

## The density of human semen and the validation of weight as an indicator of volume: a multicentre study<sup>2</sup>

Phillip L. Matson<sup>1,3,4</sup>, Kim Myssonski<sup>5</sup>, Steven Yovich<sup>6</sup>, Linda Morrison<sup>7</sup>,  
Jacquelyn Irving<sup>8</sup>, Hassan W. Bakos<sup>9,10</sup>

<sup>3</sup>Keogh Institute for Medical Research, Sir Charles Gairdner Hospital, Nedlands, Western Australia, <sup>4</sup>School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, <sup>5</sup>Canberra Fertility Centre, Deakin, ACT, Australia, <sup>6</sup>Pivot Medical Centre, Perth, Western Australia, <sup>7</sup>Sydney IVF, Sydney, New South Wales, <sup>8</sup>Queensland Fertility Group, Brisbane, Queensland, <sup>9</sup>Repromed, Dulwich, South Australia, <sup>10</sup>Discipline of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia, Australia

Received: 8 January 2010; accepted: 28 April 2010

### SUMMARY

A multi-centre study was undertaken to: a/ determine the density of human semen, and b/ assess the validity of measuring semen volume either volumetrically or gravimetrically. Semen samples from four clinical categories (azoospermia following vasectomy, azoospermia without vasectomy, oligozoospermia ( $<20 \times 10^6/\text{ml}$ ) and normozoospermia ( $\geq 20 \times 10^6/\text{ml}$ )) had similar

<sup>1</sup>Corresponding author and current address: Hollywood Fertility Centre, Hollywood Private Hospital, Nedlands 6009, Australia; e-mail: [phil.matson@hollywoodivf.com](mailto:phil.matson@hollywoodivf.com)

<sup>2</sup>on behalf of Scientists in Reproductive Technology, Fertility Society in Australia

densities (one-way ANOVA:  $F(3,180)=1.25$ , not significant), being close to 1.0 g/ml when taken to one decimal place. Measurement of semen volume was then made with either a graduated pipette or by weighing and assuming a density of 1 g/ml. A comparison of the two methods gave an excellent correlation, with a gradient of 1.0571 and a coefficient of determination ( $R^2$ ) of 0.98 ( $p<0.0001$ ). However, it was noted that the aspiration of the ejaculate in to a graduated pipette underestimated the volume by approximately 0.2 ml, but in an inconsistent manner making the use of a set correction factor inappropriate. The estimation of volume to one decimal place by weighing the collection container before and after ejaculation, assuming a density of 1 g/ml, would seem to be a viable alternative although the density of a small number of samples may deviate from this assumption. Whilst the relatively small underestimation of volume with a pipette is unlikely to have clinical significance, the known reporting of inaccurate results by a laboratory is contrary to the philosophy and key principles of quality management. *Reproductive Biology 2010 10 2: 141-153.*

**Key words:** semen analysis, ejaculate volume, semen density, semen weight, quality assurance

## INTRODUCTION

Basic semen analysis has remained an essential screening test in the assessment of human male fertility and the measurement of semen volume is an important parameter [21]. In the pioneering work of MacLeod volume was measured with graduated cylinders [20] and this simple method has been recommended in various textbooks [12, 22] and, more importantly, in the universally accepted standardized protocol produced by the World Health Organisation [28]. Another volumetric approach using graduated pipettes has also been proposed [22, 26]. However, both these methods are in marked contrast to the gravimetric use of weight of the ejaculate as an indicator of its volume as used in clinical studies [6, 10, 19] or proposed in semen analysis text books and manuals [1, 7-9].

The direct relationship between weight and volume hinges on the density of the semen being 1 g/ml. Early studies confirmed human semen to have

a specific gravity of between 1.020 and 1.040 [2, 14] or a density of 0.970 to 1.043 g/ml [5] suggesting that the density is close enough to 1.0 g/ml when working to one decimal place and that weight should be an accurate index of volume. A comparison of volumetric and gravimetric methods does not give similar results, and the use of pipettes or graduated cylinders has been shown to significantly underestimate semen volume by up to 0.5 ml due to residual fluid being left behind in the vessel [4, 5]. Indeed, some studies have actually applied correction factors because they have subsequently discovered the limitation of their volumetric method [15, 16].

