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Sperm concentration determination between hemacytometric and CASA systems: Why they can be different

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Abstract

Determination of sperm concentration is a critical component of semen analysis. Traditionally, the hemacytometer has been the standard for calibrating other technologies used to estimate sperm concentration, including photometry, Coulter counters, flow cytometry, and computer-automated semen analysis (CASA). Disposable capillary-loaded slides are commonly used in conjunction with most CASA systems currently in use. Questions have been raised regarding differences in sperm concentration measurements between CASA systems (using 20 μm disposable slides) and hemacytometry. This review explains that these differences are largely due to the Segre–Silberberg (SS) effect, which occurs during Poiseuille flow in thin, capillary-loaded slides. The SS effect can lead to errors in estimation of particle concentration, as demonstrated with latex beads and suspensions of human or porcine spermatozoa. The SS effect does not appear to have time to develop in the hemacytometer, which at 100 μm is considerably deeper than most disposable slides. Thus, hemacytometry, when properly performed, remains the gold standard for estimation of sperm concentration. When using thin (20 μm) slides with CASA systems, recognition of the appropriate compensation factor to adjust for the SS effect is critical for accuracy.

Keywords: Sperm; Semen; Concentration; Hemacytometer; CASA

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1. Introduction

Discrepancies between particle concentration estimates generated from hemacytometer counts and disposable 20 μm slides have been previously recognized [1–5]. A recent explanation of this phenomenon may effectively resolve variation of sperm concentration measurements between these two techniques [6,7]. The Segre–Silberberg (SS) effect describes flow dynamics that result in a concentration differential for particles entrained in laminar flows [8]. This effect applies to spermatozoa loaded into counting chambers by capillary action. As the interest in computer-automated semen analysis systems (CASA) increases, the SS effect must be understood to prevent errors in sperm concentration measurements.

2. Theory: Segre–Silberberg effect

Current CASA systems generally rely on disposable slides with defined-depth chambers for sperm concentration and motility, with or without morphology analyses. Chambers approximately 20 μm deep are the most common, since they provide a monolayer of cells (which is important for focal depth and image acquisition without interfering with sperm motion characteristics). Compared to the standard (100 μm deep) hemacytometer, disposable chambers are usually both more shallow and longer (from entry to exit ports); these differences form the basis for the distribution of sperm in these two types of chambers, which ultimately affect sperm concentration estimates.

Filling a relatively thin (20 μm) chamber by capillary action exposes particles suspended in the fluid to a velocity gradient, causing the particles to migrate perpendicular to the direction of flow [9]. Particles thus suspended in a laminar flow become concentrated at predictable distances from the walls of the chamber and are subsequently transported to the leading edge of the flow, resulting in a wave of higher concentration at the meniscus [6,7]. The laminar flow observed during loading of a capillary chamber is described as Poiseuille flow and can be visualized as fluid passing between two parallel sheets. Laminar Poiseuille flow results when a system is characterized by a low Reynolds number ($Re < 1$) [10]; the Reynolds number is calculated from the fluid density, viscosity, velocity, and chamber depth. For example, diluted boar semen as commonly used to load 20 μm chambers, has a Reynolds number of $Re \sim 0.02$.

Fluid is drawn into the chamber under the force of surface tension between the fluid and glass, with a meniscus forming at the leading edge. Flow velocity increases with distance from the walls (top and bottom of chamber), and reaches its peak at the center of the fluid channel. The filling velocity is proportional to chamber depth and decreases as the meniscus penetrates the chamber. Thus, the transverse velocity gradient characterizing Poiseuille flow decreases as the meniscus travels further into the chamber, and shallow chambers fill more slowly than deeper ones.

As a result of the transverse velocity gradient, particles suspended in a Poiseuille flow become concentrated at defined distances from the walls, with the preferred positions depending on particle characteristics and flow rate. Although these positions may be easier to quantify for simple particles such as freely rotating latex beads, more complex structures

such as sperm cells migrate toward their own stable positions within the flow. These stable positions, or SS planes, can be defined according to their spatial relationship to the central plane of the channel, and are symmetrical on opposite sides. As the particles collect in regions where the fluid is moving faster than the mean flow velocity, they begin to move more rapidly than the mean fluid flow rate and are subsequently transported to the leading edge of the flow, the meniscus. Thus, an area of higher particle concentration forms at the meniscus, effectively reducing the concentration in the rest of the chamber. Since the velocity gradient is independent of chamber depth, the velocity at which particles move perpendicular to the flow is relatively constant for 20 μm chambers and 100 μm hemacytometers. However, the deeper the chamber, the longer it will take particles to reach stable SS planes.

By making reasonable assumptions about the particle characteristics of a sperm cell (e.g., effectively spherical with a 2.0 μm radius) and the contact angle between the meniscus and glass walls and measuring other factors such as viscosity of diluted semen, it is possible to predict the behavior of such cells during capillary filling. For example, when filling a 20 μm chamber, boar spermatozoa reach their stable SS planes quite rapidly; most should be found within 10% of those positions after the fluid has moved only 1 mm into the chamber. Because the cells are pumped toward the meniscus as previously described, an area of reduced concentration is left behind the leading edge in the remainder of the chamber. Conversely, the 100 μm hemacytometer is five-fold deeper than the 20 μm chamber, resulting in five times more rapid filling velocity than the 20 μm slide. Although the transverse velocity is similar, the rapid flow coupled with the greater distance separating the SS planes results in no substantial segregation of spermatozoa during loading.

3. Practice: field studies with boar sperm

A compensation factor of 1.3 was derived for 20 μm four-chamber disposable slides (Leja Products, B.V., Nieuw-Vennep, The Netherlands) by applying the SS effect to diluted boar semen [6]. A CASA system (UltiMate, V.12.1; Hamilton Thorne Biosciences, Beverly, MA, USA) was programmed with the compensation factor and used in two field trials to evaluate the effectiveness of the predicted compensation factor. In both trials, Improved Neubauer hemacytometers (Fisher Scientific Inc., Pittsburgh, PA, USA) that had been depth-validated using an interferometer (Hamilton Thorne Biosciences) were used for comparative counts [11]. For Trial I, samples ($N = 12$) typical of the US boar stud industry ($\sim 40 \times 10^6$/mL) were analyzed within 6 h of processing. Trial II was conducted at a commercial boar stud (Iowa Select Farms, Iowa Falls, IA, USA), where gel-free ejaculates ($N = 47$) could be analyzed in a standard production setting. For each trial, hemacytometer counts and CASA measurements were collected by different people and submitted to a third person that compared results.

Using pooled data from both trials, the correlation coefficient between the sperm concentrations determined by the UltiMate corrected for the SS effect and hemacytometry was $r^2 = 0.936$ [7]. Not only was the correlation coefficient high, but the slope of the plot (UltiMate versus hemacytometer) was 0.984, giving an expected gradient of unity between

the two methods of sperm concentration measurement of 1.6%. Precision, accuracy and repeatability were comparable between the two sites.

4. Summary

Due to the Segre–Silberberg effect, sperm concentration in the readable area of the slide was lower than the actual concentration of the sample when diluted semen was loaded into typical 20 μm slides utilized by most CASA systems. Using a compensation factor to adjust for the SS effect was critical for accuracy. Since the SS effect is insignificant in deeper (100 μm) chambers, the hemacytometer remains the gold standard by which all other methods of estimating sperm concentration should be compared.

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This discussion is drawn from original material published in the Journal of Andrology earlier this year (2005). The reader is directed to the following articles for more detailed information: Refs. [6,7].

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