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Comparison of Three Sperm-Counting Methods for the Determination of Sperm Concentration in Human Semen and Sperm Suspensions

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Abstract

Background: Sperm counts are currently being performed using a variety of methods that produce results that do not correlate well with each other or with the current "gold standard" reference method, hemacytometer. Significant differences in the accuracy of sperm counts using these different counting methods emphasize the need for standardization and quality control procedures to ensure adequate intermethod agreement between sperm counting methods. We compared sperm counts using 3 commonly used methods—hemacytomer, Cell-VU, and Makler—applied to both semen and sperm suspensions prepared from semen.

Methods: Semen samples were obtained by masturbation from 60 outpatient volunteers. Sperm suspensions containing low, medium, and high sperm concentrations were prepared from 38 of these semen samples. The results of sperm counts on semen and sperm suspension samples were analyzed with SPSS 11.0 software.

Results: Compared with the Cell-VU method, the hemacytometer and Makler methods overestimated sperm concentration in both semen and sperm suspension samples. Sperm counts in randomly selected semen samples (n = 35), and semen samples containing low, medium, or high sperm concentrations, were higher by hemacytometer than the Makler method, due, most likely, to the dilution of semen required when counting sperm concentration using a hemacytometer.

Conclusion: The Cell-VU method of sperm counting provided the best accuracy and precision among the 3 methods we studied and may represent a better worldwide "gold standard" for sperm counts than the current hemacytometer method.

Semen analysis, especially sperm counting, is an important test performed in andrology laboratories. Due to the unavailability of external proficiency testing programs for semen analysis, it has recently been shown that there is wide variation in sperm counts between these laboratories.[1,2] Moreover, this variability is expected to adversely impact patient care when results for semen analysis parameters (ie, sperm concentration, motility, and vitality) on the same semen sample are reported as normal by one laboratory and as consistent with infertility by another laboratory.[3] If the results "consistent with infertility" are incorrect, a clinician might recommend artificial fertilization (ie, in vitro fertilization) to an infertile couple when this option is unnecessary. Such discordances can occur using other semen analysis parameters because andrologists differ in their use of these parameters when assessing fertility potential and the measurement of these parameters is not standardized,[4] despite the availability and importance of quality control (QC) procedures for these measurements.[5]

The sperm count is a basic test for assessing male fertility, and there have been calls for global standardization of this test.[6,7] Epidemiologic studies have suggested that sperm concentration has declined over the past 50 years, while some reports have suggested otherwise.[8,9] Moreover, others have shown that sperm concentration varies significantly by geographic region.[10,11] However, there is disagreement over the existence of seasonal variation in the quality of sperm.[12,13] In addition, the results of these studies are complicated by the disparities in sperm counts obtained using different methods.[14,15]

Recently, Brazil and colleagues were the first to compare the semen quality of men from 4 cities in the United States using standardized methods and strict adherance to QC measures.[16,17] In addition, Keel and colleagues previously reported the results of an external proficiency testing (PT) survey involving hundreds of andrology laboratories in the United States and samples for assessing sperm concentration, morphology, motility, and the presence of antisperm antibodies.[1] The results of this survey demonstrated that values for sperm concentration on the same semen sample varied from 3×10^6/mL to 492×10^6/mL, while the coefficient of variation (CV) for manual and computer-assisted semen analysis (CASA) sperm counting methods varied from 30% to 138%, and 24% to 99%, respectively. Moreover, other studies have shown that standardization of sperm counting methods, including the chambers used for counting, affects the imprecision of these methods because of the variability contributed by counts performed by different technologists and by the sperm concentration in different semen samples (ie, semen samples with low sperm concentrations are expected to yield higher imprecision than samples with high sperm concentrations).[14,15,18]

In 1996, Seaman and colleagues,[19] using a reference solution containing a known concentration of precalibrated latex beads, reported the results of a study of the accuracy of 4 semen counting methods (hemacytometer, Cell-VU, Makler, and Micro-Cell). The mean bead concentrations obtained using the Cell-VU and Micro-Cell methods were consistently similar to the bead concentration of the standard solution; however, the hemacytometer and the Makler methods overestimated the bead concentration of the reference solution by as much as 50%, and there was significant inter-chamber variability between counts.

