Comparison of the SwimCount Home Diagnostic Test with Conventional Sperm Analysis

Damia Castello[1*], Virginia Garcia-Laez[1], Faruk Buyru[2], Emre Bakiricioglu[3], Thomas Ebbesen[4], Anette Gabrielsen[5], Marcos Meseguer[1]

[1]Department of Clinical Embryology Valencian Institute of Infertility University of Valencia, Valencia, Spain

[2]Acibadem Fulya Hospital, IVF Laboratory, Istanbul, Turkey

[3]Unimed Center, Hakki Yeten, Kat Fulya-Istanbul, Turkey

[4]Nordik Cryobank, Andrology Laboratory, Copenhagen, Denmark

[5]Ciconia Fertility Clinic, IVF Laboratory, Aarhus, Denmark

*Corresponding author: Damia Castello, Valencian Institute of infertility, University of Valencia, Valencia, Spain. Tel: +34662919463; Email: damia.castello@ivirma.com

Abstract

The objective of this study was to use a home test kit (SwimCount®) as to sperm quality test for measure the male fertility. A total of 324 semen samples were included and analysed using Makler counting chambers and compared to home test kit readout. Before counting the number of Progressive Motile Sperm Cells (PMSC) using Makler counting chamber, 0.5mL of the sperm sample was added to the SwimCount® (SC) test device. Test results were read and categorized as low, normal or high PMSC concentration. The mean concentration of our sample was 15.5 million of PMSC per mL. Approximately 23% of the samples had a PMSC semen count per mL below the threshold of 5 mill/mL, considered by subnormal concentration by World Health Organization (WHO). An area under curve of 0.95 was obtained when the home test performance was compared with traditional semen analysis performed in standard IVF lab. An accuracy of 95% is in the range of excellent agreement. A good balance between the sensitivity and specificity were obtained at a cut off value of 10.6 mill PMSC per mL, which gave a sensitivity and specificity of the 88.1% and 93.3%, respectively. The cut of value of 10.6 million PMSC per mL was obtained in this study, correlate to 10.6/1.6 = 6.6 mill PMSC per mL, which is very close to the 5 mill/mL cut of value proposed by WHO. The results confirmed the usability of the test as a screening device for male factor infertility home kit.

Keywords: Male fertility; Semi-quantitative; Sperm analysis; Sperm count; Sperm home test; Sperm motility

Introduction

Male fertility is determined by measuring several parameters according to World Health Organization (WHO 5th ed., 2010), however the concentration of progressive motile sperm has been stablished as predictive parameter for estimating fertility in sub- and fertile couples [1,2]. According to WHO criteria, to couple that achieve a pregnancy in a maximum 1 year, semen sample should contain at least 15mill motile and immotile, spermatozoa per mL, and that 32% of these spermatozoa should be progressive motile (WHO 2010). Nowadays, semen analysis is a basic tool to investigate male factor infertility. On the basis of the spermiogram, couples are provided information on the management of the infertility treatment. Approximately 10-15 % of couples are suffering infertility, and semen analysis became the first approach for male factor infertility diagnosis. Many men find is it stressful and displeasing experience to make a semen sample in the clinic and the possibility for a home test evaluation of male factor infertility could be an interesting alternative for many men. Furthermore, the possibility to test the semen quality at home would possibly lead more men to test their semen quality at home and thus bypassing the waiting time if the semen quality is compromised. The couple could contact a fertility clinic to get help immediately and be treated while the woman is still young of age. Many people try to get pregnant at home for too long and if home semen quality test with reliable results is available, this would inevitably reduce the time to pregnancy. Poor sperm quality is a key factor behind the problems many couples experience when trying to conceive. Since

it can be inconvenient and embarrassing to visit hospital or fertility clinic to get tested, many men choose to buy at-home sperm tests.

