The impact of ESHRE basic semen analysis course: 6 years of Italian experience

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Summary

The assessment of conventional semen analysis parameters is an essential initial investigation for all the couples treated for infertility problems. Mainly, the absence of standardization in semen analysis can led a suboptimal care for childless couples.
The European Society of Human Reproduction and Embryology (ESHRE) realized a standardized training course, which has been run in different regions of the world. Particularly in Italy 6 courses have been organized. The aim of the present analysis was to examine to what extent attendance of the course resulted in any positive effect on the variability of evaluations performed by different participants.

KEY WORDS: semen analysis, standardization, course, Italy.

Introduction

Semen analysis provides crucial information on the functional status of seminiferous tubules, epididymis, prostate and seminal vesicles and the patency of the male genital tract, on which clinicians base their initial diagnosis. The assessment of conventional semen analysis parameters is an essential initial investigation for all the couples in treatment for infertility problems (1). Additionally, it is crucial in order to elucidate the effect of the treatments that may be improved the testicular function (2).
The main problem of the lack of standardization in semen analysis can led a suboptimal care for childless couples (3). Therefore, it is very

important for diagnostic laboratories [these who perform in vitro fertilization techniques (IVF) and those, which are non IVF clinics] to perform semen analysis correctly and precisely so that clinicians can correctly estimate, the probability of conception or choose the most adequate technics to be used in an IVF program for couples under investigation.

The World Health Organization (WHO) laboratory manual is recognized worldwide as the gold standard for human semen examination. The last version was published in 2010 (4).

Nevertheless, many laboratories performing basic semen analysis they do not employ the methods recommended by the WHO manual (2). Others, even performing semen analysis according to WHO manual standards are characterized by a high degree of variability. On the other hand, reducing the variability intra and among evaluators performing semen analysis is essential to participate in external quality assessment programs; participating laboratories evaluate samples from the same semen pool and receive feedback on the competence of their evaluators (5).

In order to support the development of higher quality analysis in basic semen laboratories, the Special Interest Group for Andrology (SIGA) in ESHRE has initiated the organization of courses in basic semen analysis (6). The SIGA presented the first course on Basic Semen Analysis (BSA) in 1994 that has been since then standardized and repeated in many countries mainly in Europe (Sweden, The Netherlands, Belgium, Denmark, Norway, Finland, Ukraine, Spain, Greece, Portugal, Poland) but also in other non-European countries (South Africa, Canada and the UK) in different languages (6). The subjects shown in the course have been updated regularly in response to new discoveries and publications.

Following publication of the WHO manual 5th edition (2010) (WHO5) (4), the ESHRE Subcommittee for training of the Special Interest Group for Andrology (SIGA) has evaluated potential amendments to its BSA course. Several areas have been updated and explained more in details, such as: evaluation of sperm motility, sperm morphology, abstinence, sperm concentration, sperm vitality and even the methodologies for sperm preparation (7).

The ESHRE BSA course recommendations have provide results with similar or even better quality than those recommended in WHO manual 5th edition, often with less demand on time and efforts in the laboratory (7). Based on these modifications, the handbook "A Practical Guide to Basic Laboratory Andrology" has been the reference text for the ESHRE BSA course all over the years (1).

In Italy several ESHRE BSA courses have been organized since 2012. The aim of this study was to examine whether there were any immediate effects of the exercises provided during these courses on the variability of assessments made by the different participants.

Material and methods

Development of courses

In Italy 6 courses have been organized since 2012 with a total of 120 participants (Table 1). One of the main principles of the courses was to organize them in the native language of the participants. The lectures included explanation of the methods recommended in order to minimize errors. Sometimes the WHO manual (4) recommended more than one procedure to obtain results but it was decided that participants should only learn one technique for each procedure, in order to improve standardization.

Straightway during the first day of the course a first exercise, called pre-test session, was done by all the participants. Mainly during the pre-

Table 1. Registered Basic Semen Analysis courses given according to EHSRE-SIGA in Italy.

Venue	Dates
Roma	October 8-11, 2012
Milan	October 1-4, 2013
Florence	October 7-10, 2014
Milan	May 12-15, 2015
Roma	September 27-30, 2016
Pistoia	October 3-6, 2017

test session all the participants have to evaluate concentration, motility and morphology of samples. This exercise permits the evaluation of the initial performance of each participant. Afterward, each lecture was followed by a practical session (training sessions) during which measurements of concentration and motility (total, progressive and rapid) were made on two fresh semen samples. Moreover, the theory sessions include an overview of the clinical use of the results obtained.

