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Novel device for male infertility screening with single-ball lens microscope and smartphone

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Objective: To investigate the usefulness of a novel semen analysis device consisting of a single-ball lens microscope paired with a state-of-the-art smartphone equipped with a camera.

Design: Laboratory investigation.

Setting: University research laboratory.

Patient(s): A total of 50 semen samples obtained from volunteers were analyzed for count, concentration, and motility with an 0.8-mm ball lens and three types of smartphone. Comparisons were made with results obtained with a laboratory-based computer-assisted sperm analysis (CASA) system.

Intervention(s): None.

Main Outcome Measure(s): Sperm concentration; sperm motility.

Result(s): Sperm concentration counted with a ball lens and each smartphone showed a very strong correlation with the CASA results. Likewise, sperm motility calculated with our device showed significant correlations to CASA. If eight spermatozoa or fewer were found on the field of view of an iPhone 6s, the semen specimens were considered to be below the lower reference limit for sperm concentration of World Health Organization 2010 guidelines (15 x 10^6 spermatozoa/mL). The sensitivity was 87.5%, and specificity was 90.9%.

Conclusion(s): Smartphones have great potential to analyze semen because they are portable, contain excellent digital cameras, and can be easily attached to a microscope. A single-ball lens microscope is inexpensive and easy to use for acquiring digital microscopic movies. Given its small size and weight, the device can support testing for male fertility at home or in the field, making it much more convenient and economical than current practice. This single-ball lens microscope provides an easy solution for global users to rapidly screen for male infertility. (Fertil Steril® 2016; - : - : - . ©2016 by American Society for Reproductive Medicine.)

Key Words: Infertility, semen analysis, sperm concentration, sperm motility

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertsterrforum.com/koborismartphone-semen-analysis/

Worldwide reports suggest that infertility affects 15%–20% of couples of reproductive age, and approximately 50% of these are accountable to the male partner (1). Although men with infertility represent a significant percentage of the infertile population, public awareness of this fact is limited at best. Literature and the other media have often neglected the male component of reproduction other than its sexual nature (2). Wider access to male infertility assessment may help to resolve this deficiency.

Semen analysis is the key element in diagnosing the reproductive potential of a man with fertility in question. In current practice men must use a clinic or other hospital facility to have their semen analyzed. Once at the clinic, they are asked to produce a semen sample, which is then analyzed by staff at the clinic. Many subjects are not comfortable with this procedure, which they often find embarrassing and expensive (3).

A Dutch scientist, Antonie van Leeuwenhoek, first discovered the spermatozoon in 1677 using a single-ball lens microscope that he had invented. He is considered to be "the father of optic microscopy" and bacteriology (4). He constructed a magnificent ball lens microscope with which he was able to observe very tiny objects in detail. He became the first to observe protozoa, red blood cells, the sperm of animals, and bacteria (5). The development and deployment of ball lenses have recently indicated their usefulness in a number of medical areas (6). Here...
we report on a novel semen analysis device consisting of a van Leeuwenhoek’s single-ball lens microscope paired with a state-of-the-art smart phone equipped with a camera of the kind found in nearly all commercial smart phones.

**MATERIALS AND METHODS**

**Ball Lens Microscope**
We developed a Leeuwenhoek’s microscope constructed with a single-ball lens of 0.8 mm in diameter (Hirosofu Japan) inserted into a plastic jacket that attaches to a commercial smart phone. For a ball lens, basic measures of optical performance can be described in terms of the ball radius (r), index of refraction (n), effective focal length (EFL), and magnification (MAG). As the distance of distinct vision is 250 mm, for an 0.8-mm diameter ball lens:

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EFL = \frac{n \times r}{2(n - 1)} \approx 0.45
\]

\[
MAG = \frac{250}{EFL} \approx 555
\]

The approximate magnification provided by this ball lens was 555 times. As the ball lens becomes smaller, the magnification becomes larger. We chose the 0.8-mm ball lens because this magnification was adequate to see sperm size. This method uses ambient illumination as its light source and does not require the incorporation of a dedicated light source. In this study, semen samples were backlit by a small single light-emitting diode flashlight. An image of sperm that had been enlarged by a single-ball lens was photographed in the smartphone camera. The picture was recorded for 3 seconds by the charge-coupled device image sensor of a smartphone camera.