This data suggested marked differences in the accuracy and reliability of sperm counts using these methods and emphasized the need for standardization and QC of sperm counting methods.

In recent years, our group has been working on standardization and QC procedures for semen analysis testing. Using standardized solutions containing low and high concentrations of precalibrated latex beads, our studies confirmed the results of Seaman and colleagues that the bead concentration obtained using the Cell-VU method matched the reference value more closely than the concentration obtained using the hemacytometer and Makler sperm counting methods, which overestimated bead concentration.[20-22] However, differences in the viscosity of the bead solution versus human semen may limit the value of this solution as a QC material for sperm counting methods.

The hemacytometer is recommended in the World Health Organization (WHO) manual for sperm counts, along with appropriate QC procedures. However, several other sperm counting methods are in use by andrology laboratories, including DROP, Standard Count, Cell Vision, MicroCell, 2X-CEL, Makler, JCD, and Burker methods using either disposable or reusable chambers (eg, Cell-VU).[23-25] Using these methods, Mahmoud and colleagues[25] compared bead counts obtained from 10 chambers using a reference solution containing calibrated latex beads. The results showed that only the Standard Count, MicroCell, Cell-VU, and Cell Vision methods provided bead counts within the acceptable range of values for the reference solution. Standard Count, MicroCell, and Cell Vision methods use disposable chambers loaded by capillary action, and several investigators have reported results using the MicroCell sperm counting method.[26-28] Although investigators have shown the MicroCell sperm counting method provides better accuracy and precision than other methods, the combination of the MicroCell method with CASA was the most accurate and precise method for assessing sperm concentration (and motility) compared with sperm counts obtained using the Makler and hemacytometer sperm counting methods.[26,27] A subsequent investigation, however, showed that higher sperm concentrations were obtained in the distal end of the MicroCell chamber compared with the proximal end in regions of used (9.9 mm from the entrance of the chamber) and new (8.0 mm from the entrance of the chamber) MicroCell chambers distal to the opening of the chamber. This finding explains, in part, the reported underestimate of sperm count using the MicroCell, with its 20 µm depth chamber loaded by capillary action, compared with results obtained using the hemacytometer.[29]

Because the comparability of sperm counts between different laboratories is critically dependent on the method considered the "gold standard" for comparing counts, we compared the accuracy and precision of counts obtained by 3 commonly-used sperm-counting methods—hemacytometer, Cell-UV, and Makler—using both semen samples and sperm suspensions prepared from them containing low, moderate, or high sperm concentrations.

Materials and Methods

We purchased a hemacytometer (Qiujing, Shanghai, China), Cell-VU (Millennium Sciences, New York), and Makler (Sefi-Medical Instrument, Haifa, Israel) sperm-counting devices. Semen samples were obtained from 60 outpatients by masturbation during their visit to the Department of Andrology, Nanjing Jinling Hospital, China. Semen samples were liquefied at 37°C for 30 minutes and followed by semen analysis. Sperm suspensions were prepared by washing semen samples twice with normal saline (NS)(0.9% NaCl) and adjusting the samples to the required sperm concentration by dilution with NS.

Sperm counting was performed using the hemacytometer, Cell-VU, and Makler methods.