Percentage of normal morphology and live sperm were obtained in each session using slides previously fixed and stained by the organizers. The teratozoospermia index (TZI) was also calculated. The TZI is an overall indicator of the level of teratozoospermia, equal to the mean number of abnormalities per abnormal spermatozoon (8). The results of the assessment were always discussed the day after. At the end of the course, there were two examinations: one written (theory) and one practical (assessment of the unknown fresh samples). In order to obtain a diploma from ESHRE, a participant had to pass both written and practical examination.

The results from the pre-test, training and examination session were compared with the results obtain by the "experts" (by the *course teachers*). In general, each participant of the course assessed two samples during pre-test, training and examination session (a mean of 6 samples per course edition). Unfortunately, sometimes, occasional participants failed to show a result because the lack of time due to minimal laboratory experience.

Statistical methods

Analytical variation of concentration, vitality, motility (progressive and total motility), morphology and TZI was expressed by means of the coefficient of variation (CV = 100 x standard deviation/ mean). Accuracy was estimated calculating the mean difference (*d*) between the mean of experts and participants for each sample. To investigate any change in accuracy and analytical variability among participant groups over the course: from the pre-test to the last day of the examination, we compared the mean CV and *d* obtained in each sessions (pre-test, train-

ing and examination) (two samples per session, that means 6 samples per course) using the Kruskal-Wallis test.

Results

Figure 1 shows a significant linear trend for decreasing variability among participants in the determination of concentration and morphology and TZI, as measured by changes in mean CV obtained for each sample from pre-test, training and examination sessions.

Moreover, a reduction in the variability among participants in the evaluation of the progressive motility of sperm has been observed (Figure 1), being statistically significant in the mean CV among the participants in the rapid progressive motility (Pre-test: 80.3 ± 12.0%; Training: 56.9 ± 10.1%; examination: 50.2 ± 7.2%; p<0.05).

For vitality and total motility, no significant changes were observed among the three groups, mostly due to the low CVs obtained in pre-test sessions.

Regarding the accuracy (Figure 2) a decrease in the difference between the expert means and participant means for all the seminal parameters analyzed except in the total motility was observed.

Discussion

The variability of concentration measurement observed in the pre-test session (CV=40.4%) was very high; this parameter was found higher compared to the variability observed for the centers participating to the European and Spanish External Quality Assessment program (EQAP) (5). The lack of experience of some participants in the use of the Neubauer improved chamber may be the reason for these results.

