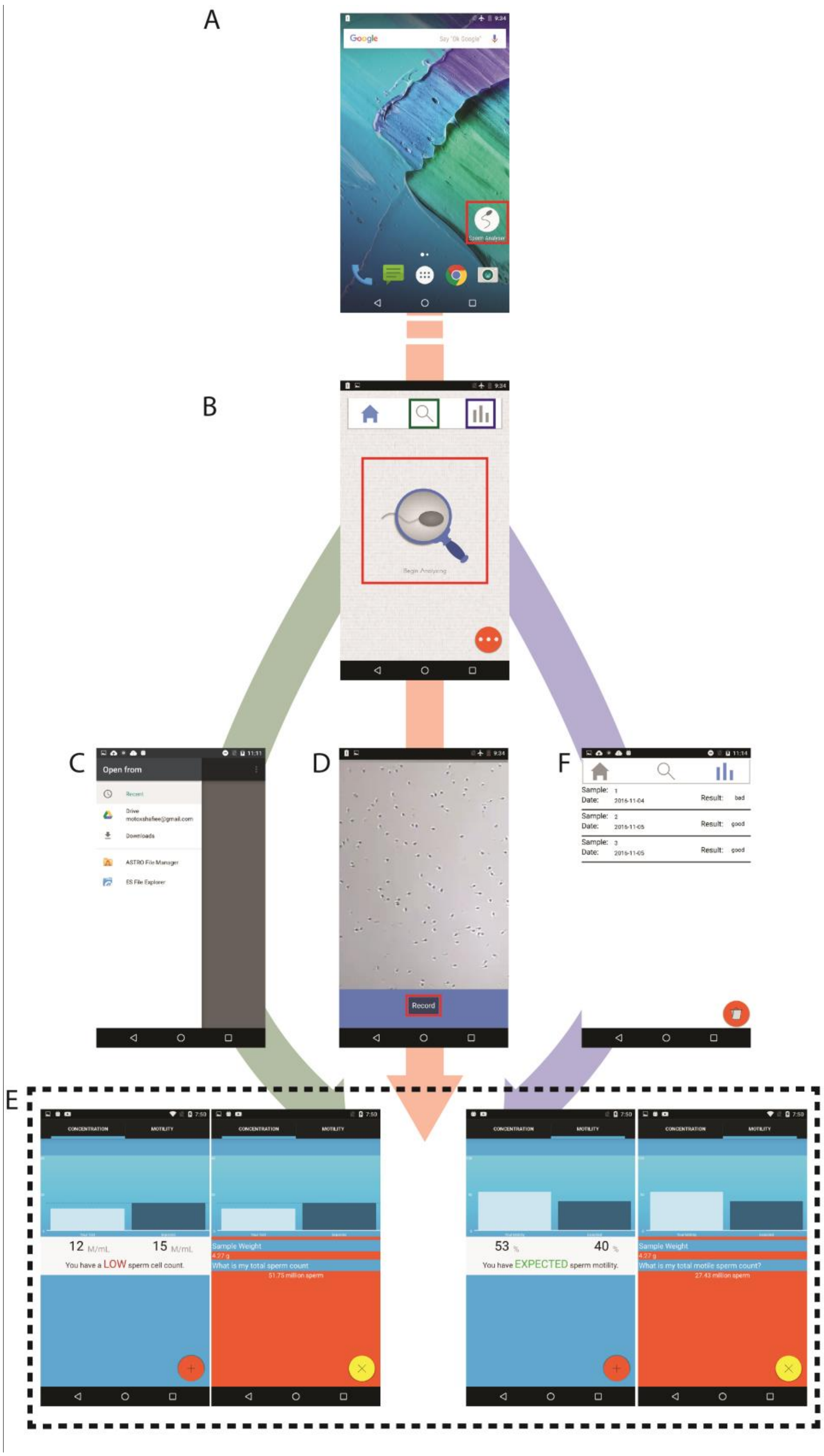


Fig. S4. Smartphone application flow. The schematic shows the process flow of the Android application developed for the smartphone-based semen analyzer. (A) The smartphone application can be selected on the home screen of the cellphone. The smartphone application icon is shown within the red box in this figure. (B) The home screen of the application shows where the different navigation options can be accessed. The schematic has been color coded to show the different possibilities. Green option leads to (C), where pre-recorded videos can be analyzed. (D) The application allows the smartphone camera to get real time access to the sample. It records and analyzes the semen sample and yields the results. The button also accesses the miniaturized weight scale via Bluetooth for semen sample volume measurement. (E) The user will first receive a simple qualitative (positive/negative) result based on the measured values for sperm concentration and motility. The patient will receive one of the following qualitative result reports: (i) You have normal sperm count and motility; (ii) You have low sperm count; (iii) You have low sperm motility; (iv) You have low sperm count and motility. Message (i) is related to negative semen samples and messages (ii, iii, and iv) are related to positive semen samples. Quantitative results for sperm concentration and motility of the sample can also be seen. Additional information can be accessed by pressing the '+' button. The sample weight and the total sperm and total motile sperm count are provided. The blue option leads to (F) where one can access the test result history.

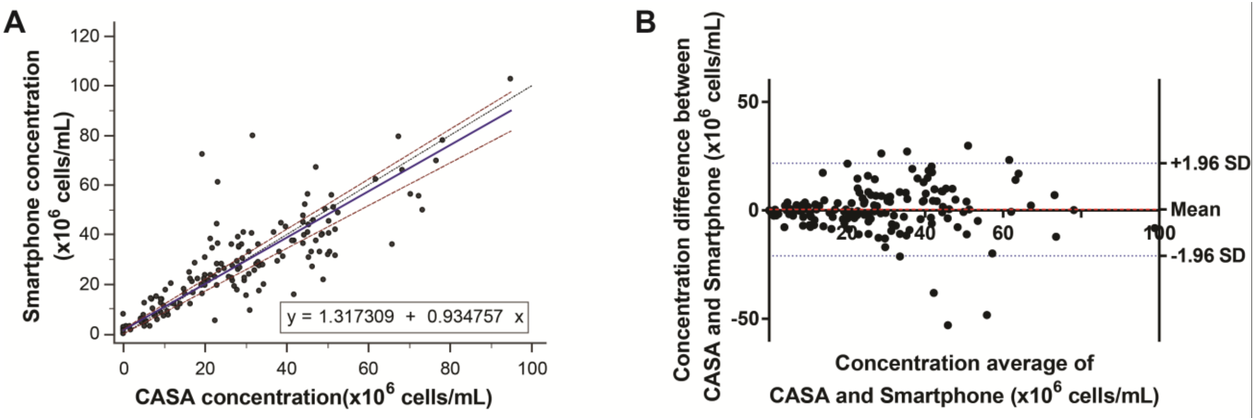


Fig. S5. Device performance for samples with concentrations below 100 million sperm/ml. (A) Passing-Bablok and (B) Bland-Altman analysis to compare sperm concentration measurement results obtained from the smartphone-based semen analyzer and CASA for patient samples with sperm concentrations below 100 million sperm/mL (n=156). The solid blue line represents the regression line, the dotted black line represents the identity line, and the two dotted red lines represent the regression line's confidence band in the Passing-Bablok figure (A). The red dotted line in the Bland-Altman figure (B) is the mean difference of the methods, and the blue dotted lines represent the 95% limits of agreement.

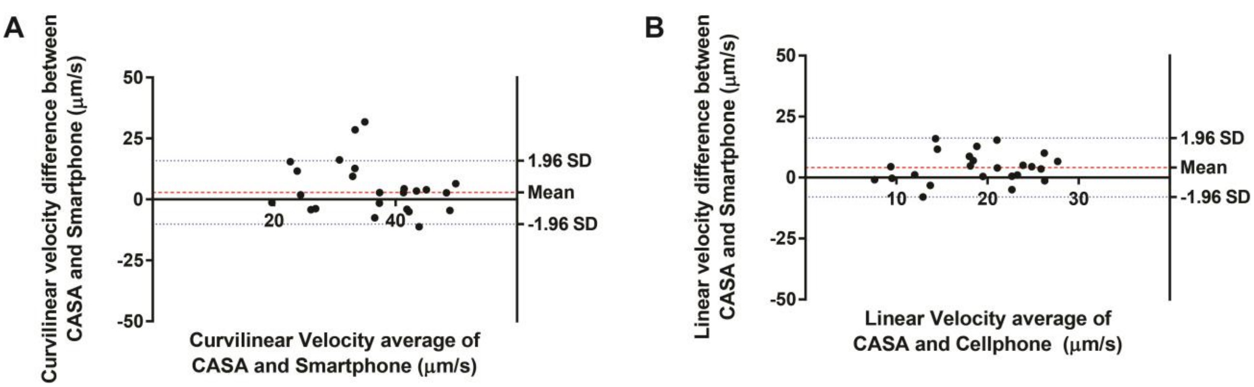


Fig. S6. Bland-Altman analysis for the measurement of sperm curvilinear (VCL) and linear velocities (VSL). (A) Comparison of sperm VCL measured by CASA and the smartphone-based semen analyzer (n=24). The mean bias and standard deviation were 4.618 and 10.63, respectively, with the limits of agreement ranging between -16.22 and 25.46. (B) Comparison of sperm VSL measured by CASA and the smartphone-based semen analyzer (n=24). The mean bias and standard deviation were 4.112 and 6.153, respectively, with the limits of agreement ranging between –7.948 and 16.17. The red dotted line is the mean difference of the methods, and the blue dotted lines represent the 95% limits of agreement in both figures. Two data points from the original data set were eliminated from the analysis as outliers.