1. Hemacytometer. We followed the method for sperm counting by hemacytometer recommended by the WHO.[23] Semen or sperm suspension was diluted with sperm-counting (SC) diluent (sodium acid carbonate-formaldehyde solution) and counted according to the procedure indicated in the WHO laboratory manual.[23]

2. Cell-VU. The Cell-VU sperm-counting method involves the use of a dual-chamber glass slide and 2 pieces of 0.5-mm thick coverslip containing a laser-etched grid on the reverse side. The grid area is 1 mm × 1 mm and is divided into 100 smaller squares, each of which is 0.1 mm × 0.1 mm. The chamber has a reported depth of 20 µm. Semen or sperm suspension samples were vortexed prior to sperm counting according to the manufacturer's instructions. Briefly, a 4 µL volume of semen or sperm suspension was loaded into the left and right chambers and the number of sperm counted based on those sperm which touched the upper and left sides instead of the lower and right sides of each square of a counting chamber. To ensure consistent counting, a minimum of 200 sperm were counted in each chamber.

3. Makler chamber. The Makler chamber was designed specifically for the determination of sperm concentration and motility in undiluted semen. It has a reported depth of 0.01 mm. The grid area in the center of the coverslip is 1 mm × 1 mm and is divided into 100 smaller squares, each of which is 0.1 mm × 0.1 mm. A 5 µL volume of semen or sperm suspension was loaded into the chamber and the number of sperm counted as indicated above for the Cell-VU method.

Comparison of sperm counts by hemacytometer and Cell-VU chambers. To ensure the comparability of sperm counts between the two sections of the hemacytometer and Cell-VU slides, we randomly selected 3 hemacytometers and 3 Cell-VU slides for counting the sperm concentration in 60 semen samples (10 samples per each of the 3 hemacytometers or Cell-VU slides with each sample loaded into the upper and lower chambers of the hemacytometers or the left and right sections of the Cell-VU slides).

Imprecision of sperm-counting methods using human semen samples. Human semen samples containing low ($<20 \times 10^6$ sperm/mL), medium ($20–100 \times 10^6$ sperm/mL), or high ($>100 \times 10^6$ sperm/mL) were selected randomly from the pool of 60 semen samples, and 30 replicate counts were performed on each semen sample (low, medium, high) using the hemacytometer, Cell-VU, and Makler methods.

Imprecision of sperm-counting methods using sperm suspension samples. Three semen samples were chosen randomly, washed twice with NS, centrifuged at 500 × *g* for 5 minutes, adjusted to low ($<20 \times 10^6$ sperm/mL), medium ($20–100 \times 10^6$ sperm/mL), or high ($>100 \times 10^6$ sperm/mL) sperm counts by appropriate dilution with SC diluent, and 15 replicate counts performed on each suspension (low, medium, high) using the hemacytometer, Cell-VU, and Makler methods.

Sperm counts in semen samples versus sperm suspensions prepared from these semen samples. Thirty-five semen samples, selected randomly from the pool of 60 semen samples, and the sperm suspension samples prepared from these semen samples, were counted using the hemacytometer, Cell-VU, and Makler methods.

Volume comparison of diluted semen samples versus samples containing saline in lieu of semen. To compare the volume of diluted saline versus diluted semen samples, 0.8 mL of SC diluent was added to each of 10 graduated centrifuge tubes (5 mL, graduated in 0.05 mL increments, Huadang Factory, Jiangsu, China), followed by the addition of 0.2 mL of NS to 5 of these tubes and 0.2 mL of fresh liquefied semen to the remaining 5 graduated centrifuge tubes. All tubes were mixed and allowed to stand for 5 minutes at room temperature. The volume in all 10 tubes was recorded separately and independently by 3 technicians, and the mean of the 3 volume determinations on each tube was calculated.

Statistical analysis. All data were analyzed with SPSS 11.0 software. Statistically-significant differences between mean sperm counts from different counting methods (ie, hemacytometer, Cell-VU, or Makler) or chambers/sections were determined by paired t-test using a *P* value of <0.05 as the indicator of statistical significance. The CV for sperm counts by different counting methods was also determined.

Results

Comparison of sperm counts by Hemacytometer and Cell-VU chambers. There was no significant difference in sperm counts obtained on human semen samples (n = 60) between the lower and upper chambers of the hemacytometer, or between the left and right sections of the Cell-VU slide, and these counts were highly correlated (r = 0.925–0.996) (**Table 1**).