**Smartphone Camera**
Three types of smartphones were used in this study: iPhone 5s (iOS 8, 8-megapixel [MP] camera, 1080p high-definition [HD] video, 30 frames per second; Apple), iPhone 6s (iOS 9, 12-MP camera, 1080p HD video, 60 frames per second; Apple), and LG Optimus Exceed2 (Android 4.4, 5 MP, 800 x 480 of video resolution). When the iPhone 5s and iPhone 6s were used, a

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**FIGURE 1**

(A) The mobile phone microscopy apparatus with an 0.8-mm ball lens enveloped in a plastic jacket and attached to the iPhone 6s FaceTime camera. (B) The technique for observing semen sample using a ball lens and LG Optimus Exceed 2 with a single light-emitting diode flashlight providing illumination. (C) The sperm could be observed clearly with a ball lens and iPhone 5s. (D) Diagram of this ball lens and smartphone camera.

front camera (FaceTime camera, 1.2-MP camera, 720p HD video) was adopted for investigation (Fig. 1).

**Semen Preparation**

A total of 50 human semen samples were obtained by masturbation into a sterile cup from volunteers between November 2015 and January 2016. After the samples liquefied for approximately 30 minutes, the samples were mixed well by pipette and transferred to a calibrated tube for volume determination. This device comprised two pieces: a lens part and a polyethylene sheet part. A 10-μL aliquot was transferred on a 50-μm transparent polyethylene sheet, which was next attached by magnetic force to the microscope. This device

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**FIGURE 2**

(A) The number of spermatozoa on the field of view counted with iPhone 6s and ball lens microscope showed significant correlations with semen concentration of CASA (n = 45, r = 0.89, P < .001). (B) Semen motility calculated with iPhone 6s and ball lens microscope showed significant correlations with the result of CASA (n = 45, r = 0.88, P < .001). (C) The calculated total motile count of a sample with iPhone 6s and the ball lens demonstrated significant correlation to CASA (n = 45, r = 0.92, P < .001). (D) The sperm count with iPhone 5s showed significant correlations with CASA (n = 45, r = 0.89, P < .001). (E) The sperm motility with iPhone 5s showed significant correlations with CASA (n = 45, r = 0.82, P < .001). (F) The calculated total motile count of a sample with iPhone 5s and the ball lens demonstrated significant correlation to CASA (n = 45, r = 0.92, P < .001). (G) The sperm count with LG Optimus Exceed 2 showed significant correlations with CASA (n = 45, r = 0.84, P < .001). (H) The sperm motility with LG Optimus Exceed 2 showed significant correlations with CASA (n = 45, r = 0.89, P < .001). (I) The calculated total motile count of a sample with LG Optimus Exceed 2 and the ball lens demonstrated significant correlation to CASA (n = 45, r = 0.91, P < .001).

did not need to use the semen chamber owing to the narrow depth of field of the image. The distance between the single-ball lens and the smartphone camera was 1.4 mm. We can reuse them with a new polyethylene sheet. The phone was not stained by the semen sample with appropriate use.

Motile and static sperm were counted manually on an enlarged personal computer screen (MacBook Pro Retina 13-inch; Apple) connected to the smartphone once the video clips were taken. Only whole spermatozoa with head and tail on the personal computer screen were counted. Spermatozoa with progressive motility as well as those with nonprogressive motility were considered as motile sperm. We did not count the spermatozoa that were out of focus. Motility was calculated as the motile sperm count divided by the total sperm count. Three frames of sperm were counted for each specimen, and the results were averaged. We compared the replicate values to determine whether they were acceptably close within an error of 30%. If so, we proceeded with calculations; if not, new aliquots were prepared and assessed (Supplemental Video, available online). We analyzed semen samples both with our device and computer-assisted sperm analysis (CASA) software (SCA HUMAN edition version 5.2; MICROPTIC). The CASA analysis was performed on separate 5-μL aliquots on a glass slide. We tested this device by one person.

Statistical Analyses
Spearman’s rank test was used to test the correlations of semen concentration and motility for each sample specimen between the smartphone camera and the CASA. All statistical analyses were performed with the Statcel 3 program (OMS Publishing), and differences were considered significant at \( P < .05 \). This study was approved by the institutional review boards of Dokkyo Medical University Koshigaya Hospital (Koshigaya, Japan) and the University of Illinois at Chicago.

RESULTS
Semen Analyses
Five semen samples that contained more than \( 100 \times 10^6 \) sperm/mL were excluded owing to too many sperm (more than 50 sperm) to count on the field of view (FOV) of screen. The concentrations of 14 samples were \(< 15 \times 10^6/\text{mL} \) spermatozoa, 17 samples were between 15 and \( 50 \times 10^6/\text{mL} \) spermatozoa, and 14 samples were between 50 and \( 100 \times 10^6/\text{mL} \) spermatozoa measured by CASA.

The relationship of each sperm specimen count in the FOV on the smartphone screen with a ball lens and CASA results are shown in Figure 2. Sperm concentration counted with the ball lens smartphone device demonstrated strong correlation with CASA results \((P < .001)\). Sperm motility recorded with the ball lens also demonstrated significant correlation to CASA \((P < .001)\). In addition, the calculated total motile count of a sample with the ball lens demonstrated significant correlation to CASA \((P < .001)\).

