

# Semen analysis workshops in India and Africa: the vital role of training and external quality control programmes

D.R. FRANKEN

Faculty Health Science, University of Stellenbosch, Tygerberg Hospital, Tyberberg 7505, South Africa.

Correspondence at: drf@sun.ac.za

## Abstract

Two hundred and fifty seven individuals from 16 African and Indian andrology laboratories attended several semenology workshops from 1997 to 2013. The external quality control programme in Africa indicated the ability of participants to maintain the technology skills following a hands-on training. The pre-training sessions of the workshops indicated a total lack of knowledge how to perform a semen analysis according to the WHO manual. This was the case for morphology, motility and concentration. The results of this report underline the vital role of training as well as external quality control programmes.

**Key words:** Andrology, developing countries, external quality control, semen analysis, training.

## Introduction

The analysis of human semen especially in developing countries remains the cornerstone of the male fertility work-up schedule, and therefore the laboratory technician's quality assurance should be mandatory (Ombelet et al., 1995). Although many laboratories claim to use the World Health Organization's manual for the analysis of human semen as a guideline, a survey concluded that only 5% of United Kingdom laboratories adhered to the current WHO rules for the evaluation of sperm morphology. This was also the case for staining, classification and sampling techniques (Riddell et al., 2005). These reports are partly responsible for the concerns expressed that analysis of human semen has become a neglected test and should be regarded as a technique of the past (McDonough, 1997; Chong et al., 1983). On the other hand Jequier concluded that semen analysis needs only to be performed competently without the need for costly and time-consuming forms of quality assurance (Jequier 2005; 2010). We have demonstrated that in order to maintain the reading skills to evaluate morphology, hands-on training is mandatory (Franken and Aneck-Hahn, 2008; Franken et al., 2000; Franken et al., 2003a; 2003b).

The Tygerberg's andrology unit interest in sperm morphology started in the early 70's, when emphasis was placed on the importance of well-defined criteria to assess normality (Van Zyl et al., 1990; Menkveld et al., 1990; Mortimer and Menkveld, 2001). During these early years Van Zyl et al. (1980) suggested a morphology threshold value for *in vivo* fertilization of 10%. During the last two decades, the clinical significance of sperm morphology as predictor of *in vitro* and *in vivo* fertilization has been supported by a vast number publications (Kruger et al., 1986; Enginsu et al., 1991; Ombelet et al., 1994; Eggert-Kruse et al., 1995; 1996). The current overview aimed to evaluate the role of hands-on training on the technical skills of andrology technologists as well as the importance of an external quality control programme.

## Materials and Methods

Two hundred and fifty seven individuals from African and Indian andrology laboratories attended several semenology workshops from 1997 to 2013. Eighty seven individuals from 16 Sub-Sahara African countries, i.e. Benin, Cameroon, Ethiopia, Kenya, Nigeria, South Africa, Tanzania, Uganda, Tunisia, Zambia, Tunisia, Ghana, Sudan, Egypt,

Senegal and Zimbabwe were invited to participate in semenology workshops at Tygerberg Hospital. The African workshops were presented in conjunction with the World Health Organization's Special Programme of Research, Development and Research Training in Human Reproduction (HRP, Geneva, Switzerland) aimed not only to provide training opportunities for individuals in reproductive health on the one hand, but also to strengthen the service providing capacity of the region. Furthermore, 170 individuals from Indian andrology laboratories attended workshops presented in Guwahati (Institute of Human Reproduction), Mumbai, Delhi, Indore and Bangalore (Academy of Clinical Embryologists).

The format of these workshops included a pre- and post-training sessions. During the pre-training session delegates were provided with Hemacolor (Merck Chemicals, Cat no 1.11661/1) stained slides to record the percentage normal forms. Sperm concentration and motility were evaluated on fresh semen samples provided to each delegate. All the results were collected and stored in our workshop data basis. The pre-training results were regarded as a base line value to be used to calculate the learning after the post training session. Following the pre-training session delegates were lectured on the methodologies for sperm morphology, sperm concentration, progressive motility and vitality according the methods described in the WHO 1999 and WHO 2010 manuals.

