
















































































Standards in semen examination: publishing reproducible and reliable data based on high-quality methodology









Lars Björndahl ^{1,*†}, Christopher L.R. Barratt ^{2,†},
David Mortimer ^{3,†}, Ashok Agarwal ⁴, Robert J. Aitken ⁵,
Juan G. Alvarez ^{6,7}, Natalie Aneck-Hahn ⁸, Stefan Arver ¹,
Elisabetta Baldi ⁹, Lluís Bassas ¹⁰, Florence Boitrelle ^{11,12},
Riana Bornman ¹³, Douglas T. Carrell ^{14,15}, José A. Castilla ^{16,17},
Gerardo Cerezo Parra ¹⁸, Jerome H. Check ^{19,20},
Patricia S. Cuasnicu ²¹, Sally Perreault Darney ^{22,23},
Christiaan de Jager ²⁴, Christopher J. De Jonge ²⁵,
Joël R. Drevet ²⁶, Erma Z. Drobniš ²⁷, Stefan S. Du Plessis ²⁸,
Michael L. Eisenberg ^{29,30}, Sandro C. Esteves ^{31,32,33},
Evangelini A. Evgeni ^{34,35}, Alberto Ferlin ³⁶, Nicolas Garrido ³⁷,
Aleksander Giwercman ³⁸, Ilse G.F. Goovaerts ³⁹,
Trine B. Haugen ⁴⁰, Ralf Henkel ^{41,42}, Lars Henningsohn ^{43,44},
Marie-Claude Hofmann ⁴⁵, James M. Hotaling ⁴⁶,
Piotr Jędrzejczak ⁴⁷, Pierre Jouannet ⁴⁸, Niels Jørgensen ^{49,50},
Jackson C. Kirkman Brown ^{51,52,53}, Csilla Krausz ⁵⁴,
Maciej Kurpisz ⁵⁵, Ulrik Kvist ¹, Dolores J. Lamb ⁵⁶,
Hagai Levine ⁵⁷, Kate L. Loveland ⁵⁸, Robert I. McLachlan ⁵⁹,
Ali Mahran ^{60,61}, Liana Maree ⁶², Sarah Martins da Silva ²,
Michael T. Mbizvo ⁶³, Andreas Meinhardt ⁶⁴,
Roelof Menkveld ⁶⁵, Sharon T. Mortimer ^{3,66},
Sergey Moskovtsev ^{67,68}, Charles H. Muller ⁶⁹,
Maria José Munuce ⁷⁰, Monica Muratori ⁷¹,
Craig Niederberger ^{72,73}, Cristian O'Flaherty ⁷⁴,
Rafael Oliva ^{75,76}, Willem Ombet ^{77,78}, Allan A. Pacey ^{79,80},
Michael A. Palladino ⁸¹, Ranjith Ramasamy ⁸², Liliana Ramos ⁸³,
Nathalie Rives ⁸⁴, Eduardo Rs Roldan ⁸⁵, Susan Rothmann ⁸⁶,
Denny Sakkas ⁸⁷, Andrea Salonia ^{88,89},
Maria Cristina Sánchez-Pozo ⁹⁰, Rosanna Sapiro ⁹¹,
Stefan Schlatt ⁹², Peter N. Schlegel ⁹³,
Hans-Christian Schuppe ⁹⁴, Rupin Shah ⁹⁵,
Niels E. Skakkebak ⁴⁹, Katja Teerds ⁹⁶, Igor Toskin ⁹⁷,

[†]These authors contributed equally to this study.