The use of volumetric methods to measure semen volume is widespread despite warnings of their inaccuracy as mentioned above. In these times of accountability through accreditation, laboratories are coming under increased pressure to demonstrate the use of validated methods in order to comply with minimum standards [27]. Whilst a difference in a fraction of a millilitre when assessing semen volume may not alter clinical management, there is a mandatory requirement for medical laboratories to verify the quality of the results provided to requesting clinicians [24] and also to determine the uncertainty of the results reported [23, 24]. The present study was therefore undertaken to determine the validity or otherwise of measuring semen volume either volumetrically or gravimetrically, in an attempt to help laboratories choose the most appropriate methodology for measuring accurately the volume of human semen at semen analysis. This was done by investigating: a/ the density of human semen extended to include the full range of samples of different clinical categories (i.e. azoospermia following vasectomy, azoospermia without vasectomy, oligozoospermia ( $<20 \times 10^6/\text{ml}$ ) and normozoospermia ( $\geq 20 \times 10^6/\text{ml}$ )), and b/ the relationship between weight and volume to confirm or refute the validity of a volumetric method.

## **MATERIALS AND METHODS**

Data were collected as a collaborative multicentre study by members of the scientific group – Scientists in Reproductive Technology (SIRT) within the Fertility Society of Australia. Laboratories participating were located at the

Canberra Fertility Centre (Deakin, Australian Capital Territory), Keogh Institute for Medical Research (Nedlands, Western Australia), Pivet Medical Centre (Leederville, Western Australia), Repromed (Dulwich, South Australia), Sydney IVF (Sydney, New South Wales) and Queensland Fertility Group (Brisbane, Queensland). For all centres, semen was collected by masturbation as part of patients' routine assessment and management. All protocols and equipment are validated as part of the quality management system required by the Australian accrediting bodies NATA (National Association of Testing Authorities, Rhodes, New South Wales) and RTAC (Reproductive Technology Accreditation Committee, Port Melbourne, Victoria). All weights were measured to four decimal places using an analytical balance.

### **Semen density**

The density of semen was determined in the first series of 184 semen samples. After complete liquefaction, 0.5 ml semen was accurately dispensed into a pre-weighed container, the whole weighed and the weight of the semen being the difference between the two. The density in g/ml was then calculated as the weight (g) multiplied by 2. A volume of 0.5 ml was selected to be in the middle of the working range of a 1 ml pipette, and to allow inclusion of samples with small volumes. Sperm concentration was determined using either a Makler chamber or hemocytometer [28], and samples categorized according to the WHO criteria [28] as azoospermia following vasectomy, azoospermia without vasectomy, oligozoospermia ( $<20 \times 10^6/\text{ml}$ ) and normozoospermia ( $\geq 20 \times 10^6/\text{ml}$ ).

### **Estimating weight by volume**

A comparison of gravimetric and volumetric methods was undertaken on a second series of 60 semen samples ranging in sperm concentration from 0 to  $164 \times 10^6/\text{ml}$ . All collection vessels were pre-weighed before being given to the patient. After semen collection and subsequent complete liquefaction, the weight of the whole ejaculate in the vessel was determined and the volume calculated assuming a density of 1.0 g/ml. The volume was then

measured to the nearest 0.1 ml by drawing the whole ejaculate carefully in to a graduated pipette.

### Statistical analysis

Group values were expressed as means and standard error of the mean ( $\pm$ SEM), and comparisons of group values made using one-way ANOVA [13]. The linear regression and correlation between the volumes measured volumetrically or gravimetrically was calculated by least squares using Excel 2004 for Mac (v11.5), and a comparison of the two methods made using a Bland-Altman plot [3]. Statistical significance was taken at  $p < 0.05$ .