During the morphology training sessions PowerPoint projections of numbered spermatozoa were used to emphasize the criteria for a spermatozoon to be classified as normal. This session included a consensus training session during which high quality micro-photographic images of numbered sperm were projected on a large screen. Individual spermatozoa were discussed during group sessions to underline the specific aberrations responsible for the sperm to be classified as abnormal (Figure 1).

Following the training sessions, 19 individuals from 12 laboratories were enrolled to a external quality control programme over a period of 40 months (Figure 5). This QC system entailed the following: On a quarterly basis each enrolled laboratory received a set of two pre-stained Papanicolaou slides that contained sperm obtained from normo-, terato- and/or severe teratozoospermic sperm samples. Each slide was evaluated for percentage normal cells by the reference laboratory prior to shipment.

Participants had to record the percentage normal cells for these slides and forward the results to Tygerberg Hospital, where all the information is stored in a data base. The "correct" results according

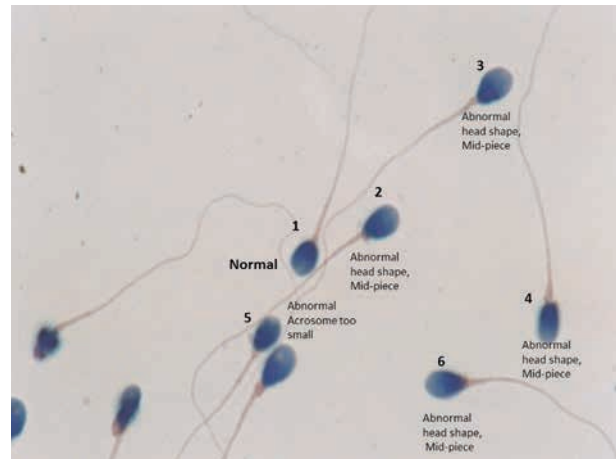


Fig. 1. — High quality micro-photographic images of numbered sperm to be evaluated by andrology technologists.

to the reference laboratory, i.e. the percentage normal forms present on each of the slides were subsequently supplied to the participating laboratory.

For a spermatozoon to be considered normal the sperm head, neck, mid-piece and the tail must be within the limits and guidelines described in the WHO 2010 manual for the analysis of human semen. The head should be oval in shape. Allowing for the slight shrinkage that fixation and staining induce; the length of the head should be 4.0-5.0 $\mu$ m and the width 2.5-3.5 $\mu$ m. The length-to-width ratio should be 1.50 to 1.75. Estimation of the length and width of the spermatozoa were made with an ocular micrometer. There should be a well-defined acrosomal region comprising 40-70% of the head area. The midpiece should be slender, less than 1 $\mu$ m in width, about one and a half times the length of the head, and attached axially to the head. The tail should be straight, uniform, thinner than the mid-piece, uncoiled and approximately 45 $\mu$ m long (WHO, 1999). This classification scheme requires that all "borderline" forms be considered abnormal (Menkveld et al., 1990).

#### *External quality control programme*

From July 1999 to January 2003, each participating laboratory received 14 slide sets (28 slides) over a 40 month period. The first set of slides was shipped 3 months after the training course; a total of 286 slides were sent to the 12 participating laboratories (19 individuals).

#### *SD-score*

Due to the fact that the morphological slides used for evaluation of trainee standards were random samples from different individuals, standardization

was needed with respect to an index that is independent of the morphological level. The count for strict criteria for normal morphologic sperm is a binomial random variable and the variance of this variable is dependent on the mean. Let  $p$  denote the morphology score (%) for a slide. The standard deviation of this outcome is  $\sqrt{p(100 - p)}$  which clearly shows the dependence.