A significant reduction of the variability was observed after the course; moreover this reduction was lower than the one obtained in the previous EQAPS. The use of the same counting chamber during the course it could have had an impact in the significant reduction of the variability, indeed differences in the precision has
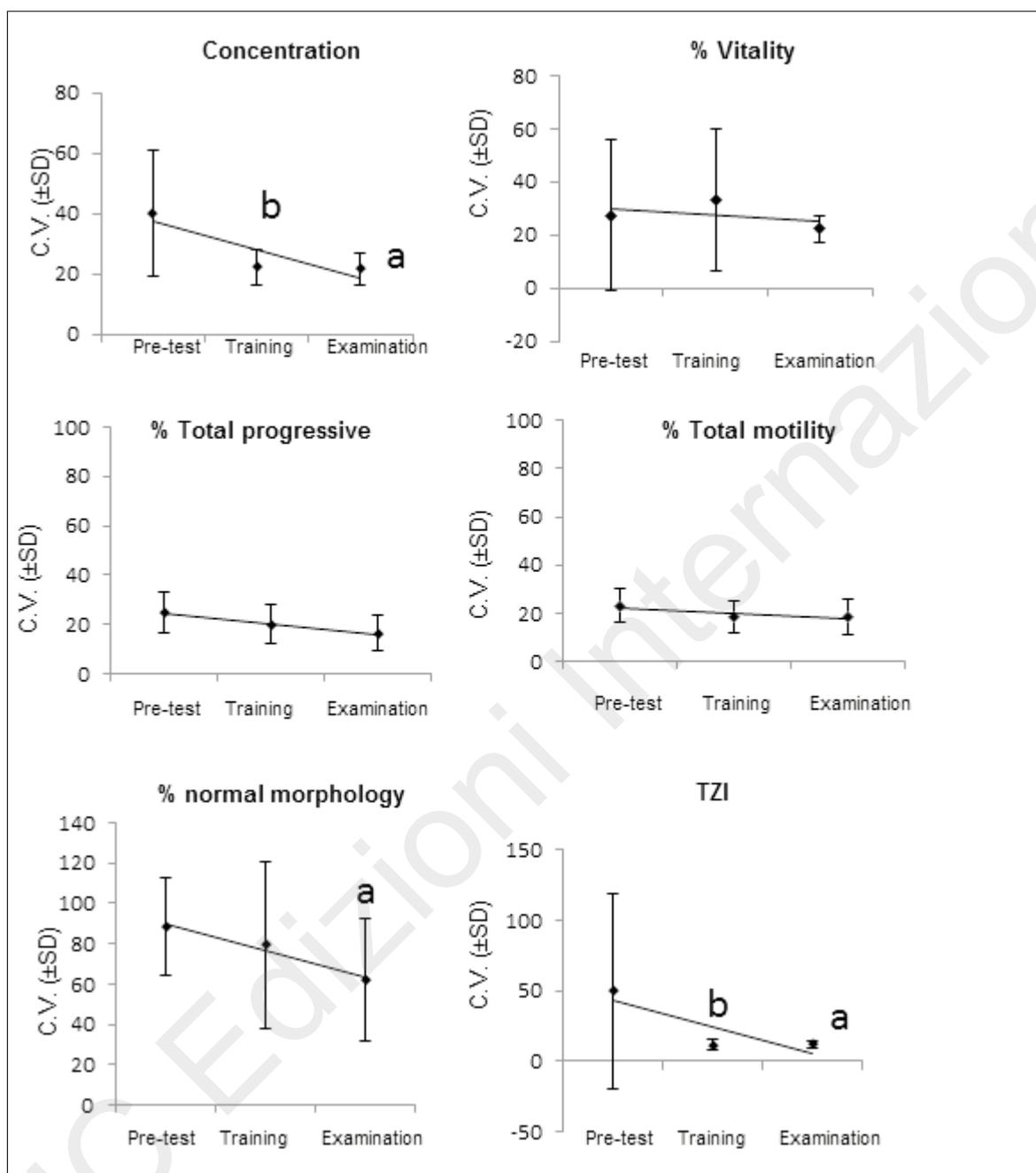
Figure 1 - Coefficient of variation obtained in pre-test, training and examination sessions (mean ± standard deviation). a, $p<0.05$ pre-test vs examination; b, $p<0.005$ pre-test vs training.

been described with the use of different counting chambers for the determination of the sperm concentration (9-11). *The significant variability reduction may be attributed to the use the same counting chamber by all the participants.* Indeed, differences in the precision has been described with the use of different counting chambers for the determination of the sperm concentration (4).

