| **Supplemental table**: Semen analysis has performed according to World Health Organization (2010) and in besides the checklist by Björndahl, Barratt, Mortimer & Jouannet (2016) was performed. | |
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| PATIENTS: | |
| For clinical studies: The patient population (e.g. patients, volunteers, students) has been declared in the manuscript, together with the recruitment method and inclusion and exclusion criteria. | ✓ |
| If used in the manuscript, the term 'male factor' must be completely defined. | ✓ |
| GENERAL ASPECTS: | |
| Patients were instructed to maintain 2–7 days of sexual abstinence before collecting a sample for investigation. | The protocol of this study was performed (1 and 4 days of ejaculatory abstinence) |
| Patients were informed about the importance of reporting any missed early ejaculate fractions, and men's answers were noted on the laboratory form. | ✓ |
| For specimens not collected at the laboratory, patients were instructed to avoid cooling or heating of the semen sample during transport to the laboratory. | Seminal samples were collected only at the andrology laboratory |
| Samples were kept at 37°C before initiation of and during the analysis in case of sperm motility assessment. | ✓ |
| For samples collected adjacent to the laboratory, analysis was initiated after completion of liquefaction and within 30 min after ejaculation. If this was not done—and more importantly when some of the samples are collected in the laboratory and others are collected at home—it should be checked that this did not influence the data (and, if yes, that this effect must be included as a confounding factor in the statistical analysis). | Seminal samples were collected only at the andrology laboratory |
| Liquefaction was first checked within 30 min after ejaculation. | ✓ |
| Volume was determined either by weighing or using a wide-bore volumetric pipette. | ✓ |
| Viscosity was measured using either a wide-bore pipette or a glass rod. | ✓ |
| All staff members who performed the analyses have been trained in basic semen analysis (ESHRE Basic Semen Analysis Course—or equivalent—and further in-house training) and participate regularly in internal quality control. | ✓ |

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| If more than one method can be recommended for a particular characteristic (e.g. to measure volume), only one should be used in a given study. | ✓ |
| SPERM CONCENTRATION ASSESSMENT:<br>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: fixate). | ✓ |
| Only standard dilutions were used (1:50, 1:20, or 1:10). | ✓ |
| Sperm concentration was assessed using haemocytometers with improved Neubauer ruling. | ✓ |
| 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. | ✓ |
| Sperm counting was done using phase contrast microscope optics (200–400×). | ✓ |
| Comparisons were made between duplicate counts, and counts re-done when the difference exceeded the acceptance limits. | ✓ |
| Typically at least 200 spermatozoa were counted in each of the duplicate assessments. | ✓ |
| SPERM MOTILITY ASSESSMENT:<br>Motility assessments were performed at 37°C+0.5°C. | ✓ |
| Motility assessments were done using phase contrast microscope optics (200–400×). | ✓ |
| Sperm motility was classified using a four-category scheme: rapid progressive, slow progressive, non-progressive, and immotile (WHO 1999; Björndahl *et al.* 2010; Barratt, Björndahl, Menkveld & Mortimer 2011). | Sperm motility was classified in progressive, non-progressive and immotile) |
| Motility assessments were done in duplicate and compared; counts were re-done on new preparations when the difference between duplicates exceeded the acceptance limits. | ✓ |
| The wet preparation was made with a drop of 10µl and a 22 x 22 mm coverslip to give a depth of 20µm (must be at least 10 µm, but not too deep so as to allow spermatozoa to move freely in and out of focus; typically ca. 20 µm). | ✓ |

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| At least 200 spermatozoa were assessed in each duplicate motility count. | ✓ |
| At least 5 microscope fields of view were examined in each duplicate count. | ✓ |
| SPERM MORPHOLOGY ASSESSMENT:<br>Tygerberg Strict Criteria were used for the evaluation of human sperm morphology. Note: 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. | Kruger strict criteria |
| 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. | ✓ |
| At least 200 spermatozoa were assessed in each ejaculate. | ✓ |
| Assessments were done under high magnification (×1000– 1250) using a 100× high resolution oil immersion objective and bright field microscope optics. | ✓ |
| OTHER FINDINGS:<br>The presence of abnormal clumping (aggregates and agglutinates) was recorded. | Not observed in this study. |
| Abnormal viscosity was recorded. | Not observed in this study |
| The presence of inflammatory cells was recorded and reported if more than 1 million/ml. | ✓ |
| ANALYSING DATA:<br>The actual duration of sexual abstinence (in ‘hours’ or ‘days’) was recorded for each sample and included in the data reported in the manuscript. | ✓ |
| As a minimum in clinical studies, semen volume, sperm concentration, total number of spermatozoa/ejaculate, and abstinence time are given to reflect sperm production and output; only samples identified as having been collected completely can be included in the study. | ✓ |
| Confounding factors have been considered for statistical analysis: e.g. abstinence time and age, to evidence secular | Study was paired and abstinence time was the focus |

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| or geographical variations in sperm concentration or sperm count. | of this study |
| If appropriate, optional biochemical markers for prostatic, seminal vesicular and epididymal secretions were analysed and reported both as concentration and total amount. | Among them, only epididymal secretion was analyzed. |
| Signs of active infection/inflammation were noted and considered in the analysis of data in the study (e.g. inflammatory cells, impaired sperm motility, possibly also antisperm antibodies and reduction of secretory contributions). | ✓ |

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