In fact, several sperm qualities have been developed in the last years for sperm quality assessment test. Sperm quality could be measured with Babystart FertilCount® (Babystart Ltd., Tipton-London), or SpermCheck Fertility® (Charlottesville, USA) offering to fast information only about the total concentration of spermatozoa in the semen sample. Other home test as Test-Point Male Fertility® (Imhotep Medical Group, Netherlands), determine normal or less than normal activity. A different approach could be Micra Home Semen Analysis (ZeraGrowing Ltd, South Korea) consisting of a microscope for measuring concentration and motility, but subjective for a quantification of sperm quality analysis.

However, the existing home tests do not accurately assess male infertility since they only measure total sperm count and sperm quality not. SwimCount® (MotilityCount ApS, Denmark) is the first home test which allows you to get a reliable answer about your chance of making a woman pregnant. SwimCount® (SC) works by measuring concentration of the PMSC, which is the most important key factor in achieving pregnancy [3]. The aim of home test for male factor infertility have never been to replace the full evaluation of male fertility that have to be performed by fertility/andrology professionals. Any abnormal result will have to be further analysed by a professional, but using SC could enable men to be investigated further by specialists if the PMSC concentration is low.

Based on parameters of standard of sperm quality analysis, to home test adapted to give right information has to measure concentration and motility at the same time. SC is a semi-quantitative (Conformité Européene) CE marked home test kit for male fertility. SC-Test measures and informs the end-user the progressive motile sperm cells in the semen sample. Progressive motile sperm cells are the best predictor form male fertility [3].

The present multi-center retrospective study which describe the clinical validation of SC, home semen quality test for analysis of motility and concentration of PMSC in the semen sample.

Material and Methods

Principles of the Assay

Sperm Sample is rapid home test developed for detecting the sperm quality based on the number of PMSC Sample is collected by the patient in home or in the clinic, keeping at room temperature (22°C - 24°C) not more than 1 hour until preparing the sample.

The SC-Test device uses the basic technique of the count-up procedure. The device is composed of two macro-chambers e.g. one placed above the other. The two chambers are separated by a filter with a pore size of 10 μm. The PMSC, with intact DNA and morphology pass actively through this filter [4].

After adding the sample to the inlet well (below the separation filter), the device is activated by pushing the slider forward of the test device. By doing so a solution consisting of Phosphate Buffered Saline (PBS) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) on top of the filter, allowing the PMSC to migrate from the raw semen sample through the filter and into the analysis chamber. When activating the device pushing the handle forward, the substrate (PBS and MTT combined) is released into the swim up chamber. In the analysis chamber the dye enters the PMSC and to mitochondria enzyme cleaves the dye to a purple/blue insoluble product. A total of 30 minutes is required for complete dyeing of PMSC, then this color reaction is used to identify the number of PMSC within the sample (Figure 1).
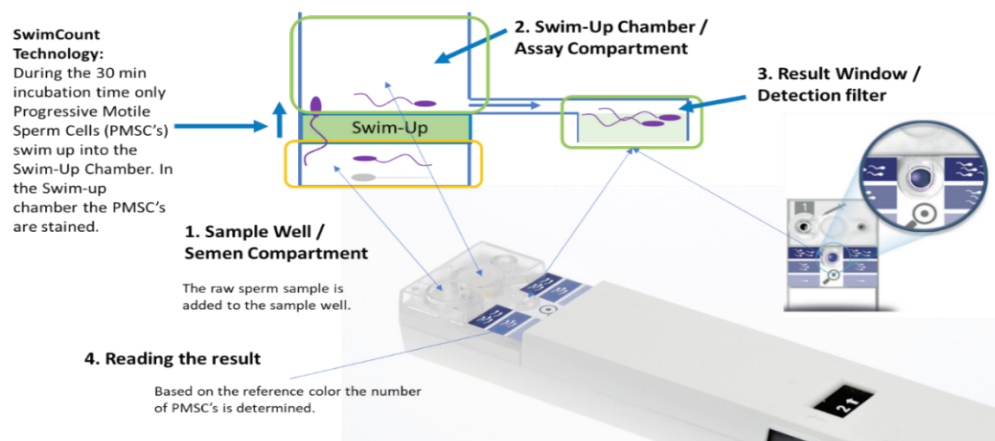
Figure 1: Images of the components of the SC device. 1. Sample well 2. Swim-Up Chamber/Assay Compartment 3. Result window/Detection Filter. 4 Reading result.