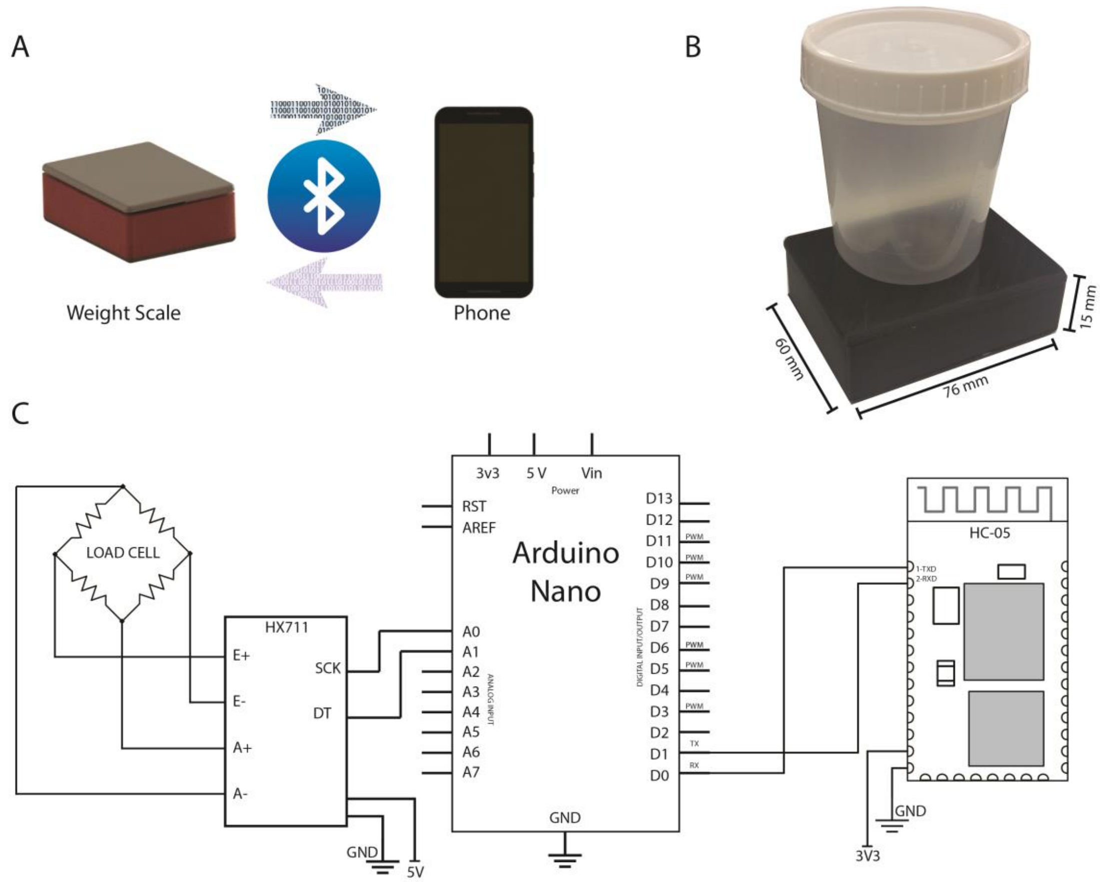


Fig. S7. Weight scale schematic and design. (A) The schematic represents the weight scale and its communication with the smartphone through a Bluetooth connection. It can send data to the smartphone and be controlled wirelessly. (B) An actual image of the 3D printed wireless weight scale with a standard collection cup as reference. (C) A circuit diagram of the weight scale and its components.

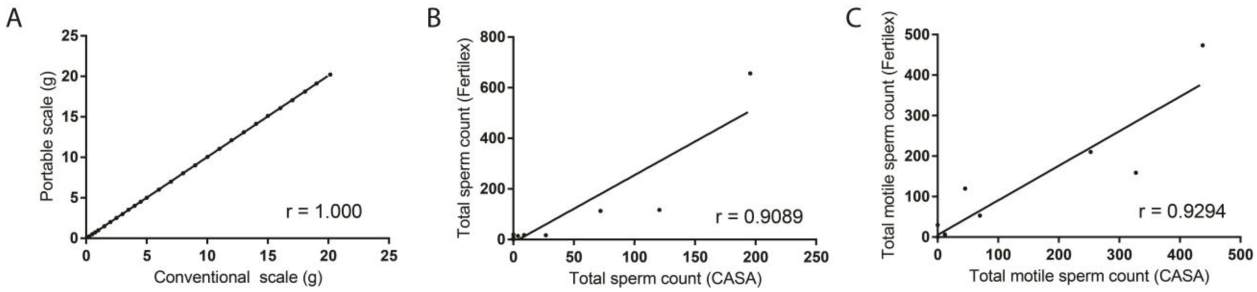


Fig. S8. Weight scale performance in estimating the total count and total motile count. (A) The linear correlation between the smartphone-based weight scale module and a conventional laboratory-based weight scale (EP64 Explorer Pro, Ohaus) on measuring the weight of water samples in microtubes (n=27). The total sperm count (B) and total motile sperm count (C) of semen samples measured by the smartphone-based platform and CASA were compared (n=14). The Pearson's correlation coefficient calculated showed high agreement.

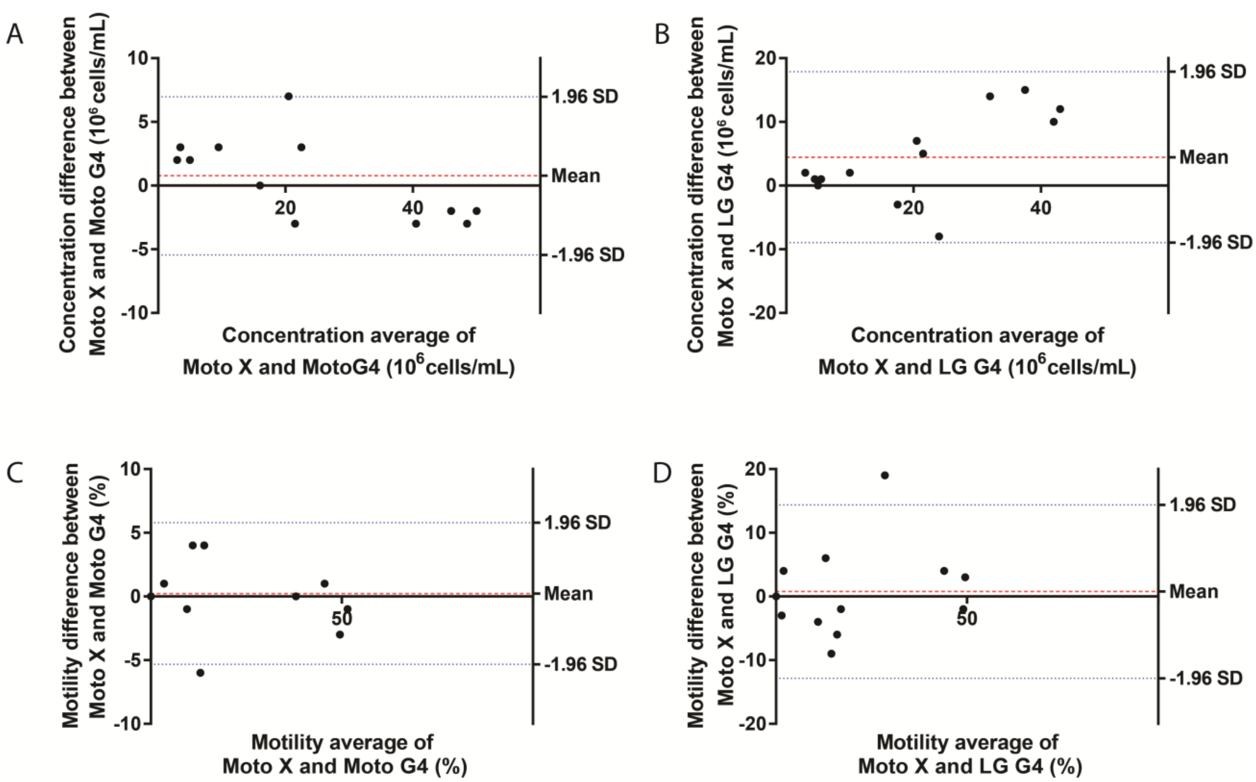


Fig. S9. Bland-Altman analysis of the three phones. Bland-Altman analysis on the sperm concentration measurement results obtained using Moto G4 (A) and LG G4 (B) compared to the results obtained by Moto X (n=13). Bland-Altman analysis on the sperm motility measurement results obtained using Moto G4 (C) and LG G4

(D) compared to the results obtained by Moto X (n=13). The red dotted line is the mean difference of the smartphones, and the blue dotted lines represent the 95% limits of agreement in the Bland-Altman figures.