Lower Reference Limit for Sperm Concentration
The World Health Organization (WHO) 2010 manual for semen analysis defines a lower reference limit for sperm concentration of \( 15 \times 10^6/\text{mL} \) spermatozoa \((7)\). The risk of having detectable oligozoospermia \((< 15 \times 10^6/\text{mL} \) spermatozoa) was 83.3% when the sperm count on FOV of iPhone 6s was eight spermatozoa or less and 9.0% when the sperm count was greater than eight sperm. Likewise, the risk of oligozoospermia was 83.3% when the sperm count on FOV of iPhone 5s was five spermatozoa or less and 12.1% when the sperm count was greater than five sperm. The risk of oligozoospermia was 75.0% when the sperm count on FOV of LG Optimus Exceed 2 was five spermatozoa or less and 12.1% when the sperm count was greater than five sperm. Consequently, if we observed eight spermatozoa or less on FOV of iPhone 6s, five spermatozoa or less on FOV of iPhone 5s and LG Optimus, the specimen was considered to be less than the reference limit of the WHO 2010 manual. The sensitivity was 83.3% and specificity 90.9% with the iPhone 6s; the sensitivity was 90.9% and specificity 88.2% with the iPhone 5s; and the sensitivity was 75.0% and specificity 87.8% with the LG Optimus Exceed 2 (Table 1).

DISCUSSION
In the 17th century, Antonie van Leeuwenhoek developed the technology to make very small lenses of high quality for use in single-ball lens handheld microscopes. With these, he made a number of important discoveries in biology, including the existence of spermatozoa. He reported that he had observed a multitude of “small animals” (sperm) which he named “animalcules” \((4)\). The magnification of van Leeuwenhoek’s glass ball lens was 270 times, and we were able to achieve 555 times magnification with a smartphone camera owing to the smaller lens and greater index of refraction.

Cellphone usage and global mobile network coverage have been expanding rapidly over the past few years; global cellphone subscriptions in 2015 exceed 96.8%, and subscriptions in developing countries grew from 30% in 2006 to 90% in 2014 \((8)\). Smartphones have great potential to support medical devices because they are ubiquitous, portable, have extensive computing power, are connected to the Internet, contain excellent digital cameras, and can be easily attached to a microscopic device \((9)\). Smartphones are used as point-of-care devices for diabetes care, mobile thermography, and perioperative management \((10–12)\). Understandably, smartphones are becoming an integral part of the global healthcare system, with the use of smartphone cameras to capture clinically relevant images growing rapidly in recent years.
years. The development of smartphone microscopy has also benefitted from the availability of low-cost miniature microscope components, such as a ball lens [6]. For example, this technology is now an important part of the diagnosis and control of bacterial and parasitic infections, such as tuberculosis, malaria, and Bacillus anthracis [13–15]. Smartphones cameras with more than 5 megapixels are capable of nearly distortion-free imaging over a broad range of magnifications, including those relevant for single-cell imaging [16].

Smartphone adapters for digital photomicrography were developed in the past. However, these adapters require a conventional microscope that is large and cumbersome [9]. We developed in the past. However, these adapters require a conventional microscope that is large and cumbersome [9]. We describe a single-ball lens microscope that is inexpensive and easy to use for acquiring digital microscopic movies [17]. This ball lens is economical at approximately US $7 at the time of this writing. Given its small size and weight, the device can support testing for male fertility at home or in the field, making it much more convenient and economical than current practice.

A disadvantage of the ball lens device is the pincushion field, making it much more convenient and economical than current practice. In addition, the focal distance of this type of lens is tenths of millimeters separated from the surface of the lens. Nonetheless, we could observe sperm easily with this ball lens device because of their characteristic form and motility, and the count and motility data we collected accurately paralleled the results obtained with a laboratory-based CASA system. Furthermore, this microscopy system had a high degree of sensitivity and specificity for diagnosis to the lower reference limit for sperm concentration by the WHO 2010 manual. The results of our study indicate that smartphone semen analysis with a ball lens microscope can indeed provide reliable and repeatable images suitable for preliminary diagnostic use.

Microscopy using smartphones offers additional capabilities such as telediagnosis, store-and-forward imaging, live-streaming of video, and the development of software enabling complex image analyses [19]. We are currently developing device extensions to make it easier to focus on a semen sample, to obtain better-quality images and to quantify semen status automatically. The ball lens microscope provides an easy solution for global users to observe digital semen movies at home. This screening method offers the potential to expand the public understanding of male infertility and its diagnosis.

REFERENCES