Under the assumption that the reference laboratory's morphology reading is the gold standard the index is the following standardised score:

$$\text{Standard deviation (SD) score} = \frac{\text{Trainee score} - \text{Reference laboratory score}}{\text{SD Reference laboratory}}$$

The Tygerberg mean SD-scores were calculated of all the reference slides 1 and 2 that were dispatched to laboratories were  $10.9 \pm 6.7\%$  and  $6.7 \pm 6.5\%$  normal forms.

Each participant was evaluated on the SD-score for his/her level of agreement with the reference value. In order to understand the role of human error we choose 2 limits of error namely the  $\pm 0.5\text{SD}$  and  $\pm 0.2\text{SD}$ -scores. We used the limit  $\pm 0.2\text{SD}$  in a previous study (Franken et al., 2003a) since it corresponded with the 25<sup>th</sup> and 75<sup>th</sup> percentile of the SD scores after the training. However, the present study includes the  $\pm 0.5$  SD limits, since the period of

evaluation was almost twice as long as the previous report.

Using the SD-scores we were able to record the sperm morphology reading ability (skills) of each trained participant over a 40 month period measured among 14 test slide sets. The individual SD-scores obtained from the pre-and post-training sessions as well as the results recorded doing the follow-up continuous quality control programme can be plotted against the period of evaluation. We classified the partaking individuals into 5 categories based on their sperm morphology reading skills (Figure 5).

During motility training PowerPoint projected video clips were used to record the different categories of sperm motility. Likewise, Neubauer counting chamber grids containing different numbers of sperm were used to train the delegates (Figure 2).

Sperm vitality was recorded using a one-step eosin nigrosin stain (Bjorndahl et al., 2003). Slides were prepared during the workshop and scored for the percentage dead (red stained) and live (unstained) sperm (Figure 3)

## Results

For sperm morphology we used the results reported by the delegates during the pre-and post-training sessions to establish the learning curve. The post mean is equal to -0.19 with 95% confidence interval

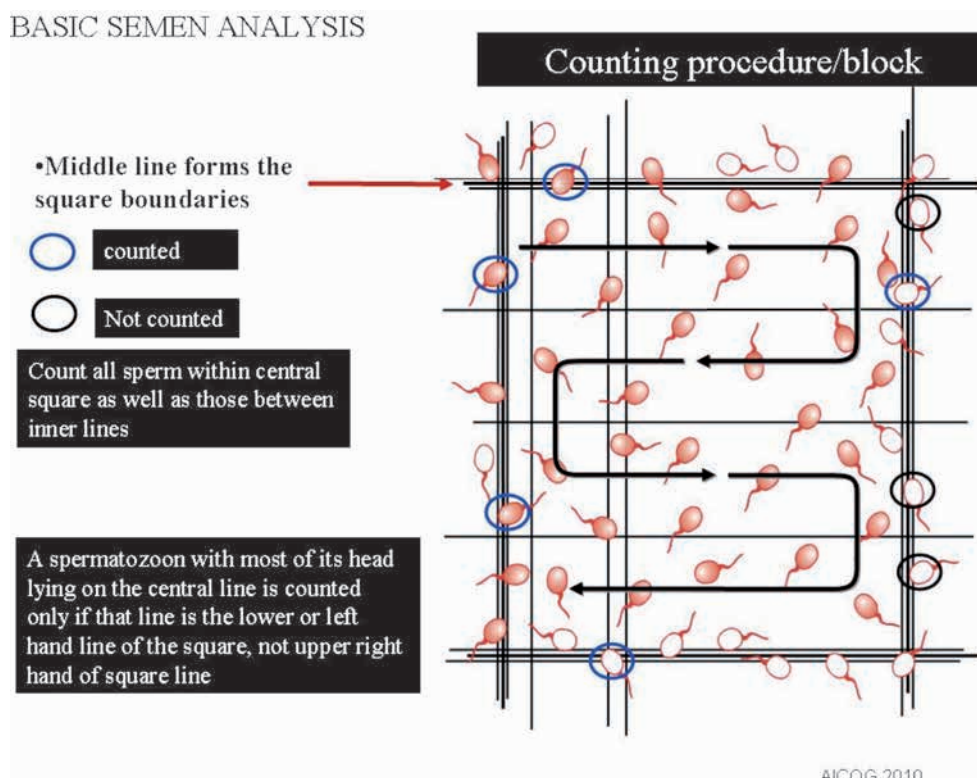
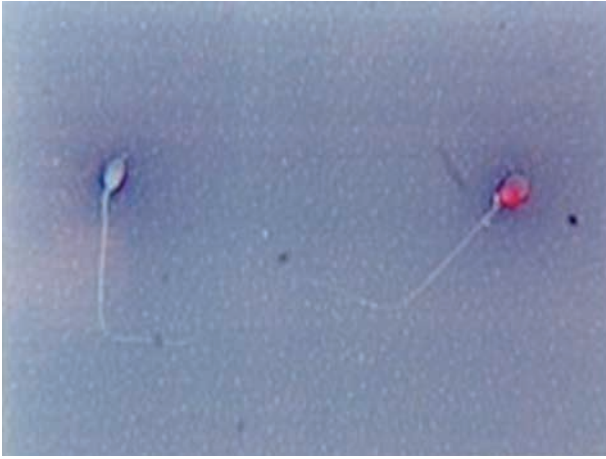