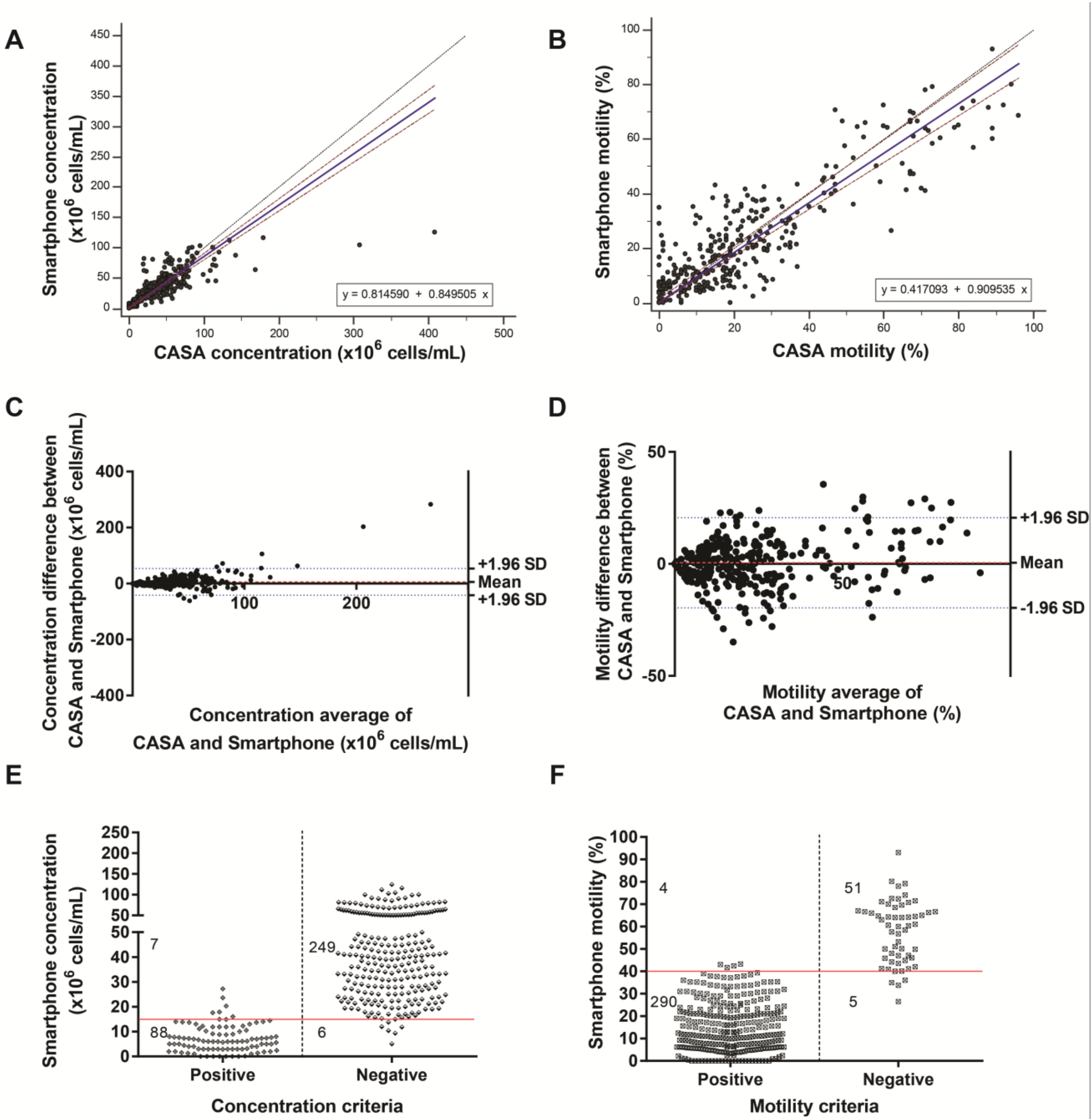
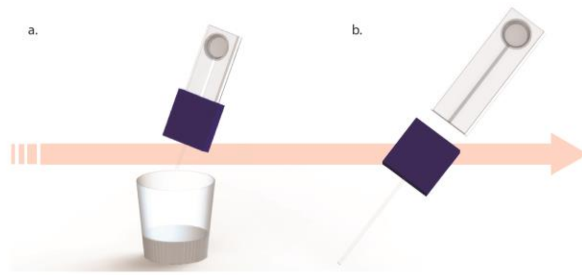


Fig. S10. Overall agreement and diagnostic performance of the smartphone-based semen analyzer. Passing-Bablok analysis to compare (A) sperm concentration and (B) motility as measured by the smartphone-based semen analyzer and CASA for all patient semen samples. The solid blue line represents the regression line, the solid black line represents the identity line, and two dotted red lines represent the confidence band in the Passing-Bablok figures. Bland-Altman analysis to compare (C) sperm concentration and (D) sperm motility as measured by the smartphone-based semen analyzer and CASA for all patient semen samples. The red dotted line in the Bland-Altman figures is the mean difference of the methods, and the blue dotted lines represent the 95% limits of agreement. Scatter plots (E) and (F) represent the qualitative performance of the device in measuring sperm concentration and motility, respectively. The solid red lines represent the threshold values for each criterion. The positive and negative in (E) represent sperm concentration below and above 15 million sperm/mL measured by CASA, respectively. The positive and negative in (F) represent sperm motility below and above 40% measured by CASA, respectively.

Steps of operation of the smartphone-based device

(i) Wait for 20 minutes for semen sample liquefaction at room temperature.

(ii) Load the disposable device with the sample.



a) Press the rubber bulb and insert the disposable device's tip into the sample cup. Release the rubber bulb to draw the sample into the microchip.

b) Discard the disposable capillary tip by pulling it off the microchip.

(iii) Attach the optical hardware accessory to the smartphone and insert the disposable device into the opening slot of the cellphone accessory.

(iv) Initialize the smartphone application, select the magnifying glass on the screen, and click "Record". The semen analysis results will appear on the screen of the smartphone.

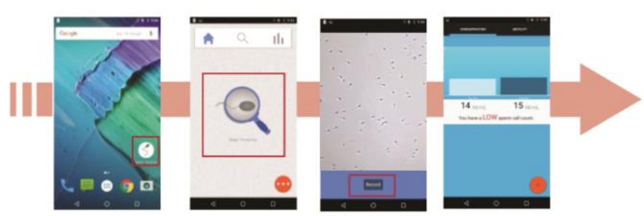


Fig. S11. Steps of operation for semen testing using the smartphone-based semen analyzer. The above figure is a replica of the instructions provided to the untrained users.

Material costs

Item	**Cost (USD)**	**Total (USD)**
3D printed attachment		**3.59**
Lenses	1	
Polylactic filament	1.2	
LED	0.09	
Battery	0.6	
Wires and switches	0.7	
Microchip		**0.86035**
PMMA	0.175	
DSA	0.00035	
PDMS bulb	0.1	
Polylactic filament	0.025	
Glass slide	0.17	
Capillary Tip	0.39	
Total		**4.45035**

Table S1. Table of costs. Manufacturing material costs for hardware attachment and microchip to perform one semen analysis with the smartphone-based semen analyzer. These estimations were calculated based on retail pricing of the materials.

Sample processing times (seconds)

Sample 1	10	*Sample 81*	6	*Sample 161*	0	*Sample 241*	3	*Sample 321*	2
Sample 2	1	*Sample 82*	1	*Sample 162*	0	*Sample 242*	6	*Sample 322*	2
Sample 3	7	*Sample 83*	1	*Sample 163*	0	*Sample 243*	3	*Sample 323*	1
Sample 4	3	*Sample 84*	1	*Sample 164*	0	*Sample 244*	3	*Sample 324*	2
Sample 5	1	*Sample 85*	0	*Sample 165*	1	*Sample 245*	3	*Sample 325*	1
Sample 6	2	*Sample 86*	0	*Sample 166*	1	*Sample 246*	3	*Sample 326*	2
Sample 7	2	*Sample 87*	3	*Sample 167*	1	*Sample 247*	3	*Sample 327*	2
Sample 8	1	*Sample 88*	4	*Sample 168*	2	*Sample 248*	2	*Sample 328*	1
Sample 9	2	*Sample 89*	1	*Sample 169*	2	*Sample 249*	2	*Sample 329*	2
Sample 10	3	*Sample 90*	40	*Sample 170*	4	*Sample 250*	3	*Sample 330*	1
Sample 11	2	*Sample 91*	1	*Sample 171*	1	*Sample 251*	3	*Sample 331*	2
Sample 12	3	*Sample 92*	3	*Sample 172*	1	*Sample 252*	2	*Sample 332*	1
Sample 13	2	*Sample 93*	4	*Sample 173*	1	*Sample 253*	3	*Sample 333*	2
Sample 14	1	*Sample 94*	3	*Sample 174*	3	*Sample 254*	3	*Sample 334*	1
Sample 15	2	*Sample 95*	22	*Sample 175*	4	*Sample 255*	2	*Sample 335*	1
Sample 16	3	*Sample 96*	6	*Sample 176*	4	*Sample 256*	3	*Sample 336*	1
Sample 17	2	*Sample 97*	3	*Sample 177*	4	*Sample 257*	5	*Sample 337*	9
Sample 18	2	*Sample 98*	1	*Sample 178*	3	*Sample 258*	3	*Sample 338*	1
Sample 19	2	*Sample 99*	3	*Sample 179*	3	*Sample 259*	4	*Sample 339*	1
Sample 20	1	*Sample 100*	4	*Sample 180*	3	*Sample 260*	3	*Sample 340*	2
Sample 21	3	*Sample 101*	2	*Sample 181*	3	*Sample 261*	2	*Sample 341*	1
Sample 22	4	*Sample 102*	8	*Sample 182*	2	*Sample 262*	3	*Sample 342*	1
Sample 23	3	*Sample 103*	6	*Sample 183*	2	*Sample 263*	2	*Sample 343*	2
Sample 24	1	*Sample 104*	1	*Sample 184*	2	*Sample 264*	2	*Sample 344*	1
Sample 25	2	*Sample 105*	3	*Sample 185*	3	*Sample 265*	2	*Sample 345*	1
Sample 26	6	*Sample 106*	3	*Sample 186*	3	*Sample 266*	4	*Sample 346*	1
Sample 27	5	*Sample 107*	2	*Sample 187*	3	*Sample 267*	5	*Sample 347*	3
Sample 28	14	*Sample 108*	3	*Sample 188*	4	*Sample 268*	3	*Sample 348*	1