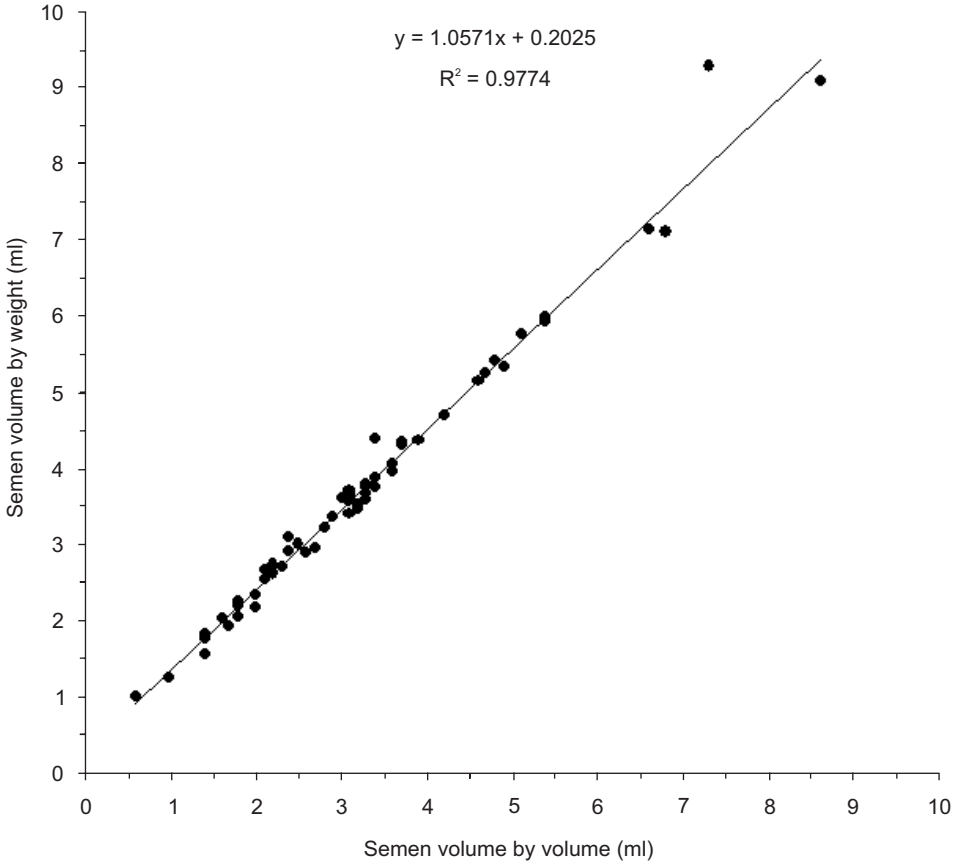
## RESULTS

The density of semen results are shown in Table 1. All the four clinical categories of semen had similar densities (one-way ANOVA:  $F(3,180)=1.25$ , not significant) being close to 1.0 g/ml when taken to one decimal place (overall mean $\pm$ SEM being  $1.0184 \pm 0.0029$  g/ml). A comparison of the volume of 60 semen samples measured by gravimetrically and volumetrically is shown in Figure 1. There was an excellent correlation, with a gradient of 1.0571 and a coefficient of determination ( $R^2$ ) of 0.98 ( $p < 0.0001$ ). However, there was

*Table 1.* The density of semen from samples that were found at analysis to be azoospermic following vasectomy, azoospermic without a vasectomy, oligozoospermic or normozoospermic