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Herman Tournaye ⁹⁸, **Paul J. Turek** ⁹⁹,
Gerhard van der Horst ^{100,101,102}, **Monica Vazquez-Levin** ¹⁰³,
Christina Wang ^{104,105}, **Alex Wetzels** ¹⁰⁶,
Theodosia Zeginiadou ^{107,108}, and **Armand Zini** ¹⁰⁹

¹ANOVA, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden ²Reproductive Medicine Research Group, Division of Systems Medicine, School of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK ³Oozoa Biomedical Inc., West Vancouver, BC, Canada ⁴Case Western Reserve University, Moreland Hills, OH, USA ⁵Priority Research Centre for Reproductive Science, Faculty of Science and Faculty of Health & Medicine, University of Newcastle, Callaghan, NSW, Australia ⁶Centro Androgen, La Coruña, Spain ⁷Harvard Medical School, Boston, MA, USA ⁸Department of Urology, University of Pretoria, Pretoria, South Africa ⁹Department of Experimental and Clinical Medicine, University of Florence, Florence, Tuscany, Italia ¹⁰Andrology Department, Laboratory of Andrology and Sperm Bank, Fundació Puigvert, Barcelona, Spain ¹¹Department of Reproductive Biology, Fertility Preservation, Andrology, CECOS, Poissy Hospital, Poissy, France ¹²Paris Saclay University, UVSQ, INRAE, BREED, Jouy-en-Josas, France ¹³School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa ¹⁴Andrology and IVF Laboratory, Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA ¹⁵Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA ¹⁶GAMETIA Biobank, Granada, Spain ¹⁷Hospital Universitario Virgen de las Nieves and Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain ¹⁸LAFER Sperm Bank, Tuxpan 10-606, Roma Sur, C.P. 06760, Cuauhtémoc, Mexico City, Mexico ¹⁹Robert Wood Johnson Medical School at Camden, The University of Medicine and Dentistry of New Jersey, Camden, NJ, USA ²⁰Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Cooper Hospital/University Medical Center, Melrose Park, PA, USA ²¹Instituto de Biología y Medicina Experimental (IbyME-CONICET), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina ²²US EPA, Cary, NC, USA ²³US NIH, Cary, NC, USA ²⁴Faculty of Health Science, University of Pretoria, Pretoria, South Africa ²⁵University of Minnesota Medical Center, University of Minnesota, Minneapolis, MN, USA ²⁶Université Clermont Auvergne/CNRS/INSERM-GreD Institute, Clermont-Ferrand, France ²⁷School of Medicine, University of Missouri, Columbia, MI, USA ²⁸College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates ²⁹Male Reproductive Medicine and Surgery, Stanford University School of Medicine, Stanford, CA, USA ³⁰Department of Urology, Stanford University School of Medicine, Stanford, CA, USA ³¹ANDROFERT, Andrology and Human Reproduction Clinic, Campinas, Brazil ³²Department of Surgery (Division of Urology), University of Campinas (UNICAMP), Campinas, Brazil ³³Faculty of Health, Aarhus University, Aarhus C, Denmark ³⁴CRYOGONIA Cryopreservation Bank, Athens, Greece ³⁵Laboratory of Physiology, Department of Medicine, Democritus University of Thrace, Greece ³⁶Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padova, Italia ³⁷IVI Foundation, Health Research Institute La Fe, Valencia, Spain ³⁸Department of Translational Medicine, Lund University, Malmö, Sweden ³⁹Antwerp University Hospital, Edegem, Belgium ⁴⁰Department of Life Sciences and Health, Oslo Metropolitan University, Oslo, Norway ⁴¹Department of Metabolism, Digestion & Reproduction, Imperial College London, London, UK ⁴²Department of Medical Bioscience, University of the Western Cape, Bellville, South Africa ⁴³Division of Urology, Department of CLINTEC, Karolinska Institutet, Stockholm, Sweden ⁴⁴Department of Urology, Karolinska University Hospital, Stockholm, Sweden ⁴⁵Department of Endocrine Neoplasia & Hormonal Disorders, University of Texas MD Anderson Cancer Center, Houston, TX, USA ⁴⁶Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA ⁴⁷Department of Cell Biology, Poznan University of Medical Science, Poznan, Poland ⁴⁸Université Paris Descartes, Paris, France ⁴⁹Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark ⁵⁰International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark ⁵¹Centre for Human Reproductive Science (ChRS), UK ⁵²College of Medical & Dental Sciences, University of Birmingham, UK ⁵³Birmingham Women's and Children's NHS Foundation Trust, UK ⁵⁴Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy ⁵⁵Department of Reproductive Biology and Stem Cells, Institutet of Human Genetics, Poznan, Poland ⁵⁶Brady Department of Urology, Center for Reproductive Genomics and Englander Institute for Precision Medicine, Weill Cornell Medical College, Cornell University, New York, NY, USA ⁵⁷Braun School of Public Health and Community Medicine, Hadassah Medical Center, The Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel ⁵⁸Hudson Institute, Centre for Reproductive Health, Monash University, Clayton, VIC, Australia ⁵⁹Hudson Institute of Medical Research, Centre for Endocrinology and Metabolism, Monash University, Clayton, VIC, Australia ⁶⁰Dermatology and Andrology Department, Assiut University Hospital, Assiut, Egypt ⁶¹Faculty of Medicine, Assiut University, Assiut, Egypt ⁶²Department of Medical Bioscience, University of the Western Cape, Bellville, South Africa, ⁶³Country Director, Population Council (International Programs) ⁶⁴Department of Anatomy and Cell Biology, Justus-Liebig-University of Giessen, Giessen, Germany ⁶⁵Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa ⁶⁶Division of REI, Department of Obstetrics & Gynaecology, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada ⁶⁷Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada ⁶⁸CreATe Fertility Centre, Toronto, ON, Canada ⁶⁹Male Fertility Laboratory, Department of Urology, University of Washington School of Medicine, Seattle, WA, USA ⁷⁰Laboratorio de Medicina Reproductiva, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina ⁷¹Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Florence, Italy ⁷²Department of Urology, UIC College of Medicine, IL, USA ⁷³Department of Bioengineering, UIC College of Engineering, IL, USA ⁷⁴Department of Surgery (Urology Division), McGill University, Montréal, QC, Canada ⁷⁵Molecular Biology of Reproduction and Development Group, Biomedical Research Institute August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain ⁷⁶Hospital Clínic, University of Barcelona, Barcelona, Spain ⁷⁷Genk Institute for Fertility Technology, Genk, Belgium ⁷⁸Department of Obstetrics and Gynaecology, ZOL Hospitals and Hasselt University, Genk, Belgium ⁷⁹Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK ⁸⁰Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, UK ⁸¹Bloomfield College, Bloomfield, NJ, USA ⁸²Department of Urology, Miller School of Medicine, University of Miami, Miami, FL, USA ⁸³Division of Reproductive Medicine, Department of Obstetrics and Gynaecologie, Radboud UMC, Nijmegen, The Netherlands ⁸⁴Service Laboratoire de Biologie de la Reproduction-CECOS, Equipe Physiopathologie Surrénalienne et Gonadique, Unité Inserm 1239 NorDic, CHU-Hôpitaux de Rouen, UFR Santé—Université de Rouen, Rouen, France ⁸⁵Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain ⁸⁶Fertility Solutions Inc., Warrensville Heights, OH, USA ⁸⁷Boston IVF, Boston, MA, USA ⁸⁸University Vita-Salute San Raffaele, Milan, Italy ⁸⁹Division of Experimental Oncology/Unit of Urology, URI, IRCCS Ospedale San Raffaele, Milan, Italy ⁹⁰Department of Clinical Chemistry and Molecular Biology, Virgen del Rocío University Hospital, Seville, Spain ⁹¹Depto de Histología y Embriología, Facultad de Medicina, Gral. Flores, Uruguay ⁹²Centre of Reproductive Medicine and Andrology, Münster, Germany ⁹³Department of Urology, Weill Cornell Medicine, New York, NY, USA ⁹⁴Section of Andrology, Department of Urology, Pediatric Urology & Andrology, Justus-Liebig-University/University Hospital of Giessen-Marburg, Giessen, Germany ⁹⁵Lilavati Hospital & Research Centre, Mumbai, India ⁹⁶Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands ⁹⁷WHO Department of Sexual and Reproductive Health and Research