Sample 29	15	*Sample 109*	3	*Sample 189*	2	*Sample 269*	3	*Sample 349*	1
Sample 30	8	*Sample 110*	4	*Sample 190*	2	*Sample 270*	4	*Sample 350*	1
Sample 31	4	*Sample 111*	5	*Sample 191*	1	*Sample 271*	3		
Sample 32	6	*Sample 112*	14	*Sample 192*	6	*Sample 272*	2		
Sample 33	17	*Sample 113*	8	*Sample 193*	1	*Sample 273*	3		
Sample 34	14	*Sample 114*	4	*Sample 194*	5	*Sample 274*	3		
Sample 35	8	*Sample 115*	2	*Sample 195*	1	*Sample 275*	2		
Sample 36	7	*Sample 116*	20	*Sample 196*	1	*Sample 276*	2		
Sample 37	6	*Sample 117*	3	*Sample 197*	1	*Sample 277*	3		
Sample 38	3	*Sample 118*	7	*Sample 198*	1	*Sample 278*	2		
Sample 39	7	*Sample 119*	2	*Sample 199*	3	*Sample 279*	4		
Sample 40	4	*Sample 120*	17	*Sample 200*	4	*Sample 280*	5		
Sample 41	3	*Sample 121*	10	*Sample 201*	3	*Sample 281*	3		
Sample 42	3	*Sample 122*	2	*Sample 202*	4	*Sample 282*	3		
Sample 43	3	*Sample 123*	7	*Sample 203*	2	*Sample 283*	2		
Sample 44	2	*Sample 124*	1	*Sample 204*	2	*Sample 284*	4		
Sample 45	4	*Sample 125*	2	*Sample 205*	2	*Sample 285*	2		
Sample 46	3	*Sample 126*	4	*Sample 206*	3	*Sample 286*	3		
Sample 47	4	*Sample 127*	10	*Sample 207*	2	*Sample 287*	5		
Sample 48	3	*Sample 128*	9	*Sample 208*	1	*Sample 288*	3		
Sample 49	5	*Sample 129*	14	*Sample 209*	3	*Sample 289*	3		
Sample 50	42	*Sample 130*	2	*Sample 210*	2	*Sample 290*	2		
Sample 51	7	*Sample 131*	7	*Sample 211*	2	*Sample 291*	2		
Sample 52	11	*Sample 132*	3	*Sample 212*	1	*Sample 292*	2		
Sample 53	7	*Sample 133*	13	*Sample 213*	2	*Sample 293*	2		
Sample 54	6	*Sample 134*	6	*Sample 214*	1	*Sample 294*	1		
Sample 55	3	*Sample 135*	8	*Sample 215*	3	*Sample 295*	4		

Sample 56	2	*Sample 136*	7	*Sample 216*	3	*Sample 296*	4		
Sample 57	7	*Sample 137*	1	*Sample 217*	8	*Sample 297*	7		
Sample 58	6	*Sample 138*	2	*Sample 218*	3	*Sample 298*	3		
Sample 59	1	*Sample 139*	7	*Sample 219*	3	*Sample 299*	3		
Sample 60	3	*Sample 140*	11	*Sample 220*	3	*Sample 300*	3		
Sample 61	9	*Sample 141*	7	*Sample 221*	4	*Sample 301*	2		
Sample 62	13	*Sample 142*	5	*Sample 222*	3	*Sample 302*	2		
Sample 63	12	*Sample 143*	1	*Sample 223*	1	*Sample 303*	2		
Sample 64	9	*Sample 144*	20	*Sample 224*	2	*Sample 304*	4		
Sample 65	11	*Sample 145*	6	*Sample 225*	2	*Sample 305*	2		
Sample 66	12	*Sample 146*	10	*Sample 226*	2	*Sample 306*	2		
Sample 67	16	*Sample 147*	4	*Sample 227*	4	*Sample 307*	1		
Sample 68	15	*Sample 148*	8	*Sample 228*	6	*Sample 308*	1		
Sample 69	12	*Sample 149*	1	*Sample 229*	2	*Sample 309*	1		
Sample 70	36	*Sample 150*	6	*Sample 230*	4	*Sample 310*	1		
Sample 71	14	*Sample 151*	1	*Sample 231*	2	*Sample 311*	1		
Sample 72	14	*Sample 152*	8	*Sample 232*	6	*Sample 312*	2		
Sample 73	22	*Sample 153*	8	*Sample 233*	4	*Sample 313*	1		
Sample 74	22	*Sample 154*	6	*Sample 234*	3	*Sample 314*	5		
Sample 75	30	*Sample 155*	22	*Sample 235*	1	*Sample 315*	10		
Sample 76	6	*Sample 156*	12	*Sample 236*	3	*Sample 316*	2		
Sample 77	6	*Sample 157*	4	*Sample 237*	3	*Sample 317*	4		
Sample 78	5	*Sample 158*	3	*Sample 238*	2	*Sample 318*	2		
Sample 79	8	*Sample 159*	8	*Sample 239*	1	*Sample 319*	3		
Sample 80	12	*Sample 160*	0	*Sample 240*	2	*Sample 320*	1		

Table S2. Processing times for the 350 samples tested by trained and untrained users. The average time, across all samples, taken for sample processing excluding the image acquisition times (1 s) by the smartphone application was 4.48 seconds, and the median was 3 seconds. The time was calculated by the smartphone application using the smartphone's internal clock.

Raw data for cryopreserved semen samples

	Concentration ($x10^6$ cells/mL)				
Sample #	**Hemocytometer**	**Phone average**	**Measurement 1**	**Measurement 2**	**Measurement 3**
1	3.33E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00
2	3.33E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
3	1.71E-01	2.74E-01	3.08E-01	3.08E-01	2.05E-01
4	2.94E+00	2.91E+00	2.15E+00	2.77E+00	3.80E+00
5	1.03E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
6	9.23E-02	1.03E-01	1.03E-01	1.03E-01	1.03E-01
7	1.63E+00	5.13E-01	5.13E-01	7.18E-01	3.08E-01
8	4.58E+00	3.56E+00	3.39E+00	3.69E+00	3.59E+00
9	3.85E-03	1.03E-01	1.03E-01	1.03E-01	1.03E-01
10	7.41E-02	6.84E-02	1.03E-01	0.00E+00	1.03E-01
11	1.75E+00	9.58E-01	1.03E+00	1.03E+00	8.21E-01
12	5.59E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
13	4.71E-01	3.08E-01	3.08E-01	3.08E-01	3.08E-01
14	6.52E+00	2.15E+00	2.15E+00	2.15E+00	2.15E+00
15	1.09E-01	3.08E-01	3.08E-01	3.08E-01	3.08E-01
16	1.03E+00	1.23E+00	1.23E+00	1.23E+00	1.23E+00
17	6.17E+00	6.02E+00	5.75E+00	5.95E+00	6.36E+00
18	6.71E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
19	3.35E-01	1.37E-01	2.05E-01	1.03E-01	1.03E-01
20	1.93E+00	1.40E+00	1.44E+00	1.54E+00	1.23E+00
21	1.19E+01	1.19E+01	1.22E+01	1.28E+01	1.06E+01
22	1.82E-01	2.05E-01	3.08E-01	2.05E-01	1.03E-01
23	7.29E-01	4.79E-01	5.13E-01	7.18E-01	2.05E-01

24	2.50E+00	2.57E+00	3.18E+00	3.18E+00	1.33E+00
25	6.53E-01	4.79E-01	5.13E-01	4.10E-01	5.13E-01
26	1.96E+00	1.37E+00	1.44E+00	1.44E+00	1.23E+00
27	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
28	6.82E-02	2.05E-01	1.03E-01	3.08E-01	2.05E-01
29	1.08E+00	6.16E-01	6.16E-01	7.18E-01	5.13E-01
30	7.48E+00	4.41E+00	5.44E+00	4.00E+00	3.80E+00
31	4.44E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
32	1.67E-01	1.71E-01	1.03E-01	2.05E-01	2.05E-01
33	7.65E-01	2.39E-01	5.13E-01	1.03E-01	1.03E-01
34	5.46E+00	3.45E+00	3.59E+00	3.69E+00	3.08E+00
35	4.17E-02	6.84E-02	0.00E+00	2.05E-01	0.00E+00
36	3.75E-01	1.71E-01	1.03E-01	2.05E-01	2.05E-01
37	1.27E+00	1.27E+00	1.23E+00	1.33E+00	1.23E+00
38	5.79E+00	2.60E+00	2.15E+00	3.49E+00	2.15E+00
39	6.82E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
40	3.96E-01	4.79E-01	6.16E-01	4.10E-01	4.10E-01
41	1.40E+00	4.10E-01	5.13E-01	3.08E-01	4.10E-01
42	4.07E+00	2.02E+00	1.33E+00	2.87E+00	1.85E+00
43	4.17E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
44	3.33E-01	2.05E-01	3.08E-01	2.05E-01	1.03E-01
45	1.15E+00	1.09E+00	8.21E-01	1.23E+00	1.23E+00
46	4.77E+00	5.81E+00	5.85E+00	5.54E+00	6.05E+00
47	3.23E+01	3.21E+01	3.20E+01	3.22E+01	3.22E+01
48	3.76E+01	4.22E+01	4.19E+01	4.23E+01	4.23E+01
49	3.18E+01	3.41E+01	3.43E+01	3.40E+01	3.40E+01
50	3.18E+01	2.72E+01	2.79E+01	2.68E+01	2.68E+01
51	2.91E+01	2.91E+01	2.91E+01	2.92E+01	2.92E+01
52	3.49E+01	2.72E+01	2.73E+01	2.72E+01	2.72E+01

53	3.34E+01	2.66E+01	2.66E+01	2.65E+01	2.65E+01
54	4.50E+01	4.67E+01	4.63E+01	4.69E+01	4.70E+01
55	3.92E+01	4.76E+01	4.76E+01	4.76E+01	4.76E+01
56	3.28E+01	5.97E+01	5.91E+01	6.00E+01	6.00E+01

Table S3. Raw data of cryopreserved samples collected for measuring sperm concentration. The values reported under 'phone average' are the average of measurements 1, 2, and 3. The average of the coefficient of variation between these repeated measurements was 4.47%.