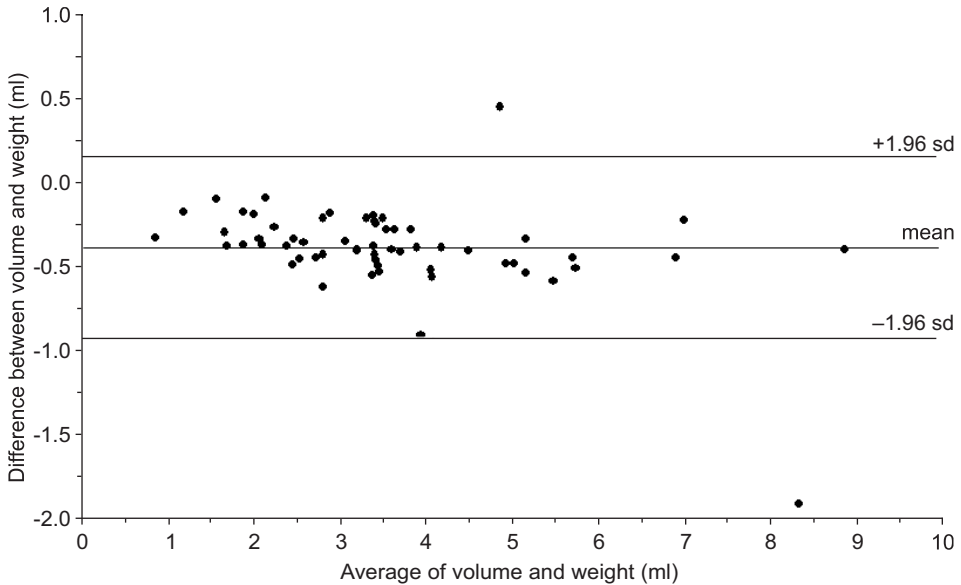
Semen category	n	Density (g/ml) (mean $\pm$ SEM)	Density range (g/ml)
Azoospermia (with vasectomy)	10	$1.0409 \pm 0.0051$	1.0100-1.0580
Azoospermia (without vasectomy)	4	$1.0247 \pm 0.0083$	1.0128-1.0492
Oligozoospermia	39	$1.0158 \pm 0.0056$	0.9510-1.1152
Normozoospermia	131	$1.0173 \pm 0.0036$	0.8096-1.0858

normozoospermic:  $\geq 20 \times 10^6$  sperm per ml); oligozoospermic:  $< 20 \times 10^6$  sperm per ml; one-way ANOVA:  $F(3,180)=1.25$ , not significant



*Figure 1.* The correlation between the volume of semen measured with a pipette and the inferred volume based on weight (assuming a density of 1 g/ml) of 60 semen samples.

a difference between the two methods such that extrapolation of the line of best fit showed the volume of semen left after drawing all up in to a pipette was 0.2025 ml, whilst an examination of the individual samples showed an underestimation (mean $\pm$ SEM) by the volumetric method of  $0.40\pm 0.03$  ml. The individual differences are shown in Figure 2 plotted against the mean of the two methods. Whilst the summary statistics show an overall underestimate when measured volumetrically (mean bias = -0.40 ml; coefficient of repeatability = 0.53 ml), there is a wide range in the difference between



*Figure 2.* A Bland-Altman plot showing the difference between the volume measured by either weight or volume. This difference is plotted against the mean of the two methods.

volumetric and gravimetric methods ranging from +0.43 ml to -1.91 ml suggesting a single correction factor would be inappropriate.

## DISCUSSION

Semen analysis is plagued by a lack of standardization in its performance and reporting [17]. Whilst small differences in the reported semen volume may not affect the clinical management of an infertile man, medical laboratories have a mandatory responsibility to validate their methodology to ensure accuracy and provide an indication of the uncertainty of their results to aid clinicians with interpretation [11, 23, 24]. The measurement of volume may be difficult to control as evidenced by the lack of inclusion in external quality assurance schemes [18], and it would seem inappropriate to use methods that are known to contain major errors. The measurement of

semen volume with pipettes or graded cylinders has been shown previously to give an underestimate [5] and yet the practice is widespread presumably by virtue of being recommended by authoritative texts aimed at reducing inaccuracy and unreliability [22, 28]. The present study has therefore made a systematic appraisal of weight as an alternative as previously promoted by Eliasson [7, 9] and others [1].