Regarding motility, we observed a reduction of the variability among the participants in the determination of the percentage of rapidly progressive spermatozoa supporting the utility of the course. Similar results has been obtained in the Spanish and European EQAP (5). Nevertheless, the variability in the total motility was very low compared to the rapidly progressive motility, as it has been described by other
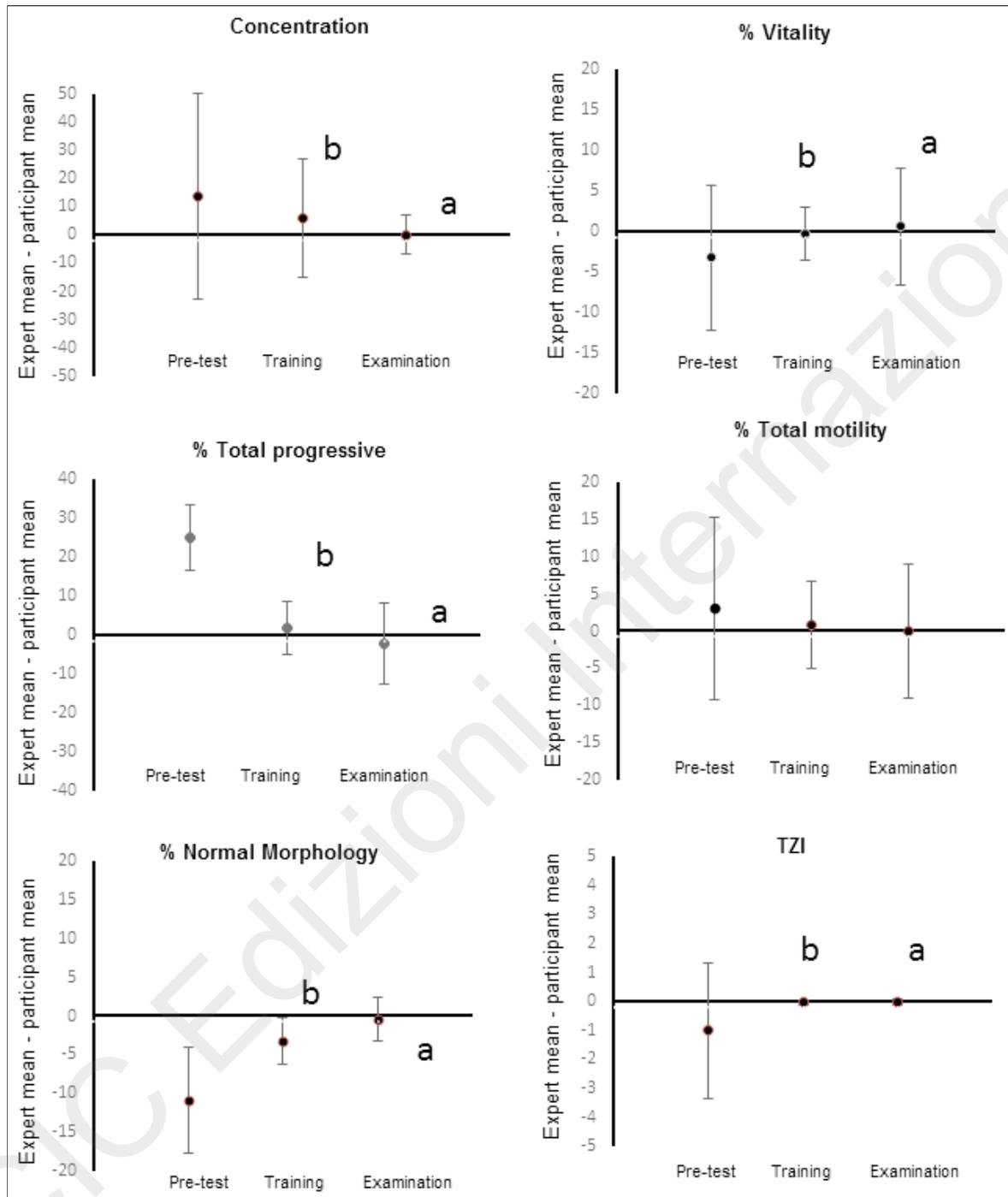
Figure 2 - Differences between the mean of experts and participants in pre-test, training and examination sessions (mean ± standard deviation). a, p<0.05 pre-test vs examination; b, p<0.005 pre-test vs training.

authors (12, 13). Our results demonstrated that it is possible a proper evaluation of the percentage of rapidly progressive motility spermatozoa. The importance in the clinic of this value has been demonstrated (14) and we think that this parameters should be analyzed as the SIGA-

ESHRE recommend (7).

For vitality, we have not observed a significant reduction of the variability among the participants after the course and this may be due to the lower variability observed in the pre-test session. The results observed in the examination

session are very similar to those described by Keel and collaborators (11) but very different to those achieved by Walter (1992) (12) that ranged between 42 to 90%. This discrepancy may be due in the case of Walker and cols they used cryopreserved semen samples and in our case and in the study of Keel and cols fix slides prepared with fresh semen was used (11).

High variability in the determination of the normal spermatozoa was observed, moreover this variability was significantly decreased within the course, reaching values similar to those observed in the Spanish and European EQAP (5). The variability of determination of sperm morphology could be due to the difference in the fixation and staining techniques used (11, 15). In our case, we used the same fixed and stained samples, suggesting that the variability was mainly due to the different morphology criteria used (16).

In the case of the TZI calculation, we observed a great decreased mainly due to the high variability observed in the pre-test. We observed that many participants did not know this value and they did not know how to calculate it, despite the recommendations for its use by the OMS manual (4). The high precision of the value at the end of the course among the participant makes it a valid parameter for its use in daily clinical practice (17).

One of the main factors that may be responsible for the variability among the participants may be the inclusion in the course of the standard procedures following ESHRE-SIGA recommendations (1), that are not fully concordant, with those recommended in the most recent WHO laboratory manual (WHO5). These particular areas of discourse have been reviewed by Barratt el al (7).

The improvement of the accuracy between the participants in the majority of the parameters studied after the BSA course, can directly improve the patient care of couples with reproductive desire because improved the validity of the analytic results. Our results are in concordance with the ones obtained by Björndahl et al. (2002) (6). The authors used the concordance defined as the proportion of results within ± 10% of the expert result, and our study present

the mean difference between the expert mean and participant mean.