Fig. 2. — Diagram of a Neubauer counting grid illustrating the counting methodology of sperm



**Fig. 3.** — Vitality stained spermatozoa depicting dead (red stained) and live (unstained) sperm.

of -0.98 to 0.61. Since this interval spans 0 it shows that the mean morphology reading after training by the participants was not significantly different from 0 (Figure 4).

*Poor reading skills*

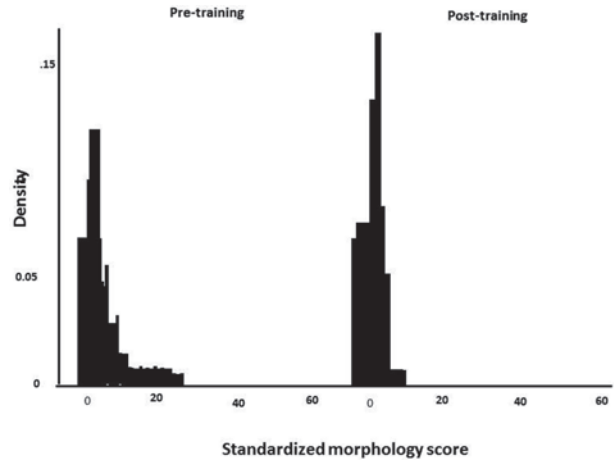
If  $\leq 50\%$  readings, recorded over the evaluation period were inside the limits of error i.e. the  $\pm 0.5SD$ -score, marginal reading standards were assumed.

*Marginal reading skills*

If 51-60% readings recorded over the evaluation period were within the  $\pm 0.5SD$ -score, marginal-to-good reading skills were assumed.

*Good reading skills*

If 60-69% of the readings, recorded over the evaluation period, fell inside the limits of error i.e. the  $\pm 0.5SD$ -score, good reading standards are assumed.