Raw patient data (trained users)

SAMPLE #	**Total motility (%)**		**Concentration ($x10^6$ cells/mL)**		**Curvilinear velocity (µm/s)**		**Linear velocity (µm/s)**		**Time (s)**
	CASA	Phone	CASA	Phone	CASA	Phone	CASA	Phone	Phone
1	9.00	35.21	307.60	104.77	23.95	28.20	12.10	15.30	10
2	18.00	17.11	5.20	12.20	38.90	22.65	20.50	15.72	1
3	53.00	64.56	408.00	124.74	39.70	44.72	23.80	22.76	7
4	6.00	12.00	41.90	37.44	19.00	20.25	9.35	9.65	3
5	67.00	47.14	6.50	2.88	36.55	38.13	21.90	14.95	1
6	6.00	5.56	11.00	7.35	30.40	14.94	11.60	7.16	2
7	52.00	36.00	9.40	13.85	32.80	40.34	19.70	19.27	2
8	10.00	11.29	6.90	13.73	47.60	19.06	20.30	8.66	1
9	2.00	6.25	7.80	8.03	25.30	23.42	12.55	11.43	2
10	0.00	13.85	38.70	44.73	0.00	23.62	0.00	13.05	3
11	73.00	79.24	134.10	111.86	38.25	49.49	25.60	26.93	2
12	68.00	69.62	178.90	115.88	46.65	51.23	31.00	24.37	3
13	37.00	13.16	5.70	5.04	49.70	47.03	28.70	13.36	2

14	75.00	60.36	142.30	87.59	47.05	43.09	27.65	24.08	1
15	61.00	64.22	67.50	79.63	39.85	43.92	22.95	22.40	2
16	44.00	33.83	28.30	34.69	45.15	41.70	20.20	25.14	3
17	35.00	20.62	27.90	29.29	37.65	28.20	22.35	13.64	2
18	3.00	6.31	23.40	25.42	50.85	19.04	22.25	6.31	2
19	1.00	0.00	27.80	17.66	92.50	21.44	44.30	9.96	2
20	6.00	4.76	4.30	6.03	29.60	18.00	7.15	8.04	1
21	66.00	41.30	116.00	70.07	43.60	39.23	26.40	21.39	3
22	81.00	71.15	77.90	91.95	53.15	46.66	31.25	21.22	4
23	11.00	19.71	23.20	61.23	24.95	28.74	8.90	16.90	3
24	71.00	41.13	9.30	8.76	42.70	39.91	23.05	19.08	1
25	35.00	20.14	24.10	27.97	39.65	26.97	25.20	12.44	2
26	68.00	47.08	168.70	62.74	38.75	36.00	27.05	22.63	6
27	16.00	17.43	4.50	4.48	N/A	N/A	N/A	N/A	5
28	8.00	15.94	30.80	33.17	N/A	N/A	N/A	N/A	14
29	9.00	10.41	27.30	19.05	N/A	N/A	N/A	N/A	15
30	12.00	5.82	30.90	9.33	N/A	N/A	N/A	N/A	8
31	8.00	5.17	16.50	11.83	N/A	N/A	N/A	N/A	4
32	52.00	53.22	37.70	29.26	N/A	N/A	N/A	N/A	6
33	3.00	3.40	34.30	19.93	N/A	N/A	N/A	N/A	17
34	28.00	5.00	20.10	23.61	N/A	N/A	N/A	N/A	14
35	25.00	2.20	22.40	5.07	N/A	N/A	N/A	N/A	8
36	7.00	18.00	13.90	11.89	N/A	N/A	N/A	N/A	7

37	2.00	4.85	30.00	15.31	N/A	N/A	N/A	N/A	6
38	0.00	4.14	0.00	7.80	N/A	N/A	N/A	N/A	3
39	18.00	24.55	46.30	27.15	N/A	N/A	N/A	N/A	7
40	36.00	28.37	49.00	21.81	N/A	N/A	N/A	N/A	4
41	37.00	33.28	47.30	67.19	N/A	N/A	N/A	N/A	3
42	6.00	11.00	5.10	6.10	N/A	N/A	N/A	N/A	3
43	5.00	9.89	11.70	20.29	N/A	N/A	N/A	N/A	3
44	0.00	3.88	1.70	1.34	N/A	N/A	N/A	N/A	2
45	23.00	5.90	9.30	12.26	N/A	N/A	N/A	N/A	4
46	47.00	40.95	16.10	24.81	N/A	N/A	N/A	N/A	3
47	13.00	11.73	9.20	13.71	N/A	N/A	N/A	N/A	4
48	3.00	0.89	33.30	17.31	N/A	N/A	N/A	N/A	3
49	24.00	31.08	41.90	15.64	N/A	N/A	N/A	N/A	5
50	18.00	39.00	31.70	80.00	N/A	N/A	N/A	N/A	42
51	20.00	33.33	29.70	21.21	N/A	N/A	N/A	N/A	7
52	17.00	20.50	29.70	22.51	N/A	N/A	N/A	N/A	11
53	0.33	7.35	73.20	49.93	N/A	N/A	N/A	N/A	7
54	0.00	1.81	21.73	34.41	N/A	N/A	N/A	N/A	6
55	0.67	24.55	20.37	21.39	N/A	N/A	N/A	N/A	3
56	0.00	34.88	26.77	21.23	N/A	N/A	N/A	N/A	2
57	0.33	2.51	46.03	33.13	N/A	N/A	N/A	N/A	7
58	0.00	3.43	48.07	33.15	N/A	N/A	N/A	N/A	6
59	13.67	5.32	5.13	2.99	N/A	N/A	N/A	N/A	1

60	28.67	7.82	19.97	19.50	N/A	N/A	N/A	N/A	3
61	47.33	49.87	50.57	33.01	N/A	N/A	N/A	N/A	9
62	46.33	46.08	49.47	50.18	N/A	N/A	N/A	N/A	13
63	44.00	45.89	39.83	43.20	N/A	N/A	N/A	N/A	12
64	43.67	44.86	43.97	47.63	N/A	N/A	N/A	N/A	9
65	36.67	21.55	36.77	40.85	N/A	N/A	N/A	N/A	11
66	33.67	21.17	45.13	40.90	N/A	N/A	N/A	N/A	12
67	44.67	50.15	44.47	52.19	N/A	N/A	N/A	N/A	16
68	44.00	40.11	72.40	55.48	N/A	N/A	N/A	N/A	15
69	29.00	25.60	43.97	44.82	N/A	N/A	N/A	N/A	12
70	54.67	60.65	94.77	102.93	N/A	N/A	N/A	N/A	36
71	27.33	37.53	45.40	43.74	N/A	N/A	N/A	N/A	14
72	27.33	31.71	46.43	45.88	N/A	N/A	N/A	N/A	14
73	34.67	30.17	76.67	69.67	N/A	N/A	N/A	N/A	22
74	22.00	24.72	45.17	56.20	N/A	N/A	N/A	N/A	22
75	23.67	19.95	61.77	62.39	N/A	N/A	N/A	N/A	30
76	24.67	33.64	48.83	40.13	N/A	N/A	N/A	N/A	6
77	12.67	12.09	24.77	26.25	N/A	N/A	N/A	N/A	6
78	12.33	11.09	21.40	23.63	N/A	N/A	N/A	N/A	5
79	17.33	11.60	20.10	30.92	N/A	N/A	N/A	N/A	8
80	14.00	18.90	23.20	36.19	N/A	N/A	N/A	N/A	12
81	14.33	38.56	32.13	25.53	N/A	N/A	N/A	N/A	6
82	7.00	13.33	0.60	2.98	N/A	N/A	N/A	N/A	1

83	0.00	0.00	0.00	1.81	N/A	N/A	N/A	N/A	1
84	0.00	0.00	0.00	0.04	N/A	N/A	N/A	N/A	1
85	0.00	16.59	0.00	1.42	N/A	N/A	N/A	N/A	0
86	0.00	0.00	0.00	2.44	N/A	N/A	N/A	N/A	0
87	62.00	26.50	22.83	19.48	N/A	N/A	N/A	N/A	3
88	49.67	57.60	8.17	14.87	N/A	N/A	N/A	N/A	4
89	12.00	6.22	1.53	3.16	N/A	N/A	N/A	N/A	1
90	25.00	29.86	19.37	72.30	N/A	N/A	N/A	N/A	40
91	17.00	5.50	9.87	11.14	N/A	N/A	N/A	N/A	1
92	21.67	18.61	19.87	20.62	N/A	N/A	N/A	N/A	3
93	30.33	8.65	33.93	27.66	N/A	N/A	N/A	N/A	4
94	4.33	7.58	28.10	25.72	N/A	N/A	N/A	N/A	3
95	16.33	22.74	51.07	55.89	N/A	N/A	N/A	N/A	22
96	18.33	37.13	70.23	56.15	N/A	N/A	N/A	N/A	6
97	12.00	4.27	8.23	5.78	N/A	N/A	N/A	N/A	3
98	18.67	6.07	13.87	11.93	N/A	N/A	N/A	N/A	1
99	12.33	11.27	19.80	22.19	N/A	N/A	N/A	N/A	3
100	14.33	11.76	65.90	36.08	N/A	N/A	N/A	N/A	4
101	9.67	7.75	19.30	15.94	N/A	N/A	N/A	N/A	2
102	19.67	21.33	50.37	40.33	N/A	N/A	N/A	N/A	8
103	10.67	10.61	17.47	28.50	N/A	N/A	N/A	N/A	6
104	22.33	29.96	7.23	10.28	N/A	N/A	N/A	N/A	1
105	10.00	31.92	19.00	19.40	N/A	N/A	N/A	N/A	3