When using gravimetric methods, the lack of a correction factor would demand that the specific gravity (i.e. ratio of mass to the mass of an equal volume of water at 4°C) or density (g/ml) be 1 or 1g/ml, respectively. Whilst earlier studies confirmed the specific gravity to be close to 1 [2, 14], they were limited by poor detail of how the specific gravity was determined, the treatment of semen as a single fluid irrespective of the clinical background, and low numbers (e.g. only six semen samples; [14]). A more recent study described a range of densities of 0.970 to 1.043 g/ml [5], but here they only studied fertile men. The present study has for the first time described the densities of semen of four categories according to the sperm concentration, namely azoospermia (with or without vasectomy), oligozoospermia ( $<20 \times 10^6/\text{ml}$ ) and normozoospermia ( $\geq 20 \times 10^6/\text{ml}$ ). There was no difference between the groups but it should be noted that the range (tab. 1) did contain a few outliers from a minimum density of 0.8096 to a maximum of 1.1152. It would appear from the present study that weight would give a good approximation of volume for the majority of samples but that error will occur with a minority due to biological differences in the composition of the seminal fluid. This contrasts with errors in volumetric measurement of semen volume which occur due to failure to aspirate or transfer all of the ejaculate leaving some behind [5]. A difference between pipetted volume and volume estimated by weight has been previously described in the order of 0.4 ml [4] and 0.5 ml [5], and correction factors of 0.1 ml [16] and 0.49 ml [15] have been added retrospectively to samples with volume measured volumetrically because of this perceived shortfall. The present study showed that linear regression estimated that approximately 0.2 ml was remaining after aspiration of the ejaculate (fig. 1) but that there was an average difference of 0.4 ml when looking at the mean of individual samples. Presumably, the physi-



cal properties of the plastic containers will affect surface tension and the amount of residual semen left after aspiration. The wide range of suppliers worldwide means that a simple correction factor should not be applied as that would in itself introduce more error.

There are inevitably benefits and limitations with any method used, and a summary comparing volumetric and gravimetric methods to measure semen volume is given in Table 2. The cost of the volumetric method is that of disposable graduated pipettes and is on-going whilst a gravimetric method is an up-front cost of a simple balance measuring to one or two decimal places plus any maintenance. Whilst these costs will vary according to the workload and discounts available on consumables and the availability of an existing balance, a preliminary appraisal at a medium-sized unit without an existing balance would indicate that the overall costs of the two methods are not dissimilar. However, there are a number of advantages to measuring semen gravimetrically. Firstly, it eliminates the underestimation which may occur as a result of semen clinging to the sides of the container [5]. Secondly, it may reduce error associated with pipetting viscous samples. Thirdly, it has previously been recommended that the last few drops of semen should not be forcibly expelled as this may create droplets or aerosol [25, 28], which in itself may create an inherent underestimation of volume. Measuring semen gravimetrically may eliminate this potential occupational health and safety issue especially with samples of increased viscosity. Nevertheless, there are a few limitations to measuring the semen gravimetrically. It has been previously reported that this approach may overestimate semen volume if name labels are inadvertently placed on the container after it has been weighed and the sample produced [4], and that individual containers vary sufficiently in weight to make the weighing before and after sample collection necessary [4]. Also, a gravimetric approach may not be suitable where the containers are not issued by the analyzing laboratory as the weight of the empty container would not be possible to obtain. Finally, the density of some semen samples may not be close to 1 g/ml as shown in the present study (tab. 1) making the estimation of volume by weight unreliable as these samples cannot be identified under routine circumstances, although these would be very small in number.