Imprecision of sperm-counting methods using human semen samples. Sperm counts were significantly higher ($P<0.001$) in all 3 semen samples (low, medium, high) by hemacytometer versus Cell-VU and Makler methods, and imprecision (CV, %) ranged from 9.5, 12.8%; 6.2, 14.5%; and 12.6, 22.7%, respectively (**Table 2**).

Imprecision of sperm-counting methods using sperm suspension samples. Sperm counts were significantly higher for the low sperm concentration sample between Makler and hemacytometer methods and significantly lower for the medium and high sperm concentration samples between Cell-VU and hemacytometer methods, while imprecision (CV, %) ranged from 10.8, 17.7% (hemacytomer); 7.1, 16.0% (Cell-VU); and 11.7, 22.6% (Makler) (**Table 3**).

Sperm counts in semen samples versus sperm suspensions prepared from these semen samples. A highly-significant difference ($P<0.001$) in sperm counts was observed between semen and sperm suspension counts obtained by the Makler method compared with the hemacytometer method, with a higher sperm count obtained on sperm suspension by the Makler method ($51.6 \pm 31.7 \times 10^6$/mL) versus the hemacytometer method ($39.7 \pm 23.1 \times 10^6$/mL). In addition, a lower count was obtained on semen by the Cell-VU method ($54.3 \pm 39.6 \times 10^6$/mL) versus the Makler method ($78.9 \pm 59.2 \times 10^6$/mL) (**Table 4**).

Volume comparison of diluted semen samples versus samples containing saline in lieu of semen. When 0.2 mL of semen was added to 0.8 mL of SC diluent, the final volume of the mixture was lower than that obtained when 0.2 mL of NS was added to 0.8 mL of SC diluent and the same results were obtained using 5 different semen samples (**Figure 1**).

Discussion

As expected, there was no significant difference in sperm counts on semen obtained between chambers or sections of the hemacytometer or Cell-VU slides, respectively, or between these

Table 1_Comparison of Semen Sperm Counts[1] in Chambers of the Hemacytometer and Cell-VU Devices

	Sperm Count (Mean ± SD), x 10^6/mL					
	Hemacytometer No.			Cell-VU No.		
Chamber	1	2	3	1	2	3
Lower/Left	50.8 ± 18.8	58.2 ± 37.4	50.5 ± 33.7	52.8 ± 35.9	61.5 ± 50.2	54.5 ± 49.6
Upper/Right	49.0 ± 18.2	56.0 ± 34.8	50.6 ± 32.5	50.4 ± 33.8	59.4 ± 46.3	50.5 ± 43.0
r	0.925	0.969	0.988	0.968	0.994	0.996

[1]*Using a total of 60 semen samples with 10 different semen samples used to load both the upper and lower chambers of 3 hemacytometers and the left and right sides of 3 Cell-VU sperm counting slides. SD, standard deviation; No., number; r, correlation coefficient between sperm counts (n = 10) obtained on the lower and upper chambers per hemacytometer or the left and right sides per Cell-VU device.*

Table 2_Imprecision Data[1] Using Semen Samples

	Sperm Count (Mean ± SD), x 10^6/mL					
	Hemacytometer		Cell-VU		Makler	
Sperm Concentration	Mean ± SD	CV, %	Mean ± SD	CV, %	Mean ± SD	CV, %
Low	25.9 ± 3.3	12.8	11.0 ± 1.6*	14.5	17.5 ± 2.2*	12.6
Medium	89.9 ± 8.7	9.5	47.6 ± 3.0*	6.2	59.6 ± 13.5*	22.7
High	206.7 ± 21.2	10.8	128.4 ± 14.7*	7.1	175.9 ± 19.1*	15.0

[1]*Based on 30 replicate sperm counts in each semen sample (low, medium, high).*