106	1.00	21.13	33.17	27.40	N/A	N/A	N/A	N/A	3
107	11.00	30.97	26.50	22.44	N/A	N/A	N/A	N/A	2
108	15.33	7.03	14.97	16.02	N/A	N/A	N/A	N/A	3
109	4.33	12.47	10.27	17.58	N/A	N/A	N/A	N/A	3
110	18.00	35.36	16.90	20.95	N/A	N/A	N/A	N/A	4
111	1.00	22.08	30.73	23.97	N/A	N/A	N/A	N/A	5
112	19.00	10.39	22.90	44.15	N/A	N/A	N/A	N/A	14
113	9.00	12.50	44.07	36.30	N/A	N/A	N/A	N/A	8
114	2.33	7.36	14.80	17.88	N/A	N/A	N/A	N/A	4
115	14.33	15.67	17.03	16.67	N/A	N/A	N/A	N/A	2
116	16.33	19.89	51.57	51.10	N/A	N/A	N/A	N/A	20
117	11.67	16.64	22.87	28.05	N/A	N/A	N/A	N/A	3
118	25.33	16.12	32.53	28.23	N/A	N/A	N/A	N/A	7
119	13.33	26.72	13.23	10.52	N/A	N/A	N/A	N/A	2
120	23.00	41.72	50.23	45.27	N/A	N/A	N/A	N/A	17
121	1.33	6.17	26.60	36.07	N/A	N/A	N/A	N/A	10
122	5.33	2.41	12.37	11.87	N/A	N/A	N/A	N/A	2
123	12.67	10.51	29.30	30.50	N/A	N/A	N/A	N/A	7
124	4.00	13.70	4.93	7.39	N/A	N/A	N/A	N/A	1
125	21.33	9.33	22.53	22.88	N/A	N/A	N/A	N/A	2
126	6.67	7.30	18.23	21.99	N/A	N/A	N/A	N/A	4
127	14.00	20.46	28.13	38.97	N/A	N/A	N/A	N/A	10
128	23.67	39.39	41.43	37.38	N/A	N/A	N/A	N/A	9

129	14.33	16.81	21.27	38.24	N/A	N/A	N/A	N/A	14
130	10.67	6.44	6.73	14.42	N/A	N/A	N/A	N/A	2
131	9.67	29.06	26.77	27.19	N/A	N/A	N/A	N/A	7
132	4.33	21.53	25.87	22.75	N/A	N/A	N/A	N/A	3
133	29.33	21.13	52.63	48.62	N/A	N/A	N/A	N/A	13
134	8.33	6.65	28.63	26.63	N/A	N/A	N/A	N/A	6
135	36.33	27.50	47.27	37.50	N/A	N/A	N/A	N/A	8
136	17.33	16.73	45.43	30.67	N/A	N/A	N/A	N/A	7
137	11.33	5.46	7.57	7.65	N/A	N/A	N/A	N/A	1
138	10.00	11.50	10.83	9.16	N/A	N/A	N/A	N/A	2
139	1.00	9.14	39.13	30.95	N/A	N/A	N/A	N/A	7
140	35.67	25.62	48.63	39.83	N/A	N/A	N/A	N/A	11
141	29.67	27.89	51.83	31.54	N/A	N/A	N/A	N/A	7
142	18.00	10.19	34.27	27.88	N/A	N/A	N/A	N/A	5
143	14.33	4.88	9.93	7.30	N/A	N/A	N/A	N/A	1
144	19.00	20.00	68.27	66.00	N/A	N/A	N/A	N/A	20
145	12.00	14.31	50.07	31.73	N/A	N/A	N/A	N/A	6
146	7.33	10.35	44.23	39.80	N/A	N/A	N/A	N/A	10
147	10.33	11.00	32.97	29.00	N/A	N/A	N/A	N/A	4
148	13.67	15.00	51.97	42.00	N/A	N/A	N/A	N/A	8
149	6.00	1.00	4.73	6.18	N/A	N/A	N/A	N/A	1
150	2.33	11.00	33.10	36.00	N/A	N/A	N/A	N/A	6
151	7.33	2.59	7.93	7.27	N/A	N/A	N/A	N/A	1

152	2.67	8.00	29.47	41.00	N/A	N/A	N/A	N/A	8
153	17.33	13.00	28.93	36.00	N/A	N/A	N/A	N/A	8
154	17.00	9.32	38.10	32.82	N/A	N/A	N/A	N/A	6
155	14.33	24.00	78.20	78.00	N/A	N/A	N/A	N/A	22
156	19.33	11.84	31.43	40.51	N/A	N/A	N/A	N/A	12
157	20.67	10.35	16.43	22.25	N/A	N/A	N/A	N/A	4
158	6.67	4.10	20.80	21.49	N/A	N/A	N/A	N/A	3
159	18.00	15.59	28.37	35.22	N/A	N/A	N/A	N/A	8
160	0.00	0.00	0.00	1.54	N/A	N/A	N/A	N/A	0
161	0.00	0.00	0.00	0.00	N/A	N/A	N/A	N/A	0
162	0.00	0.00	0.00	1.00	N/A	N/A	N/A	N/A	0
163	0.00	0.00	0.00	0.15	N/A	N/A	N/A	N/A	0
164	0.00	0.00	0.00	1.00	N/A	N/A	N/A	N/A	0

Table S4. Raw data collected from clinical specimens at MGH fertility clinic tested by trained users. The average processing time excluding the image acquisition time (1s) was 6.63 seconds, and the median time was 4 seconds. The time was calculated by the smartphone application using the cellphone's internal clock.

CASA repeatability values (sperm concentration)

Sample 53	93.12	*Sample 83*	100.00	*Sample 113*	95.99	*Sample 143*	95.29
Sample 54	97.55	*Sample 84*	100.00	*Sample 114*	94.67	*Sample 144*	96.02
Sample 55	93.49	*Sample 85*	100.00	*Sample 115*	90.36	*Sample 145*	97.70
Sample 56	98.04	*Sample 86*	100.00	*Sample 116*	97.96	*Sample 146*	97.32
Sample 57	94.17	*Sample 87*	97.89	*Sample 117*	98.42	*Sample 147*	96.53
Sample 58	96.62	*Sample 88*	90.86	*Sample 118*	99.63	*Sample 148*	98.32
Sample 59	98.31	*Sample 89*	97.87	*Sample 119*	91.86	*Sample 149*	92.65
Sample 60	98.43	*Sample 90*	98.67	*Sample 120*	99.71	*Sample 150*	96.29
Sample 61	96.74	*Sample 91*	95.14	*Sample 121*	94.92	*Sample 151*	93.46
Sample 62	95.05	*Sample 92*	94.79	*Sample 122*	94.67	*Sample 152*	93.50
Sample 63	97.95	*Sample 93*	97.45	*Sample 123*	93.09	*Sample 153*	99.01
Sample 64	98.63	*Sample 94*	96.61	*Sample 124*	96.05	*Sample 154*	98.49
Sample 65	99.00	*Sample 95*	96.70	*Sample 125*	93.09	*Sample 155*	98.04
Sample 66	97.91	*Sample 96*	93.93	*Sample 126*	97.24	*Sample 156*	96.53
Sample 67	97.55	*Sample 97*	95.00	*Sample 127*	95.02	*Sample 157*	93.31
Sample 68	96.51	*Sample 98*	97.95	*Sample 128*	99.10	*Sample 158*	100.00
Sample 69	95.46	*Sample 99*	95.62	*Sample 129*	98.13	*Sample 159*	96.27
Sample 70	97.79	*Sample 100*	95.72	*Sample 130*	93.95	*Sample 160*	100.00
Sample 71	98.15	*Sample 101*	95.21	*Sample 131*	97.18	*Sample 161*	100.00
Sample 72	97.57	*Sample 102*	98.28	*Sample 132*	98.09	*Sample 162*	100.00
Sample 73	96.19	*Sample 103*	98.33	*Sample 133*	97.62	*Sample 163*	100.00
Sample 74	97.57	*Sample 104*	95.79	*Sample 134*	98.17	*Sample 164*	100.00
Sample 75	98.83	*Sample 105*	96.77	*Sample 135*	98.76		
Sample 76	99.44	*Sample 106*	98.71	*Sample 136*	96.18		
Sample 77	95.99	*Sample 107*	93.58	*Sample 137*	93.99		
Sample 78	98.78	*Sample 108*	95.11	*Sample 138*	96.12		

Sample 79	98.05	*Sample 109*	92.20	*Sample 139*	92.99		
Sample 80	95.86	*Sample 110*	92.64	*Sample 140*	97.70		
Sample 81	94.88	*Sample 111*	92.88	*Sample 141*	99.39		
Sample 82	65.11	*Sample 112*	97.40	*Sample 142*	99.05		

Table S5. Repeatability measures for the concentration parameter. The average repeatability was 96.40%.

CASA repeatability values (sperm motility)

Sample 53	50.00	*Sample 83*	100.00	*Sample 113*	90.00	*Sample 143*	86.00
Sample 54	100.00	*Sample 84*	100.00	*Sample 114*	77.78	*Sample 144*	66.57
Sample 55	50.00	*Sample 85*	100.00	*Sample 115*	97.73	*Sample 145*	92.31
Sample 56	100.00	*Sample 86*	100.00	*Sample 116*	96.08	*Sample 146*	84.62
Sample 57	50.00	*Sample 87*	99.08	*Sample 117*	94.59	*Sample 147*	88.57
Sample 58	100.00	*Sample 88*	97.16	*Sample 118*	94.58	*Sample 148*	95.35
Sample 59	75.29	*Sample 89*	81.94	*Sample 119*	91.73	*Sample 149*	85.71
Sample 60	94.51	*Sample 90*	92.31	*Sample 120*	95.83	*Sample 150*	87.50
Sample 61	95.30	*Sample 91*	91.76	*Sample 121*	66.67	*Sample 151*	91.67
Sample 62	94.10	*Sample 92*	86.84	*Sample 122*	81.61	*Sample 152*	88.89
Sample 63	93.84	*Sample 93*	93.73	*Sample 123*	91.33	*Sample 153*	92.27
Sample 64	97.04	*Sample 94*	92.86	*Sample 124*	87.39	*Sample 154*	94.44
Sample 65	92.66	*Sample 95*	98.00	*Sample 125*	98.46	*Sample 155*	94.20
Sample 66	83.02	*Sample 96*	95.41	*Sample 126*	95.24	*Sample 156*	94.15
Sample 67	96.85	*Sample 97*	79.71	*Sample 127*	96.04	*Sample 157*	93.43
Sample 68	96.64	*Sample 98*	93.33	*Sample 128*	97.26	*Sample 158*	88.32
Sample 69	90.63	*Sample 99*	93.33	*Sample 129*	94.20	*Sample 159*	96.89
Sample 70	98.41	*Sample 100*	97.73	*Sample 130*	84.03	*Sample 160*	100.00
Sample 71	94.95	*Sample 101*	88.94	*Sample 131*	88.94	*Sample 161*	100.00
Sample 72	96.87	*Sample 102*	93.12	*Sample 132*	78.29	*Sample 162*	100.00
Sample 73	92.13	*Sample 103*	89.87	*Sample 133*	90.83	*Sample 163*	100.00
Sample 74	97.44	*Sample 104*	89.11	*Sample 134*	90.43	*Sample 164*	100.00
Sample 75	98.61	*Sample 105*	94.54	*Sample 135*	94.33		
Sample 76	93.67	*Sample 106*	100.00	*Sample 136*	95.16		
Sample 77	90.48	*Sample 107*	91.67	*Sample 137*	97.14		
Sample 78	93.33	*Sample 108*	94.56	*Sample 138*	94.54		
Sample 79	92.86	*Sample 109*	86.67	*Sample 139*	100.00		