(includes the UNDP/UNFPA/UNICEF/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction—HRP), Geneva, Switzerland ⁹⁸Centre for Reproductive Medicine, Vrije Universiteit Brussel, Brussels, Belgium ⁹⁹The Turek Clinic, San Francisco, CA, USA ¹⁰⁰Medical Bioscience, University of the Western Cape, Bellville, South Africa ¹⁰¹Physiology Medical School, Stellenbosch University, Stellenbosch, South Africa ¹⁰²Department of Animal Science, Stellenbosch University, Stellenbosch, South Africa ¹⁰³IBYME, CONICET-FIBYME, Buenos Aires, Argentina ¹⁰⁴Clinical and Translational Science Institute, The Lundquist Institute, Torrance, CA, USA ¹⁰⁵Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA, USA ¹⁰⁶Fertility Laboratory, Radboud University Medical Centre, Nijmegen, The Netherlands ¹⁰⁷Thessaloniki Andrology Laboratory—Hellenic Sperm Bank, Thessaloniki, Greece ¹⁰⁸Laboratory of Histology-Embryology, Medical School, University of Athens, Athens, Greece ¹⁰⁹Division of Urology, Department of Surgery, St Mary's Hospital, McGill University, Montreal, Canada

*Correspondence address. Andrology Laboratory, ANOVA, Karolinska University Hospital and Karolinska Institutet, Norra Stationsgatan 69, level 4, S-113 64 Stockholm, Sweden. E-mail: lars.bjorndahl@ki.se  <https://orcid.org/0000-0002-4709-5807>

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ABSTRACT: Biomedical science is rapidly developing in terms of more transparency, openness and reproducibility of scientific publications. This is even more important for all studies that are based on results from basic semen examination. Recently two concordant documents have been published: the 6th edition of the *WHO Laboratory Manual for the Examination and Processing of Human Semen*, and the International Standard ISO 23162:2021. With these tools, we propose that authors should be instructed to follow these laboratory methods in order to publish studies in peer-reviewed journals, preferable by using a checklist as suggested in an Appendix to this article.

Key words: reproducibility / basic semen examination / standardized laboratory procedures / andrology / reproductive medicine / laboratory training / quality control / patient security / science development / journal requirements

Appeal to the scientific society involved in Andrology and Reproductive Medicine

As scientists are aware, there has been much discussion about the transparency, openness and reproducibility of science. This is not a new issue. Ten years ago, Begley and Ellis proposed a series of recommendations to improve the reliability of studies in preclinical cancer research (Begley and Ellis, 2012) that helped to initiate a series of developments to address and improve reproducibility. These have included more detailed reporting and transparency of methods such as the STAR Methods for *Cell Press* journals <https://www.cell.com/star-authors-guide>. Concomitant with these developments, national programmes, such as The MDAR (Materials Design Analysis Reporting) Framework, for transparent reporting in the life sciences have been launched (Macleod et al., 2021) and specific consortia have been developed to repeat key published experiments, e.g. Reproducibility Project: Cancer Biology (RP: CB) (<https://elifsciences.org/collections/9b1e83d1/reproducibility-project-cancer-biology>) (Rodgers and Collings, 2021). Furthermore, there are significant resources available such as EQUATOR guidelines (<https://www.equator-network.org/>). The clear direction of travel is to improve standards and have transparent reporting of research (Amara, 2022). There are challenges, however. For example, in the RP:CB project, insufficient information was available to repeat selected experiments published in high impact journals. Furthermore, in the experiments that could be repeated (50/193), fewer than half yielded similar results. As such, the final report of the RP: CB consortia suggested that 'it is hard to assess whether reported findings are credible' (Errington et al., 2021).