**P<0.001 versus hemacytometer sperm count. SD, standard deviation; CV, coefficient of variation*

Table 3_Imprecision Data[1] Using Sperm Suspensions

Sperm Concentration	Sperm Count (Mean ± SD), x 10^6/mL					
	Hemacytometer		Cell-VU		Makler	
	Mean ± SD	CV, %	Mean ± SD	CV, %	Mean ± SD	CV, %
Low	23.3 ± 4.1	17.7	18.0 ± 2.9*	16.0	29.3 ± 6.6*	22.6
Medium	86.7 ± 10.2	11.8	58.1 ± 7.4*	12.7	93.3 ± 10.9	11.7
High	150.4 ± 16.2	10.8	114.7 ± 8.1*	7.1	153.7 ± 23.0	15.0

[1]Based on 15 replicate sperm counts of each sperm suspension sample (low, medium, high).
**P<0.001 versus hemacytometer sperm count. SD, standard deviation; CV, coefficient of variation*

Table 4_Comparison of Sperm Counts Using Semen (n = 35) Versus Sperm Suspensions Prepared From the Semen Specimens

Sample Type	Sperm Count (Mean ± SD), x 10^6/mL		
	Hemacytometer	Cell-VU	Makler
Semen	79.3 ± 56.7	54.3 ± 39.6*	78.9 ± 59.2
Sperm suspension	39.7 ± 23.1	36.3 ± 19.0	51.6 ± 31.7*

**P<0.001 versus hemacytometer sperm count. SD, standard deviation*

2 methods of sperm counting (**Table 1**). All 3 methods provided similar imprecision using semen samples containing low sperm counts; however, the hemacytometer and Cell-VU methods provided lower imprecison at both medium and high sperm concentrations compared with the Makler method (**Table 2**) and generally similar results were obtained using sperm suspensions prepared from these semen samples (**Table 3**). In addition, mean sperm counts in semen samples (n = 35) increased in the order: hemacytometer ≥ Makler > Cell-VU, while for sperm suspensions prepared from these semen samples, mean sperm counts increased in the order: Makler > hemacytometer ≥ Cell-VU (**Table 4**). Thus, for both semen and sperm suspensions, the lowest sperm counts were obtained using the Cell-VU method. Our results for these methods, using semen and sperm suspension samples,

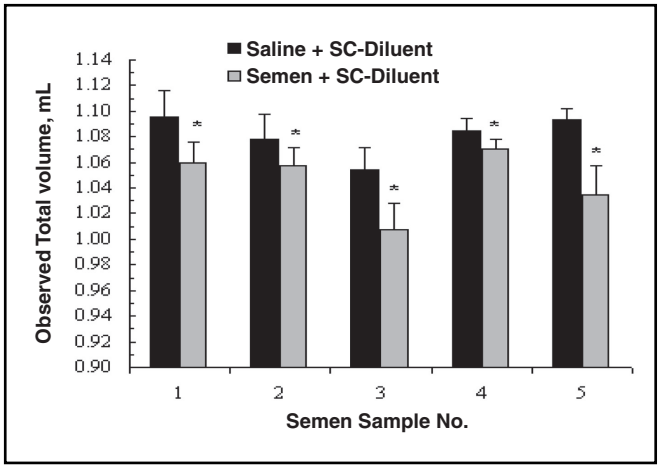

Figure 1_Observed total volume when 0.2 mL of saline or semen was mixed with 0.8 mL of SC diluent. Values represent the mean of triplicate measurements. **P*<0.05 compared with observed total volume of saline + SC diluent.

were similar to those published previously using these same methods and calibrated latex bead solutions,[19,21] suggesting that the Makler and hemacytometer methods overestimate sperm concentration.