Sample 80	93.33	*Sample 110*	92.18	*Sample 140*	97.59		
Sample 81	94.20	*Sample 111*	63.40	*Sample 141*	98.89		
Sample 82	50.00	*Sample 112*	95.00	*Sample 142*	96.89		

Table S6. Repeatability measures for the motility parameter. The average repeatability was 91.00%.

		Smartphone		
		Negative	Positive	
CASA	Negative	17	2	Specificity: 89.47%
	Positive	1	144	Sensitivity: 99.31%
				Accuracy: 98.17%

Table S7. Diagnostic results for samples tested by trained users. Contingency table for diagnosis based on both sperm concentration and motility parameters using patients' semen samples (n=164). Negative indicates semen samples with sperm concentration and motility above the WHO threshold (sperm concentration > 15 million sperm/mL and sperm motility > 40%). Positive indicates semen samples with sperm concentration or motility below the WHO threshold (sperm concentration < 15 million sperm/mL or sperm motility < 40%).

	MOTO X			MOTO G4			LG G4		
	Concentration (x 10^6 sperm/mL)	Total Motility (%)	Time (s)	Concentration (x 10^6 sperm/mL)	Total Motility (%)	Time (s)	Concentration (x 10^6 sperm/mL)	Total Motility (%)	Time (s)
Sample 1	11	9	1	8	10	7	9	13	2
Sample 2	24	13	1	17	9	6	19	19	3
Sample 3	16	16	1	16	12	6	19	10	3
Sample 4	20	16	1	23	12	6	28	18	2
Sample 5	24	10	1	21	16	5	17	19	4
Sample 6	45	46	3	47	45	11	30	42	5
Sample 7	47	48	6	50	51	8	37	50	30
Sample 8	49	51	4	51	52	10	37	48	6
Sample 9	4	0	1	2	0	3	2	0	2
Sample 10	5	0	1	2	0	4	5	3	2
Sample 11	6	4	1	4	3	4	5	0	2
Sample 12	5	0	1	2	0	4	4	0	2
Sample 13	39	38	2	42	38	11	25	19	6

Table S8. Comparison of the performance of three different phones with the smartphone-based semen analyzer. Green and blue colors represent values greater and smaller than the WHO thresholds for the corresponding semen parameter (sperm concentration of 15 million sperm/mL; sperm motility of 40%), respectively. The average times for analyzing the 13 semen samples using Moto X, Moto G4, and LG G4 were 1.85 s, 6.54 s, and 5.31 s, respectively. The median times for semen analysis were 1 s, 6 s, and 3 s for the three phones.

Raw patient data (untrained users)

	Total motility (%)		**Concentration (x10^6 sperm/mL)**		**Time (s)**
Sample #	**CASA**	**Smartphone**	**CASA**	**Smartphone**	
1	27	10	50.8	28.21	1
2	0	4.054	34	17.536	1
3	27	23.21	23.9	22.3	1
4	18	8.09	52.1	39.02	2
5	37	43.33	36.8	38.522	2
6	32	37.731	84.7	75.103	4
7	28	33.66	119.8	92.079	1
8	26	33.18	53.9	50.94	1
9	2	1	38.5	19.34	1
10	20	12.79	79.8	61.72	3
11	22	35.29	49.4	31.74	4
12	18	12.72	57.2	72.54	4
13	13	10.87	105.1	45.45	4
14	17	11.95	60.9	52.31	3
15	20	5.7	76.6	36.6	3
16	11	10	46.5	45.75	3
17	19	17.47	52.8	40.32	3
18	26	37.26	49	40.94	2
19	25	37.92	10.9	5.59	2
20	49	66.66	50.2	90.28	2

21	6	3.84	24.1	40.8	3
22	1	8.2	48.1	34.31	3
23	89	64.01	86.4	81.67	3
24	7	7.4	55.6	34.51	4
25	67	66.3	84.4	85.44	2
26	13	11.63	66.3	68.57	2
27	94	80.1724	111.7	99.64	1
28	14	24.29	56	38.48	6
29	21	26.8	70	59.13	1
30	29	34.37	116.2	44.65	5
31	25	20.26	39	39.3643	1
32	7	7.88	48.1	50.38	1
33	17	9.13	47.9	48.65	1
34	33	35.29	20.2	81.74	1
35	33	23.8	113.9	80.95	3
36	8	12.9	56.4	49.119	4
37	6	5.16	33.4	41.87	3
38	4	7.4	15.3	13.74	4
39	92	72.41	9.6	6.7	2
40	3	5.7	40.3	25.18	2
41	24	31	59.9	48.6	2
42	21	15.51	59.7	64.65	3
43	4	10.29	60.8	50.57	2

44	1	6.2	48.6	33.34	1
45	67	67	54.5	82.5	3
46	84	74	46.9	32	2
47	19	19.17	58.9	47.4	2
48	13	5.8	63.4	42.69	1
49	1	1.17	22	19.339	2
50	12	7.77	53.8	41.6	1
51	28	19.4	69.5	68.8	3
52	69	66.66	90.9	90.47	3
53	17	21.02	38.2	36.78	8
54	26	30.65	53.9	43.6	3
55	11	4.7	47.6	55.07	3
56	88	71.551	76.2	55.17	3
57	28	22.522	12.8	27.2105	4
58	73	58.477	99	55.6889	3
59	0	3.9	34	28.34	1
60	11	3.8	46.8	21.01	2
61	0	7.38	47.9	35.06	2
62	0	6	40.2	50.55	2
63	28	33.928	61.5	51.37	4
64	47	70.81	52.6	80.51	6
65	28	20	53.2	51.89	2
66	47	43.54	56.2	53.7	4

67	35	30.67	74.4	64.25	2
68	21	33.81	63.3	46.65	6
69	3	6.18	40.3	17.511	4
70	15	17.3	54.8	53.07	3
71	11	9.31	42.7	44.16	1
72	10	5.6	47.1	45.71	3
73	6	0	68.1	41.86	3
74	2	5.6	77.1	58.93	2
75	7	11.7	74.2	42.33	1
76	10	4.4	44.6	29.93	2
77	0	7.76	41.7	25.01	3
78	1	7.66	24.6	23.8815	6
79	28	37.962	60	51.4514	3
80	18	23.9	54.3	73.63	3
81	79	64.28	10.3	6.2	3
82	84	56.75	10.8	8.82	3
83	20	17.45	51.2	47.78	3
84	19	11.71	57.5	27.8	2
85	7	4.8	69.7	34.41	2
86	0	0	51	23	3
87	40	35.2	20	61.6	3
88	29	19.58	60.3	28.17	2
89	26	21.02	43.2	47.15	3

90	19	29	54	50.51	3
91	10	8.38	46.7	33.42	2
92	22	22.96	7.8	3.02	3
93	24	23.76	59.9	47.42	5
94	17	7.79	70.7	44.13	3
95	0	6.89	28.7	15.5764	4
96	55	66	80.1	99	3
97	96	68.58	108.5	62.07	2
98	19	10.74	41.5	41.5	3
99	28	42.43	69.5	62.43	2
100	2	6.194	46.9	26.26	2
101	33	15.38	8.1	7.59	2
102	43	34.92	112.2	71.66	4
103	19	6.2	50.2	42.3403	5
104	15	16.12	46.7	50.62	3
105	1	7.246	23.7	17.8144	3
106	13	7.4	48	41.15	4
107	21	9.9	46.7	47.6	3
108	19	8	61.5	32.1	2
109	19	17.2	79.3	51.45	3
110	60	64.74	84	100	3
111	24	31.1627	52.6	50.73	2
112	60	72.566	58.5	79.64	2

113	1	4.7	74.5	39.32	3
114	59	44.23	73.8	50.15	2
115	15	22.42	46.7	23.41	4
116	6	10.44	60.5	42.92	5
117	26	20.89	43.2	10.3679	3
118	4	11.28	71.7	40.23	3
119	26	24.43	49	76.97	2
120	1	5.3	14.1	23.61	4
121	15	8.48	44.2	58.01	2
122	21	22.93	50.7	56.43	3
123	16	13.86	48.1	57.25	5
124	0	0.42	58.3	16.145	3
125	0	0	18.7	22.39	3
126	1	3.62	39.7	30.9	2
127	28	19.56	68.2	57.75	2
128	9	8.6	52.1	44.79	2
129	0	0	22.1	16.48	2
130	35	21.91	55.7	21.04	1
131	2	5.9	46.9	33.21	4
132	1	19	16.9	19	4
133	15	43	47.1	56	7
134	72	63	25.8	23	3
135	71	64	36.8	20	3