In our own discipline of Andrology and Reproductive Medicine, there is a plethora of evidence to show that using non-standardized methods produces unreliable data including, for example, for human sperm concentration and sperm motility assessments. This has created significant problems for the field, making it difficult to determine the scientific accuracy of many studies and ultimately establish their real

clinical and public health impact. A recent example of this is the study of Campbell et al. where they updated the World Health Organization (WHO) semen analysis distribution values (Campbell et al., 2021). The authors reported several challenges in obtaining key information of the quality of the semen examination methods used across the studies being considered for inclusion. Standardization of semen examination has been a long-standing issue that the profession has collectively failed to address, despite the availability of proven accurate methods and robust training approaches (Björndahl et al., 2002, 2016; Barratt et al., 2011; Carrell and De Jonge, 2016; Cairo Consensus Workshop, 2020). Too many studies depending on semen analysis derived data continue to demonstrate poor robustness and rigour in semen analysis methodology (Serrano et al., 2014). When methods with a high degree of uncertainty are used, differences between normal and pathological conditions are likely to be impossible to discover since each observation, burdened by large variability due to measurement uncertainty, will have a more-or-less random result. This will cause considerable overlap in results from the different populations, making them practically inseparable.

The question for all of us working in Andrology, including Editors of journals publishing research in this field, is: What can be done to improve the situation? We believe there is currently a window of opportunity for action. The recent publication of ISO Standard 23162 for the basic examination of human semen (International Organization for Standardization, 2021) finally means that the field has *de facto* reference methods. These methods form the basis of those recommended in the new 6th edition of the WHO andrology laboratory manual (World Health Organization, 2021), which contains simple to follow and proven high-quality methods for semen examination. We propose that authors should be instructed to follow these laboratory methods in order to publish studies in peer-reviewed journals. To facilitate this, we present in the Appendix an author checklist, modified from Björndahl et al. (2016), which authors can complete and submit with their manuscript, making it simple for the journals, reviewers and readers alike to assess the quality of the semen assessment methods used,

and hence of the data being reported. We suggest that any deviation from the checklist, for purposes of testing a new reagent, different method or procedure for improving on the performance of a current recommendation, should be specified and measured against those in the checklist. If authors did not follow these methods, a separate section of the Materials and Methods should specify what differed and why, and how the variations might have impacted the accuracy of results. In other disciplines, checklists have assisted with improving the reporting of results (*Nature*, 2018; NPQIP Collaborative Group, 2019). This approach is consistent with the TOP Guidelines (Transparency and Openness Promotion; Centre for Open Science, <https://www.cos.io/initiatives/top-guidelines>; Nosek et al., 2015).

This is an important initiative. We suggest it be implemented by all journals in our discipline to help improve the transparency, openness and reproducibility of science.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

No new data were generated or included in the manuscript.

Authors' roles

L.B., C.B. and D.M. outlined the first manuscript and contacted all other authors for comments. L.B., C.B. and D.M. summarized all suggestions and finalized the manuscript that all authors have received and confirmed their participation in.

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Conflict of interest

This is an Opinion article based on the necessity for improvement of standards in the field of Andrology and Reproductive Medicine, based on the two non-profit publications, by the World Health Organization and the International Organization for Standardization. No conflicts of interest are declared. CB, as an employee of the University of Dundee, serves on the Scientific Advisory board of ExSeed Health (from October 2021, financial compensation to the University of Dundee) and is a scientific consultant for Exscientia (from September 2021, financial compensation to the University of Dundee). CB has previously received a fee from Cooper Surgical for lectures on scientific research methods outside the submitted work (2020) and Ferring for a lecture on male reproductive health (2021). CB is Editor for *Reproductive Bio Medicine Online*. DL, as an employee Weill Cornell Medicine, declares: American Board of Bioanalysis (Secretary-Treasurer:

Honorarium); Fellow Health (Equity); Roman Health (Consultant and Advisory Board) (Equity and compensation).

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Appendix

Semen analysis methodology checklist for authors, reviewers and editors (modified from Björndahl et al., 2016).