**Fig. 4.** — Histogram of standardized morphology scores pre and post-training.

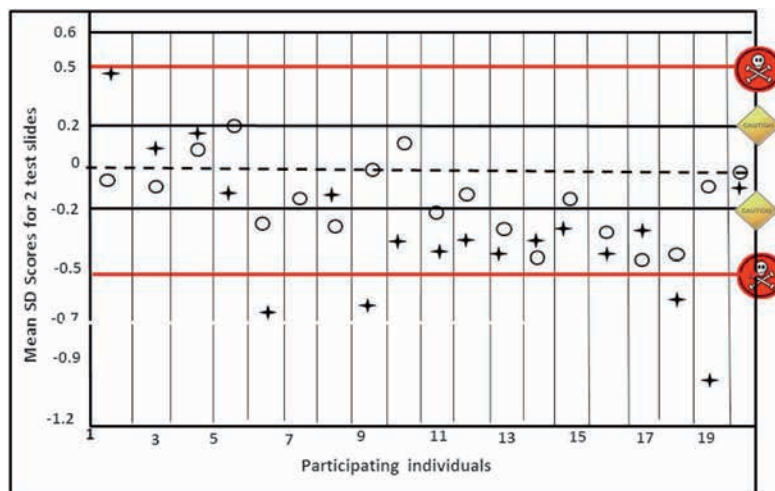
*Excellent reading skills*

If 70-79% of the readings, recorded over the evaluation period, were within the  $\pm 0.5SD$ -score, good-to-excellent reading skills were assumed.

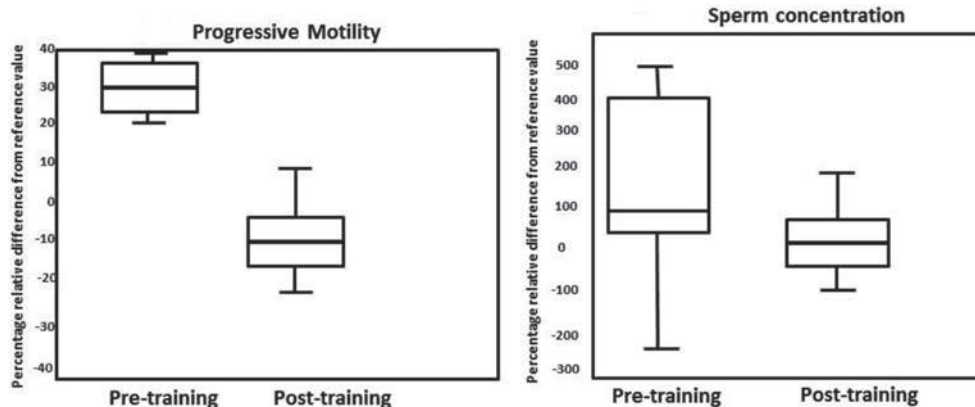
*Superior reading skills*

If  $\geq 80\%$  of the readings, recorded over the evaluation period, fell inside the limits of error i.e. the  $\pm 0.5SD$ -score, excellent sperm morphology reading skills are assumed.

The results recorded by the delegates for the percentage sperm with progressive motility and sperm concentration are depicted in Figure 6. In both motility and concentration evaluations the pre-training results were vastly overestimated. Overestimation seems to be more of a problem with high-concentration specimens (Brazil, 2010). As far as the evaluation of sperm concentration is concerned the results during the pre-training session showed a wide variation amongst the delegates.



**Fig. 5.** — The mean SD scores for 2 test slides reported by 19 individuals enrolled to an external quality control programme for sperm morphology.



**Fig. 6.** — Pre- and post-training Sperm concentration values recorded by laboratory technologists'. Box-Whisker plots illustrating the percentage difference from the reference value for sperm concentration and progressive motility during pre- and post-training sessions.

## Discussion

Laboratory technologists are often confronted with the question 'How good are we at evaluating the semen sample?' As the analysis of human semen in developing countries still remains the cornerstone of the male fertility investigation, technician quality assurance should be handled with great responsibility. Our experience has shown that the Implementation of the suggestions in the WHO 2010 manual can be regarded as a substantial challenge: it is almost impossible to learn a technique as subjective as semen evaluation from words and pictures alone. Although the WHO manual recommends an easy improved method for the hemacytometer, most laboratories in Africa and India use the Makler counting chamber. The Makler chamber is preferred since it does not need dilution of semen in most cases. This was expected since most delegates were not familiar with the use of the Neubauer counting chamber.