By pulling back the slider of the test device, the PMSC stained are removed from the Swim-up chamber and trapped on detection filter to. The substrate dyes cells that is alive (MTT is reduced in the mitochondria of living cells and change in color from yellow to blue/purple). Exclusively PMSC can swim up in the swim-up chamber, and subsequently being trapped in the result window, separating from just progressive motile and non-motile sperm cells. Color intensity is directly proportional to the concentration of the PMSC (Figure 2). The darker the color the more PMSC are present in the sample. Those spermatozoa with a higher motility will acquire deeper blue color, detecting to score for the sample color. This information is scored in the results windows, in an easy to read display.
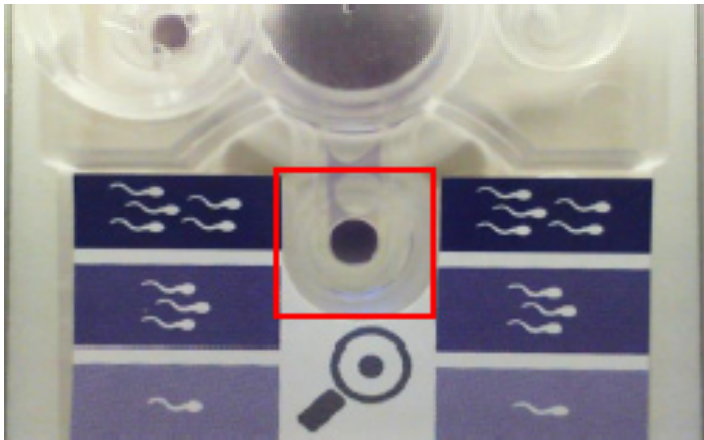
Figure 2: Window Result; grading color indicating concentration of PMSC per mL.

Study Population

This study included a total of 324 semen samples from men attending three different fertility clinics or one sperm bank for sperm analysis, between September 2016 and March 2017. The procedure and protocol for analysing were approved by an Institutional Review Board (IRB reference 1610-VLC-077-DC), which controls and approves database analyses and clinical IVF procedures for research. The whole semen sample was collected by masturbation in a sterile plastic jars or collection cups after 4 days of sexual abstinence. The use of a condom was avoided, since to possible lubricant could damage the quality of the sperm cells.

Semen Collection and Processing

The semen sample was either produced at the clinics or transported to the semen laboratory and kept at room temperature (between 22°C-24°C) not more than one hour until preparing the sample. For collecting semen sample, an aseptic recipient of the type used in andrology procedures was used (80mL sperm collection cup, Oosafe-Sparmed, Farum, Denmark).

Before analysing the semen sample, it was allowed to liquefy for at least 30 minutes and a maximum of 1 hour after producing the sample. In the laboratory, the samples were analysed macroscopically evaluating the semen odor, color, viscosity, acidity and volume. Microscopic analysis was performed to evaluate the concentration, motility, and morphology of the sample agglutination.

Analysis of Sperm Samples

The sperm concentration was evaluated by a Makler chamber (Sefi-Medical Instruments LTD., Israel), becoming the reference method for comparing with SC-Test [5,6]. A volume of 10μL was added to the Makler chamber and sperm count was determined according to the manufacturer's instructions.

The motility of the sperms was estimated as either progressive mutilate, mutilate or immotile according to WHO (World Health Organization, 2010) [7], 0.5mL of the semen sample was deposited in the SC-Test device. Following instructions of used provided manufacture, device was active and waiting 30 minutes. Result was read from the test window and was photographed. The SC was read within 5 min after finalizing the test outcome. The color intensity indicates whether the motile sperm count is above or below the WHO threshold for normal sperm (5 million motile sperms per millilitre). The darker color of the test result, the higher the concentration of progressive motile sperms in the semen sample.