The principal difference between semen and sperm suspension prepared from semen is the apparent higher viscosity of semen compared with suspension. This phenomenon is borne out by the data shown in **Figure 1** in which the observed total volume of semen + SC diluent was lower for 5 different semen samples compared with the volume based on saline + SC diluent when similar volumes of each were mixed. The formation of hydrated protein molecules after dilution of viscous semen with SC diluent might lead to the lower volume of the semen-SC diluent mixture compared with the saline-SC diluent mixture. Thus, sperm counts in diluted semen samples (eg, hemacytometer method requiring dilution of the semen) would be expected higher than counts obtained using sperm counting methods that do not require pre-dilution of the semen sample (eg, Makler method). Douglas-Hamilton and colleagues recently proposed a theoretical model to explain the lower sperm counts obtained using chambers loaded by capillary action, compared with the hemacytometer method.[30,31] This model was based on the Segre-Silberberg (SS) effect of fluid flow described by Poiseuille's Law. According to this model, when a semen sample is loaded into a chamber with a 20 µm depth, there is a high sperm concentration due to formation of a planum semilunatum, but a relatively low sperm concentration in other areas of the chamber, including the sperm-counting area. Thus, these authors suggested that sperm concentration by such methods should be adjsuted using a correction factor based on the 20 µm depth of the chamber.

The hemacytometer method is recommended by the WHO manual as the current "gold standard" for assessing sperm concentration;[23] however, the accuracy of this method has been questioned.[26,32] The Makler chamber provided the highest sperm counts, compared with the hemacytometer and Cell-VU methods, on sperm suspension samples. This chamber was invented by the Israeli scientist, Makler, in 1978, and was designed specifically for undiluted semen and has been used in many laboratories worldwide.[31] On the other hand, the Cell-VU sperm-counting method with its disposable or reusable chamber has unique advantages over other methods, especially for highly-infectious semen samples.[21] Moreover, both our studies, and those of others using precalibrated standard latex bead solutions, indicated that the accuracy and precision of sperm counts using the Cell-VU chamber exceeded those obtained using the hemacytometer and Makler methods.[19, 21]

We suggest that a standard sperm-counting method should be adopted by andrology laboratories worldwide to provide the

foundation for the reliable assessment of inter-laboratory QC for sperm counting and to ensure the accuracy, reliability, and comparability of sperm counts between these laboratories. Therefore, we recommend use of the Cell-VU slide as the new "gold standard" reference method for sperm counting. LM

Key words: Cell-VU chamber; hemacytometer; Makler chamber; sperm concentration; standardization