*Table 2.* A comparison of the benefits and limitations of using volumetric or gravimetric methods to measure semen volume

<b>Consideration</b>	<b>Method used</b>	
	<b>Volumetric</b>	<b>Gravimetric</b>
Cost	The consumable costs are on-going.	The capital cost of a balance is up-front, and the cost per sample decreases with increased numbers.
Occupational health and safety	Involves handling and pipetting, with increased risk of potential exposure to pathogens.	Collection vessel is sealed and no contact with sample.
Viscous samples	Accurate pipetting is difficult due to viscosity of the seminal fluid.	Potentially more appropriate assuming density of sample is close to 1g/ml.
Accuracy	Underestimates the volume because of residual sample sticking to the sides of the container.	More accurate as vast majority of samples have density close to 1.0 g/ml.
Sample wastage	A small volume of semen will invariably be residual in the pipette.	No pipetting, hence no wastage here. This is particularly important with valuable samples such as those with low volumes from cancer patients presenting for semen storage.
Practicality	Pipetting is very easy, and may have to be done anyway if the semen sample is to be processed for assisted reproductive techniques.	Very simple, though care must be taken to ensure the labelling of the container does not add to the weight.

It has been known for some time that volumetric methods of measuring semen volume give errors [4, 5] and yet such methods remain in common use. Similarly, the results of the current study did come as a surprise to all the laboratories participating and yet several months later only one of the six participants use a gravimetric method although several others are considering a shift. Obviously, the negligible clinical impact provides very little motivation for change, but laboratories must address this issue to satisfy requirements for accreditation as mentioned earlier, and also to know that the results reported are true and, if not, by how much they are not. The calculation of measurement uncertainty is something that medical laboratories must undertake as part of their quality management system [23, 24]. Interestingly, in Australia there is a requirement to identify both significant sources of measurement uncertainty and the opportunities for their reduction [23], and the current study and others would make it very difficult for laboratories to justify continuing to use volumetric methods.

In summary, the measurement of semen volume is prone to error. The aspiration of the ejaculate in to a graduated pipette underestimates the volume, but in an inconsistent manner making the use of a set correction factor inappropriate. The estimation of volume to one decimal place by weighing the collection container before and after ejaculation, assuming a density of 1 g/ml, would seem to be a viable alternative for the large majority of semen samples. Whilst the relatively small underestimation of volume with a pipette is unlikely to have clinical significance, the known reporting of inaccurate results by a laboratory is contrary to the philosophy and key principles of quality management.

## **ACKNOWLEDGEMENTS**

The authors would like to thank for their input and practical assistance the scientists at the following units: Canberra Fertility Centre (Sarah Rowsell), Pivet Medical Centre (James Stanger, Jesmine Wong, Saule Idelbayeva, Jason Conceicao, Christian Ottoini, Marie Herd), Repromed (Michelle Lane and Ozlem Tunc), Sydney IVF (Tyne Pollard, Paul Tyrriil, Rebecca Dorey, Nico Foley, Keren Weiss, Stephanie Nguyen, Alana Conlay, Lucy Nguyen and Joyce Tan) and Queensland Fertility Group (Andrew Noble and Keith Harrison).