The results obtained with the conventional sperm quality assessment (microscopy) was compared to the read out form the SwimCount test device and analysed using Statistical Software MedCalc version 14.12.0 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org;) 2014). Using Receiver Operating Characteristic Curve (ROC) analysis, the SC provides 95% accuracy (AUC, area under curve) when comparing the results of SC with that of conventional microscopy.
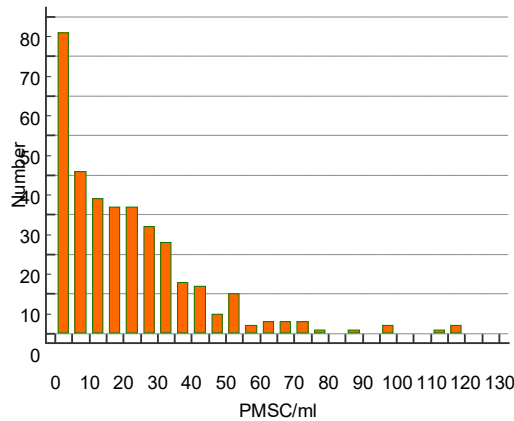
Results

A total of 324 samples were collected in the three centers participating in this multicenter study. Of these samples, 1 test was excluded from the analysis due to technical problems of the SC-Test.

Each of these samples was analysed by duplicated using Makler counting chamber, a summary of the total motile sperm concentration (the main variable of this study) is provided in the table 1.

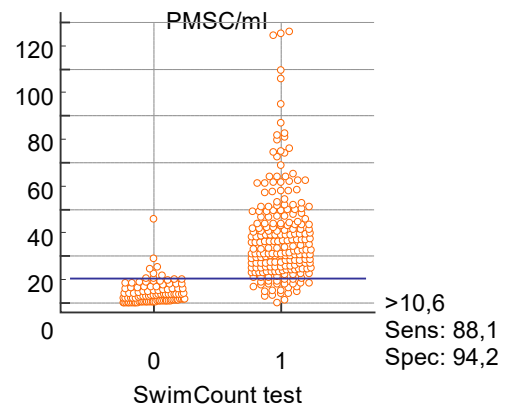| Sample Size	
Mean	20.97 (CI95%18.74-23.19)
Median	16.12
Variance	413.46
Standard Deviation	20.33

Table 1: Descriptive statistic of the 323 semen samples included in the study. Data for concentration of progressive motile sperm concentration (PMSC) per mL measured with Makler chamber is shown.

The distribution of progressive motile sperm concentration is presented in figure 3, we had a median of 16.1 million per mL. Approximately 23.5% of the semen samples had a progressive motile sperm count per mL below the threshold of 5 million/mL, which is considered as subnormal by WHO [7]. This maybe most likely explained by the fact that most of the semen samples were from men seeking fertility treatment.

Figure 4: Interactive dot diagram of the read out from the SC (x-axis) compared to the progressive motile sperm concentration (y-axis).
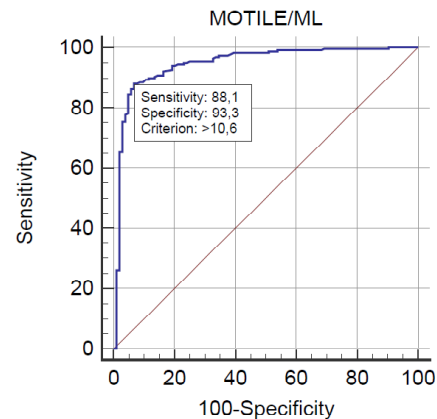

Figure 3: Distribution of the percentage of PMSC in the study, 76 of the 323 samples included had to PMSC per mL below 5 mill/mL.

After the SC-Test, operator classified the score as low or normal, depending on the color gradient observed. Of the 323 semen samples, a total of 219 (67.8%) was categorized as normal, and 104 (32.2%) was scored as poor. The best balance between the sensitivity and specificity was obtained with a cut off value around 10.6 million of PMSC per mL (Figure 4), which gave a final sensitivity and specificity of 88.1% and 93.2%, respectively.

Figure 5: ROC curve analysis of the comparison between the conventional sperm analysis using to Makler counting chamber and the read out from the SC-Test device.