1. Keel BA. Quality control, quality assurance, and proficiency testing in the andrology laboratory. *Arch Androl.* 2002;48:417–431.
2. Keel BA. How reliable are results from the semen analysis? *Fertil Steril.* 2004;82:41–44.
3. Auger J, Eustache F, Ducot B, et al. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. *Hum Reprod.* 2000;15:2360–2368.
4. Muller CH. The andrology laboratory in an assisted reproductive technologies program. Quality assurance and laboratory methodology. *J Androl.* 1992;13: 349–360.
5. Dunphy BC, Kay R, Barratt CLR, et al. Quality control during the conventional analysis of semen, an essential exercise. *J Androl.*1989;10: 378–385.
6. Franken DR. African experience with sperm morphology training courses. *Reprod Biomed Online.* 2003;7:114–119.
7. Cooper TG, Bjorndahl L, Vreeburg J, et al. Semen analysis and external quality control schemes for semen analysis need global standardization. *Int J Androl.* 2002;25:306–311.
8. Becker S, Berhane K. A meta-analysis of 61 sperm count studies revisited. *Fertil Steril.* 1997;67:1103–1108.
9. Fisch H, Goluboff ET. Geographic variations in sperm counts: A potential cause of bias in studies of semen quality. *Fertil Steril.* 1996;65:1044–1046.
10. Fisch H, Goluboff ET, Olson JH, et al. Semen analyses in 1283 men from the United States over a 25-year period: No decline in quality. *Fertil Steril.* 1996;65:1009–1014.
11. Fisch H, Ikeguchi EF, Goluboff ET. Worldwide variations in sperm counts. *Urology.* 1996;48:909–911.
12. Levine RJ, Brown MH, Bordson BL, et al. Deterioration of semen quality during summer in New Orleans. *Fertil Steril.* 1988;49:900–907.
13. Reinberg A, Smith KO, Smolensky MH, et al. Annual variation in semen characteristics and plasma hormone levels in men undergoing vasectomy. *Fertil Steril.* 1988;49:309–315.
14. Davis RO, Boyers SP. The role of digital image analysis in reproductive biology and medicine. *Arch Pathol Lab Med.* 1992;116:351–363.
15. Boone WR, Jones JM, Shapiro SS. Using videotaped specimens to test quality control in a computer-assisted semen analysis system. *Fertil Steril.* 2000;73: 636–640.
16. Brazil C, Swan SH, Drobnis EZ, et al. Standardized methods for semen evaluation in a multicenter research study. *J Androl.* 2004;25:635–644.
17. Brazil C, Swan SH, Tollner CR, et al. Quality control of laboratory methods for semen evaluation in a multicenter research study. *J Androl.* 2004;25: 645–656.
18. Neuwinger J, Behre HM, Nieschlag E. External quality control in the andrology laboratory: An experimental multicenter trial. *Fertil Steril.* 1990; 54:308–314.
19. Seaman EK, Goluboff E, BarChama N, et al. Accuracy of semen counting chambers as determined by the use of latex beads. *Fertil Steril.* 1996;66: 662–665.
20. Lu JC, Chen F, Xu HR, et al. Preliminary investigations on the standardization and quality control for the determination of acid phosphatase activity in seminal plasma. *Clin Chim Acta.* 2007;375:76–81.
21. Lu JC, Lu NQ, Huang YF, et al. Quality evaluation of three different sperm counting chambers. *Zhonghua Nan Ke Xue.* 2004;10:755–757, 760.
22. Hu YA, Lu JC, Lu NQ, et al. Comparison of four methods for sperm counting. *Zhonghua Nan Ke Xue.* 2006,12:222–224, 227.
23. World Health Organization. *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction.* 4th ed. Cambridge: Cambridge University Press; 1999:14–17.
24. Huang YF, Li PS. Urgent need for the standardization of semen analysis among clinical andrology laboratories. *Zhonghua Nan Ke Xue.* 2005;11:83–84.
25. Mahmoud AM, Depoorter B, Piens N, et al. The performance of 10 different methods for the estimation of sperm concentration. *Fertil Steril.* 1997;68: 340–345.
26. Johnson JE, Boone WR, Blackhurst DW. Manual versus computer-automated semen analyses. Part I. Comparison of counting chambers. *Fertil Steril.* 1996;65:150–155.
27. Johnson JE, Boone WR, Blackhurst DW. Manual versus computer-automated semen analyses. Part II. Determination of the working range of a computer-automated semen analyzer. *Fertil Steril.* 1996;65:156–159.
28. Johnson JE, Boone WR, Blackhurst DW. Manual versus computer-automated semen analyses. Part III. Comparison of old versus new design Microcell chambers. *Fertil Steril.* 1996;65:446–447.
29. Björndahl L, Barratt CLR. Semen analysis: Setting standards for the measurement of sperm numbers. *J Androl.* 2005;26:11.
30. Douglas-Hamilton DH, Smith NG, Kuster CE, et al. Particle distribution in low-volume capillary-loaded chambers. *J Androl.* 2005;26:107–114.
31. Douglas-Hamilton DH, Smith NG, Kuster CE, et al. Capillary-loaded particle fluid dynamics: Effect on estimation of sperm concentration. *J Androl.* 2005;26:115–122.
32. Sokol RZ, Shulman P, Paulson RJ. Comparison of two methods for the measurement of sperm concentration. *Fertil Steril.* 2000;73:591–594.