The diagrams (Fig. 4, Fig. 6) can be used as an indication of the level of technical skills and knowledge as far as the techniques for a standard semen analysis is concerned. As far as the evaluation of motility is concerned technologists overestimate motility by a large margin (Figure 6). Overestimation seems to be more of a problem with high-concentration specimens (Brazil, 2010). The results depicted in the Box Whisker plots in Figure 6 illustrate the total lack of knowledge as far as the evaluation of sperm progressive motility and concentration is concerned. Sperm motility evaluation as described by the WHO manual (WHO, 2010) has been simplified to 3 categories, namely progressively motile (PR), non-progressively motile (NP) and immotile (IM), instead of grade a, b, c or d. The results should be noted with concern as by institutions that provide semen analyses for referring clinicians. If we regard the present findings as representative of

andrology technologists' technical ability, clinicians in Third-World countries should be concerned about the diagnostic quality as far as male infertility is concerned.

The most prominent problem in morphology classification and morphology scoring is the large variation coefficient that exists between and among different technicians in different laboratories. Despite the problems associated with the preparation of slides and staining methods, the use of different classification systems and the subjective nature of visual sperm morphology assessment, we still believe in the power of this important parameter in the routine semen analysis. This is especially true for laboratories in developing countries where the lack of sophisticated diagnostic laboratories is not readily available. After training the participants read the morphology slides close to the true value. In contrast the pre- training readings were substantially biased – the Z-score of 6.5 indicates this (Figure 4).

Morphology assessment as described by the WHO 2010 remains a difficult method and the ease with which one can become proficient at performing Strict method morphology analysis is greatly overstated. Furthermore, the confidence that each laboratory that uses this method is also not realistic, For example the statement "all the borderline spermatozoa are abnormal' is confusing since the size and shape of the sperm will be used as a guide to classify the sperm as normal or abnormal. It therefore becomes very difficult to draw a line between borderline and abnormal spermatozoa (Ombelet et al., 1998; Brazil, 2010). Our experience indicated all workshop attendees assessing human sperm morphology must be aware of the risk of becoming too strict'. To emphasize the difference between normal and abnormal we have develop an interactive DVD containing high quality photographic PowerPoint images of numbered spermatozoa (Figure 1). Counting procedures are presented as images of sections

of hemacytometer grids containing spermatozoa for counting would allow technicians to compare results with the key, which again could be set by consensus of a small group.

Most of the European countries expressed the need for an external quality control programme for semen analysis (Cooper, 1996; Jørgensen et al., 1997; Ochsendorf and Beschmann, 1998; Auger et al., 2000) and further afield (WHO, 1999). The external quality programme that we installed in Africa for sperm morphology was highly successful (Figure 5). In most cases the results were within the 0.5SD score which is an indication of the delegate's ability to maintain the acquired knowledge gained during the training sessions of the workshops. Ideally a quality control programme should include evaluations for motility and concentration. Brazil (2010) suggested the use of a DVD to accompany the WHO manual and thus enhance the quality of semen analysis on a global level. Unless improvements are made, patient results will continue to be compromised and comparison between studies and laboratories will have limited merit.

Training of technicians as well as regular proficiency testing will ensure continuous communication with the referring laboratory. Proficiency testing of technician skills is of the utmost importance if andrology laboratories want to secure a professional code of conduct and clinical relevant results. The authors firmly believe that global quality control measurements in andrology laboratories will eventually become mandatory (Bjorndahl et al., 2002; Cooper et al., 1999). In order to maintain low intra- and inter-technician variation and high quality proficiency testing among laboratory technician's continuous teaching programmes should be available to all.