We represented a ROC curve analysis for comparison between the conventional sperm analysis (Makler chamber) performed in

an IVF Lab and SC-Test device, an Area Under Curve (AUC) of 0.95 was obtained (Figure 5). According to this value, agreement between traditional semen analysis and SC-Test was excellent.

Discussion

The mean aim of this study was to validate and evaluate the effectiveness of SC device as home test for semen analysis in a comfortable and not stressful environment. For this, an essential aspect of our current study was to apply to robust statistical assay for comparing the traditional method for sperm assessment used in IVF lab, with SC home test. According to guidelines of WHO (5th ed. 2010) [7], the concentration of progressively motile sperm has consistently been shown to be the most predictive factor with regard to outcome. Around 64% of studies suggest that reason chance of success with artificial insemination requires at least 5 million motile sperm and this is supported by the WHO's revised reference range for natural conception. Other home test was available on the market, but they only measure the concentration of spermatozoa in the sample, but not non-motile, dead or other cells (e.g. white blood cells) [8]. Then, these tests inform only partially about key parameters for complete knowledge of male fertility status.

With the objective of comparing the SC with the routine method for spermiogram used in main part of andrology laboratories, Makler Counting Chamber was used, spite not to be as accurate as Neubauer chamber. However, it's a rapid and usual method used in main part of laboratories for measuring sperm quality. According to the results, agreement between Makler chamber and SC-Test was established through ROC curve, and the AUC presented an accuracy of 0.95, classifying this as excellent value (>0.90). Other attempts to stablish to test for measure PMSC per mL was tried in the past [9], but only SC can be used easily at home.

The good accuracy (AUC=0.95), SC indicates agreement test could be used as self-diagnosis of male fertility providing reasonably precise information about sperm quality. At the same time, the test may provide an easy test without the stress caused by visiting a fertility center. In other case like not knowledge or curiosity about their fertility status SC represents a good tool.

Although home test represents an innovation respect traditional methods for semen analysis, any limitations are still present. In fact, this device cannot measure all parameters of a sperm analysis, as to conventional spermiogram [10]. A complete analysis of the sperm sample is required for a fertile or not fertile diagnosis, offering a detailed description of sperm parameters. Nevertheless, home-test became a rapid tool for fertility potential evaluation in privacy and comfort of home, giving an approximation of fertile situation.

Acknowledgment

References

1. Ayala C, Steinberger E, Smith DP (1996) The influence of semen analysis parameters on the fertility potential of infertile couples. J Androl 17: 718-725.

2. Zinaman MJ, Brown CC, Selevan SG, Clegg ED (2000) Semen quality and human fertility: a prospective study with healthy couples. J Androl 21: 145-153.

3. Tomlinson M, Lewis S, Morroll D, British Fertility Society (2013) Sperm quality and its relationship to natural and assisted conception: British Fertility Society guidelines for practice. Hum Fertil (Camb) 16: 175-193.

4. Asghar W, Velasco V, Kingsley JL, Shoukat MS, Shafiee H, et al. (2014) Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. ADV Healthc Mater 3: 1671-1679.

5. Cardona-Maya W, Berdugo Cadavid A (2008) Comparing the sperm concentration determined by the Makler très Neubauer chambers. AEU 32: 443-445.

6. Makler A (1980) The improved ten-micrometer chamber for rapid sperm count and motility evaluation. Fertil Steril 33: 337-338.

7. World Health Organization (2010) WHO laboratory manual for the examination and processing of human semen, 5th Edition. In: Geneva: World Health Organization, editor.

8. Klotz KL, Coppola MA, Labrecque M, Brugh VM III, Ramsey K, et al. (2008) Clinical and consumer trial performance of a sensitive immunodiagnostic home test that qualitatively detects low concentrations of sperm following vasectomy. J Urol 180: 2569-2576.

9. Bjørndahl L, Kirkman-Brown J, Hart G, Rattle S, Barratt CLR (2006) Development of novel home sperm test. Hum Reprod 21: 145-149.

10. Yu S, Rubin M, Geevarughese S, Pino JS, Rodriguez HF, et al. (2018) Emerging technologies for home-based semen analysis. Andrology 6: 10-19.