An accessible version of the checklist is available as a [supplementary file](#). This checklist is based on the ISO Standard on basic semen examination and the current WHO recommendations (“WHO6”) [1,2], and on general scientific standards. Full compliance requires that all boxes are ticked.

A deviation from this checklist does not necessarily mean that the study cannot be published, but all deviations must be declared in the Materials and Methods section of the manuscript, including their impact on accuracy and measurement uncertainty of the data, in order to allow the reader to evaluate the quality of the analyses performed. For studies not reporting all characteristics of a basic semen examination, the checklist includes the option ‘Not applicable to the study’.

Investigations that would be subject to the requirements in this checklist can roughly be classified as clinical (evaluating patient treatment, diagnostic classification or predictive powers of certain assessments), experimental (e.g. exposure of spermatozoa to different compounds or *in vitro* treatments [3,4]) or epidemiological (evaluating variations in semen characteristics or effects of exposure populations to certain compounds or other circumstances).

Any scientific rationale for not complying with the guidelines, which is not included in the Materials and Methods section of the manuscript, must be substantiated to the Editor and Reviewers.

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I. Patients

Not applicable to the study

- I.1 The studied population (e.g. patients or volunteers) has been declared in the manuscript, together with the recruitment method and inclusion and exclusion criteria. In a study concerning couples being investigated for infertility, the following is specified in the manuscript: fertility status of female partner; and primary, secondary or other level of investigation of the man.
- I.2 If used in the manuscript, the term ‘male factor’ is completely defined.

- I.3 Reference limits provided in WHO5 or 5th percentile of distribution of semen examination results in WHO6 have not been used to characterize subjects as infertile, subfertile or fully fertile.

2. General aspects

Not applicable to the study

- 2.1 Patients were instructed to maintain 2–7 days of sexual abstinence before collecting an ejaculate for investigation.
- 2.2 Patients were informed about the importance of reporting any missed early ejaculate fractions, and their responses were noted on the laboratory record.
- 2.3 For specimens not collected at the laboratory, patients were instructed to avoid cooling (under 20 °C) or heating (above 37 °C) of the semen specimen during transport to the laboratory.
- 2.4 In the laboratory, specimens were kept at 37 °C before initiation of and during the analysis in case of sperm motility assessment.
- 2.5 For specimens collected adjacent to the laboratory, analysis was initiated after completion of liquefaction and within 30 min after ejaculation. If some of the specimens were collected at the laboratory and others collected at home the influence on the data is declared and discussed in the manuscript.
- 2.6 Liquefaction was first checked within 30 min after ejaculation.
- 2.7 Volume was determined either by weighing or using a wide-bore volumetric pipette.
- 2.8 Viscosity was measured using either a wide-bore pipette or a glass rod.
- 2.9 All staff members who performed the analyses have been trained in basic semen analysis (ESHRE Basic Semen Examination Course—or equivalent—with further in-house training to establish competency), and participate regularly in internal quality control.
- 2.10 When more than one method is recommended for a particular characteristic (e.g. to measure volume), only one was used in the study. For a multicentre study, all laboratories used the same method or variable methods are declared in the manuscript.

3. Sperm concentration assessment

Not applicable to the study

- 3.1 Semen aliquot to be diluted for sperm concentration assessment was taken with a positive displacement pipette (i.e. a ‘PCR pipette’) using a recommended diluent (state which diluent: _____).
- 3.2 Only standard dilutions were used (1:50, 1:20 or 1:10, i.e. 1 + 49, 1 + 19 or 1 + 9).
- 3.3 Sperm concentration was assessed using haemocytometers with improved Neubauer ruling.
- 3.4 Haemocytometers were allowed to rest for 10–15 min in a humid chamber to allow sedimentation of the suspended spermatozoa onto the counting grid before counting.
- 3.5 Sperm counting was done using phase contrast microscope optics (200–400×).
- 3.6 Comparisons were made between duplicate counts, and counts were re-done when the difference exceeded the acceptance limits.
- 3.7 Typically at least 200 spermatozoa were counted in each of the duplicate assessments.