In order to achieve high standards of the semen analysis in the future, and be comfortable with the methods taught at the course, more individual training of the participants need to be done after the participation of the training course. For this training, it would be helpful have "standard" fixed material in the laboratories with "known" results, or even used recorded samples for the sperm motility. Moreover, an internal and external quality control should be implemented in the laboratories.

One of the most important points, in our experience, is that the participants become aware of the importance of the standardization and they increase the interest about the use of internal and external quality controls. Another important issue for the organization of future ESHRE BSA courses is that all participants will be expected to be at least familiar with some issues about sperm analysis in the laboratory, for example counting chamber, sperm morphology, etc, mainly because the main purpose of the course is to improved the quality and decrease variability in the results among and within individuals. In conclusion, it has been demonstrated that attendance on training courses can have a significant improvement on the performance of individual scientists (6).

Indeed, similar results in improvement of semen analysis have been shown after the attendance of the participants to the Italian courses. Moreover, remains unknown whether the participants will continue to perform semen analysis with the same levels of accuracy when they return to their laboratories.

Reference

1. Björndahl L, Mortimer D, Barratt C, Castilla J, Menkveld R, Kvist U, Alvarez JG, Haugen, T. Basic semen analysis. In A Practical Guide to Basic Laboratory Andrology. (2010) Cambridge: Cambridge University Press.
2. Björndahl L. Compliance with recommendations for reliable semen analysis results - a matter of importance for patients and scientific development. Andrology. 2016 Sep;4(5):771-772.
3. Björndahl L, Barratt CL, Mortimer D, Jouannet P. "How to count sperm properly": checklist for acceptability of studies based on human semen analysis. Hum Reprod.

2016 Feb;31(2):227-232.

4. World Health Organization, Department of Reproductive Health and Research. WHO laboratory manual for the examination and processing of human semen. 2010.

5. Palacios ER, Clavero A, Gonzalvo MC, Rosales A, Mozas J, Martínez L, Ramírez JP, Björndahl L, Morancho-Zaragoza J, Fernández-Pardo E, Castilla JA. Acceptable variability in external quality assessment programs for basic semen analysis. Hum Reprod. 2012 Feb;27(2):314-322.

6. Björndahl L, Barratt CL, Fraser LR, Kvist U, Mortimer D. ESHRE basic semen analysis courses 1995-1999: immediate beneficial effects of standardized training. Hum Reprod. 2002 May;17(5):1299-1305.

7. Barratt CL, Björndahl L, Menkveld R, Mortimer D. ESHRE special interest group for andrology basic semen analysis course: a continued focus on accuracy, quality, efficiency and clinical relevance. Hum Reprod. 2011 Dec;26(12):3207-3212.

8. Jouannet P , Ducot B, Feneux D, Spira A. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. Int J Androl. 1988;11:379-394.

9. Neuwinger J, Behre HM, Nieschlag E. External quality control in the andrology laboratory: an experimental multicenter trial. Fertil Steril. 1990;54:308-314.

10. Matson PL. External quality assessment for semen analysis and sperm antibody detection: results of a pilot scheme. Hum Reprod. 1995;10:620-625.

11. Keel BA, Quinn P, Schmidt Jr CF, Serafy Jr NT, Serafy Sr NT, Schalue TK. Results of the American Association of Bioanalysts national proficiency testing programme in andrology. Hum Reprod. 2000;15:680-686.

12. Walker RH. Pilot surveys for proficiency testing of semen analysis. Arch Pathol Lab Med. 1992;116:422-424.

13. Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, Matribi SE, Gony B, Keskes L, Kolbezen M, Lamarte A, Lornage J, Normal N, Pitaval G, Simon O, Virant-Klun I, Spira A, Jouannet P. Intra- and inter-individual variability in human sperm concentration, motility and vitality assesment during a wokshop involving ten laboratories. Hum Reprod. 2000;15:2360-2368.

14. Björndahl L. The usefulness and significance of assessing rapidly progressive spermatozoa. Asian J Androl. 2010 Jan;12(1):33-35.

15. Davis RO, Gravance CG. Standardization of specimen preparation, staining, and sampling methods improves automated sperm-head morphometry analysis. Fertil Steril 1993;59:412-417.

16. Ombelet W, Bosmans E, Janssen M. et al. Multicenter study on reproducibility of sperm morphology assesment. Arch Androl. 1998;4:103-114.

17. Auger J, Jouannet P, Eustache F. Another look at human sperm morphology. Hum Reprod. 2016 Jan;31(1):10-23.