## References

- 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.
- Bjorndahl L, Barratt CLR, Fraser L et al. ESHRE basic semen analysis courses 1995–99: immediate beneficial effects of standardized training. *Hum Reprod.* 2002;17:1299-1305.
- Björndahl L, Soderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining 8858 technique for human sperm vitality assessment. *Hum Reprod.* 2003;18:813-816.
- Brazil C. Practical semen analysis: from A to Z. *Asian J Androl.* 2010;12:14-20.
- Chong AR, Waiters CA, Weinreb SA. The neglected laboratory test. The semen analysis. *J Androl.* 1983;4:280-282.
- Cooper TG, Atkinson AD, Nieschlag E. Experience with external quality control in spermatology. *Hum Reprod.* 1999;14:765-769.
- Cooper TG. Implementation of quality control in the andrology laboratory. *Int J Androl.* 1996;19:67-68.
- Enginsu ME, Dumoulin CJM, Pieters MHEC et al. Evaluation of human sperm morphology using strict criteria after Diff-Quik staining: correlation of morphology with fertilization in vitro. *Hum Reprod.* 1991;6:854-858.
- Eggert-Kruse W, Schwarz H, Rohr G et al. Sperm morphology assessment using strict criteria and male fertility under in-vivo conditions of conception. *Human Reprod.* 1996;11:139-146.
- Eggert-Kruse W, Reimann-Anderson J, Rohr G et al. Clinical relevance of sperm morphology assessment using strict criteria and relationship with sperm-mucus interaction in vivo and in vitro. *Fertil Steril.* 1995;63:612-624.
- Franken DR, Aneck-Hahn N. Sperm morphology training programmes: The African Experience. *Hum Reprod Monograph.* 2008:60-63.
- Franken DR, Barendson R, Kruger TF. A continuous quality control (CQC) program for strict sperm morphology. *Fertil Steril.* 2000;74:721-724.
- Franken DR, Menkveld R, Kruger TF et al. Monitoring technologist reading skills in a sperm morphology quality control program. *Fertil Steril.* 2003a;79:1637-1643.
- Franken DR. African Experience with sperm morphology training courses. *Reproductive Medicine Online.* 2003b;7:114-119.
- Jequier AM. Is quality assurance in semen analysis still really necessary? A clinician's viewpoint. *Hum Reprod.* 2005;20:2039-2042.
- Jequier AM. Semen analysis: a new manual and its application to the understanding of semen and its pathology. *Asian J Androl.* 2010;12:11-13.
- Jørgensen N, Auger J, Giwercman A et al. Semen analysis performed by different laboratory teams: an intervariation study. *Int J Androl.* 1997;20:201-208.
- Kruger TF, Menkveld R, Stander FSH et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil Steril.* 1986;46:1118-1123.
- McDonough P. Editorial comment: Has traditional sperm analysis lost its clinical relevance? *Fertil Steril.* 1997;67:585-587.
- Menkveld R, Stander FSH, Kotze TJDW et al. The evaluation of morphologic characteristics of human spermatozoa according to stricter criteria. *Hum Reprod.* 1990;5:586-592.
- Mortimer D, Menkveld R. Sperm Morphology assessment-historical perspective and current opinions. *J Androl* 2001; 22:192-205.
- Ombelet W, Fourie leR, Vandeput H et al. Teratozoospermia and in-vitro fertilization: a randomized prospective study. *Hum Reprod* 1994;9:1479-1484.
- Ombelet W, Bosmans E, Janssen M et al. Multicenter study on reproducibility of sperm morphology assessments. *Arch Androl.* 1998;41:103-114.
- Ombelet W, Menkveld R, Kruger TF et al. Sperm morphology assessment: historical review in relation to fertility. *Hum Reprod Update.* 1995;1:543-547.
- Ochsendorf FR, Beschmann HA. Qualitätssicherung im andrologischen Labor. *Reproduktionsmedizin.* 1998;14:97-105.
- Riddell D, Pacey A, Whittington K. Lack of compliance by UK andrology laboratories with World Health Organization recommendations for sperm morphology assessment. *Hum Reprod.* 2005;20:3441-3445.
- Van Zyl JA, Kotze TJDW, Menkveld R. Predictive value of spermatozoa morphology in natural fertilization. In: Acosta AA, Swanson RJ, Ackerman SB, Kruger TF, van Zyl JA, Menkveld R eds. *Human spermatozoa in Assisted Reproduction* Williams and Wilkins 1990.
- Van Zyl JA. The infertile couple. Part. II Examination and evaluation of semen. *SAMJ.* 1980;57:485.
- World Health Organization. *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction.* Cambridge University Press; 1999.
- World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th ed. Geneva: World Health Organization; 2010.