4. Sperm motility assessment

Not applicable to the study

- 4.1 Motility assessments were performed at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.
- 4.2 Motility assessments were initiated within 30–60 min after sample collection.
- 4.3 Motility assessments were performed using phase contrast microscope optics (200–400 \times).
- 4.4 Sperm motility was classified using a four-category scheme: rapid progressive, slow progressive, non-progressive, and immotile.
- 4.5 Motility assessments were done in duplicate and compared; counts were re-done on new preparations when the difference between duplicates exceeded the acceptance limits.
- 4.6 The wet preparation was made using a drop of ___ μl and a ___ \times ___ mm coverslip to give a depth of ___ μm (must be at least 10 μm depth, but not too deep so as to allow spermatozoa to move freely in and out of focus; typically ca. 20 μm).
- 4.7 At least 200 spermatozoa were assessed in each duplicate motility count.
- 4.8 At least five microscope fields of view were examined in each duplicate count.

5. Sperm vitality assessment

Not applicable to the study

- 5.1 A validated supravital stain, appropriate to the type of microscope optics employed, was used to assess sperm vitality.
- 5.2 At least 200 spermatozoa were evaluated.
- 5.3 Assessments were done under high magnification ($\times 1000$ –1250) using a 100 \times high resolution oil immersion objective and bright field microscope optics (Köhler illumination).

6. Sperm morphology assessment

Not applicable to the study

- 6.1 Tygerberg Strict Criteria were used for the evaluation of human sperm morphology. (Another classification could be used for scientific studies with specific aims if the classification is described or referenced. Depending on the aim of the study, the evaluation of particular abnormal forms might be useful.)
- 6.2 Abnormalities are recorded for the four defined regions of the spermatozoon (head, neck/midpiece, tail and cytoplasmic residue).
- 6.3 The Papanicolaou staining method adapted for the assessment of human sperm morphology was used. For specific aims, other staining methods could be used but must then be declared and explained.
- 6.4 At least 200 spermatozoa were assessed in each ejaculate.
- 6.5 Assessments were done under high magnification ($\times 1000$ –1250) using a 100 \times high resolution oil immersion objective and bright field microscope optics (Köhler illumination).

7. External Quality Assessment (EQA)

- 7.1 The laboratory participated in EQA for the semen examination methods used to obtain data for the study.
- 7.2 Name of the EQA scheme: _____

8. Other findings

Not applicable to the study

- 8.1 The presence of abnormal clumping (aggregates and agglutinates) was recorded.
- 8.2 Abnormal viscosity was recorded.

9. Analysing data

Not applicable to the study

- 9.1 The actual duration of sexual abstinence (in 'hours' or 'days') was recorded for each specimen and included in the data reported in the manuscript.
- 9.2 As a minimum in clinical studies, semen volume, sperm concentration, total number of spermatozoa per ejaculate and abstinence time are given to reflect sperm production and output; only samples identified as having been collected completely were included in the study.
- 9.3 Confounding factors have been considered for statistical analysis: e.g. abstinence time and age, to consider secular or geographical variations in sperm concentration or sperm count.
- 9.4 If appropriate, optional biochemical markers for prostatic, seminal vesicular and epididymal secretions were analysed and reported, both as concentration and total amount.
- 9.5 Signs of active infection/inflammation were noted and considered in the analysis of data in the study (e.g. presence of non-germ line round cells, inflammatory cells, impaired sperm motility, possibly also anti-sperm antibodies or reduction of secretory contributions).

10. Data Repository

- 10.1 For the sake of transparency, all data without identification of individual patients or research participants have been saved to a trusted online repository, and there is a statement of this in the Results section of the manuscript.

Declaration by the corresponding author:

The information provided in this checklist is solemnly declared to be true.

Signature: _____ Date: _____

Name: _____

Affiliation: _____