136	19	18	13.9	14	3
137	18	31	18	25	2
138	28	9	9.6	13	2
139	13	19	10.3	15	2
140	11	21	5.4	7	4
141	16	25	5.7	6	2
142	0	0	0	0	2
143	0	0	0	0	1
144	3	16	18.4	15	1
145	80	65	43.2	32	1
146	22	36	44.3	46	1
147	71	78	25.9	19	1
148	68	61	34.1	25	2
149	18	27	11.5	13	1
150	18	23	19.4	17	5
151	19	0	10.4	3	10
152	14	21	9.8	12	2
153	5	9	6.5	10	4
154	26	15	5.8	5	2
155	23	14	4.3	4	3
156	25	21	5.3	2	1
157	24	22	7.6	14	2
158	65	51	10.1	7	2

159	34	26	6.9	6	1
160	46	40	7.8	7	2
161	20	13	6.8	3	1
162	32	20	2.8	5	2
163	0	0	0.4	5	2
164	34	29	7.4	6	1
165	0	0	0.2	6	2
166	58	50	4.4	3	1
167	70	42	5	3	2
168	89	60	16	12	1
169	22	28	81.2	34	2
170	0	0	0	0	1
171	0	0	0	2	1
172	21	11	6.9	6	1
173	19	11	5.7	3	9
174	19	20	9.3	3	1
175	67	70	9	5	1
176	27	26	8.8	5	2
177	18	15	6.9	4	1
178	29	19	3.1	4	1
179	0	0	1	4	2
180	33	30	6	9	1
181	0	0	0	0	1

182	67	48	6.8	8	1
183	89	93	16	17	3
184	0	29	41.5	32	1
185	0	0	0	2	1
186	0	0	0	0	1

Table S9. Raw data collected from clinical specimens at MGH fertility clinic tested by untrained users. The average processing time excluding the image acquisition time (1s) was 2.56 seconds, and the median time was 2 seconds. The time was calculated by the smartphone application using the cellphone's internal clock.

		Smartphone		
		Negative	Positive	
CASA	Negative	22	2	Specificity: 91.67%
	Positive	3	159	Sensitivity: 98.15%
				Accuracy: 97.31%

Table S10. Diagnostic results for samples tested by untrained users. Contingency table for results based on both sperm concentration and motility parameters for patient semen samples (n=186). Negative indicates semen samples with sperm concentration and motility above the WHO threshold (sperm concentration > 15 million sperm/mL and sperm motility > 40%). Positive indicates semen samples with sperm concentration or motility below the WHO threshold (sperm concentration < 15 million sperm/mL or sperm motility < 40%).

CASA replicates

	Total motility (%)			**Concentration (x10^6 sperm/mL)**		
Sample #	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Sample 53	1	0	0	62.4	78	79.2
Sample 54	0	0	0	21	21.4	22.8
Sample 55	0	2	0	23.2	18.8	19.1
Sample 56	0	0	0	27.2	27.4	25.7
Sample 57	0	1	0	51.6	42.2	44.3
Sample 58	0	0	0	46	46.8	51.4
Sample 59	5	20	16	5.1	5.3	5
Sample 60	27	32	27	19.4	20	20.5
Sample 61	51	48	43	53.9	49.5	48.3
Sample 62	41	47	51	53.7	49.9	44.8
Sample 63	49	39	44	41.5	39	39
Sample 64	45	41	45	43.8	43	45.1
Sample 65	42	36	32	37.5	36.5	36.3
Sample 66	39	42	20	43.8	44.6	47
Sample 67	47	45	42	46.7	43.3	43.4
Sample 68	43	47	42	77.2	68.2	71.8
Sample 69	26	35	26	39.8	45.7	46.4
Sample 70	53	55	56	93.2	99	92.1
Sample 71	25	30	27	44.7	47.1	44.4
Sample 72	27	26	29	45.7	48.7	44.9
Sample 73	29	39	36	70.6	79.5	79.9
Sample 74	21	23	22	45.2	43.2	47.1
Sample 75	23	24	24	62	60.4	62.9
Sample 76	28	23	23	48.3	49.2	49

Sample 77	14	10	14	25.9	25.7	22.7
Sample 78	11	14	12	20.9	21.8	21.5
Sample 79	20	16	16	20.5	20.5	19.3
Sample 80	13	16	13	24.3	24.1	21.2
Sample 81	16	13	14	29.5	35.4	31.5
Sample 82	0	21	0	0.7	1.1	0
Sample 83	0	0	0	0	0	0
Sample 84	0	0	0	0	0	0
Sample 85	0	0	0	0	0	0
Sample 86	0	0	0	0	0	0
Sample 87	62	63	61	22	22.8	23.7
Sample 88	50	52	47	7.2	7.5	9.8
Sample 89	17	11	8	1.5	1.5	1.6
Sample 90	29	24	22	19.8	19.4	18.9
Sample 91	14	19	18	10.6	8.9	10.1
Sample 92	20	28	17	18.4	19.2	22
Sample 93	27	34	30	35.7	32.9	33.2
Sample 94	4	5	4	27.9	29.9	26.5
Sample 95	16	16	17	53.9	51.4	47.9
Sample 96	18	20	17	61.6	72.1	77
Sample 97	16	6	14	7.5	9	8.2
Sample 98	16	20	20	13.8	13.4	14.4
Sample 99	14	12	11	21.5	19.5	18.4
Sample 100	14	14	15	61.3	71.4	65
Sample 101	8	9	12	21.2	18.7	18
Sample 102	22	20	17	48.6	51.3	51.2
Sample 103	13	10	9	17.6	16.9	17.9

Sample 104	26	24	17	6.6	7.6	7.5
Sample 105	9	10	11	19	17.9	20.1
Sample 106	1	1	1	33.6	33.6	32.3
Sample 107	10	10	13	29.7	26.4	23.4
Sample 108	15	14	17	13.5	16.1	15.3
Sample 109	3	5	5	12	9.5	9.3
Sample 110	20	19	15	17.3	14.4	19
Sample 111	0	2	1	35.2	27.2	29.8
Sample 112	20	17	20	24.1	22.1	22.5
Sample 113	8	11	8	45.6	40.4	46.2
Sample 114	3	1	3	14.4	16.4	13.6
Sample 115	14	14	15	13.4	18.8	18.9
Sample 116	17	17	15	53.7	50.3	50.7
Sample 117	13	11	11	22.5	23.6	22.5
Sample 118	23	28	25	32.3	32.7	32.6
Sample 119	14	11	15	14.2	14.6	10.9
Sample 120	24	24	21	50.2	50	50.5
Sample 121	2	0	2	24.3	29.2	26.3
Sample 122	7	3	6	11.1	13.5	12.5
Sample 123	12	11	15	25.2	32.6	30.1
Sample 124	4	3	5	4.6	4.9	5.3
Sample 125	21	21	22	24.9	23.4	19.3
Sample 126	6	7	7	18.8	17.2	18.7
Sample 127	15	14	13	31	27.3	26.1
Sample 128	23	25	23	41.4	40.8	42.1
Sample 129	16	14	13	22	21.2	20.6
Sample 130	14	7	11	7.6	6.3	6.3

Sample 131	12	8	9	26.2	28.3	25.8
Sample 132	6	5	2	24.9	26.1	26.6
Sample 133	35	28	25	50.8	52	55.1
Sample 134	10	8	7	29.7	28.1	28.1
Sample 135	39	38	32	48.4	47	46.4
Sample 136	19	17	16	45.9	48.3	42.1
Sample 137	11	11	12	8.1	6.6	8
Sample 138	10	9	11	10.5	11.7	10.3
Sample 139	1	1	1	38.8	44.4	34.2
Sample 140	34	37	36	50.8	46.9	48.2
Sample 141	30	30	29	52.4	51.8	51.3
Sample 142	17	18	19	34.1	33.8	34.9
Sample 143	12	19	12	9.6	10.9	9.3
Sample 144	11	8	38	63.4	68.2	73.2
Sample 145	10	13	13	48.3	52.3	49.6
Sample 146	6	6	10	41.8	45.6	45.3
Sample 147	9	13	9	31	35.1	32.8
Sample 148	15	13	13	53	50.2	52.7
Sample 149	4	7	7	4.7	4.1	5.4
Sample 150	3	2	2	35.4	32.9	31
Sample 151	8	8	6	6.9	8.1	8.8
Sample 152	3	3	2	28.5	26.5	33.4
Sample 153	17	15	20	29	28.4	29.4
Sample 154	16	16	19	39	38.3	37
Sample 155	14	13	16	81	78	75.6
Sample 156	21	17	20	30.9	33.6	29.8
Sample 157	18	21	23	14.2	16.9	18.2

Sample 158	8	7	5	20.8	20.8	20.8
Sample 159	18	19	17	30.2	28.5	26.4
Sample 160	0	0	0	0	0	0
Sample 161	0	0	0	0	0	0
Sample 162	0	0	0	0	0	0
Sample 163	0	0	0	0	0	0
Sample 164	0	0	0	0	0	0

Table S11. CASA triplicate measurements at MGH fertility clinic for 112 samples.

Sperm concentration	# of samples	Mean % variation
<5 M/ml	10	86.7%
5-10 M/ml	34	9.9%
11-20 M/ml	104	4.4%
21-50 M/ml	338	2.5%
51-100 M/ml	351	1.8%
100-200 M/ml	188	1.3%
>200 M/ml	42	1.9%
Sperm motility	**# of samples**	**Mean % variation**
<5 %	24	8.5%
5-10 %	209	4.6%
11-20 %	179	3.0%
21-50 %	668	2.8%
> 50 %	163	2.7%

Table S12. Measurement of CASA accuracy against manual analysis. Semen samples with various sperm concentration ranges were tested for sperm concentration and motility by the CASA system in parallel to manual semen analysis performed using a hemocytometer.

Movie S1. Video of a semen sample recorded with the smartphone-based semen analyzer. Data collected from this video were used to calculate sperm motility, concentration, and velocity parameters.