## REFERENCES

1. Auger J, Kunstmann JM, Czyglik F, Jouannet P **1995** Decline in semen quality among fertile men in Paris during the past 20 years. *New England Journal of Medicine* **332** 281-285.
2. Belonoschkin B **1949** *Procreation in humans (in German)*. Sjobergs, Stockholm.
3. Bland JM, Altman DG **1986** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1** 307-310.
4. Brazil C, Swan SH, Drobnis EZ, Liu F, Wang C, Redmon JB, Overstreet JW **2004** Standardized methods for semen evaluation in a multicenter research study. *Journal of Andrology* **25** 635-644.
5. Cooper TG, Brazil C, Swan SH, Overstreet JW **2007** Ejaculate volume is seriously underestimated when semen is pipetted or decanted into cylinders from the collection vessel. *Journal of Andrology* **28** 1-4.
6. Eliasson R **1965** Effect of frequent ejaculations on the composition of human seminal plasma. *Journal of Reproduction and Fertility* **9** 331-336.
7. Eliasson R **1971** Standards for investigation of human semen. *Andrologie* **3** 49-64.
8. Eliasson R **1973** Parameters of male fertility. In: *Human Reproduction: Conception and Contraception*, 39-51. Eds Hafez E, Evans T. Harper & Rowe, Hagerstown.
9. Eliasson R **2003** Basic semen analysis. In: *Current Topics in Andrology*, 35-89. Ed Matson P. Ladybrook Publishing, Perth.
10. Eliasson R, Lindholmer C **1972** Distribution and properties of spermatozoa in different fractions of split ejaculates. *Fertility and Sterility* **23** 252-256.
11. Feinberg M, Laurentie M **2006** A global approach to method validation and measurement uncertainty. *Accreditation and Quality Assurance: Journal for Quality, Comparability and reliability in Chemical Measurement* **11** 3-9.
12. Freund M, Peterson RN **1976** Semen evaluation and fertility. In: *Human semen and fertility regulation in men*, 344-354. Eds Hafez E. The CV Mosby Company, Saint Louis.
13. Glantz SA **1992** *Primer of biostatistics.*, Third edition. McGraw-Hill Inc, Singapore.
14. Huggins C, Scott WW, Heinen JH **1942** Chemical composition of human semen and of the secretions of the prostate and seminal vesicles. *American Journal of Physiology* **136** 467-473.
15. Iwamoto T, Nozawa S, Yoshiike M, Hoshino T, Baba K, Matsushita T, Tanaka SN, Naka M, Skakkebaek NE, Jorgenson N **2006** Semen quality of 324 fertile Japanese men. *Human Reproduction* **21** 760-765.
16. Jørgenson N, Auger J, Giwercman A, Irvine DS, Jensen TK, Jouannet P, Keiding N, Le Bon C, MacDonald E, Pekuri AM, Scheike T, Simonsen M, Suominen J, Skakkebaek NE **1997** Semen analysis performed by different laboratory teams: an intervariation study. *International Journal of Andrology* **20** 201-208.
17. Keel BA **2004** How reliable are results from the semen analysis? *Fertility and Sterility* **82** 41-44.
18. Keel BA, Quinn P, Schmidt Jr C, Serafy Jr N, Searfy Sr N, Schalue T **2000** Results of the American Association of Bioanalysts national proficiency testing programme in andrology. *Human Reproduction* **15** 680-686.

19. Lindholmer C, Thulin L, Eliasson R **1975** Semen characteristics before and after ligation of the left internal spermatic veins in men with varicocele. *Scandinavian Journal of Urology and Nephrology* **9** 177-180.
20. MacLeod J **1950** The male factor in fertility and infertility: an analysis of ejaculate volume in 800 fertile men and in 600 men in infertile marriage. *Fertility and Sterility* **1** 347-361.
21. Matson PL **1997** Clinical value of tests for assessing male infertility. *Bailliere's Clinical Obstetrics and Gynaecology* **11** 641-654.
22. Mortimer D **1994** *Practical Laboratory Andrology*. Oxford University Press, Oxford.
23. NPAAC **2007** Requirements for the estimation of measurement uncertainty. National Pathology Accreditation Advisory Council, Commonwealth Department of Health and Ageing, Canberra.
24. NPAAC **2009** Medical laboratories – particular requirements for quality and competence. National Pathology Accreditation Advisory Council, Commonwealth Department of Health and Ageing, Canberra.
25. Schrader SM **1989** Safety guidelines for the andrology laboratory. *Fertility and Sterility* **51** 387-389.
26. Silverberg KM, Turner T **2009** Evaluation of sperm. In: *Textbook of Assisted Reproductive Technologies: Laboratory and Clinical Perspectives*, 39-52. Eds Gardner DK, Weissman A, Howles CM, Shoham Z, Third edition. Informa Healthcare, London.
27. Sjöblom C, Keck C **2009** The ART laboratory in the era of ISO 1000 and GLP. In: *Textbook of Assisted Reproductive Technologies: Laboratory and Clinical Perspectives*, 25-37. Eds Gardner DK, Weissman A, Howles CM, Shoham Z. Third edition. Informa Healthcare, London.
28. WHO **1999** *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction.*, Fourth edition. Cambridge University Press, Cambridge.

