003: Material and procedures for sperm analysis

Pre-analytical procedures

Before the conscript arrives

- Prepare 11 printed labels with the study number of the conscript and the date
- Place them on the corresponding tubes and prepare the following kits and sheets:
  - **Kit for the medical doctor**
    - 1 sterile spermo-sampler (Veridial) (Remember to weight it before!)
    - 1 urine collection container
    - 2 blood tubes for blood withdrawal
  - **Kit for the lab**
    - 2 Falcon tubes (2 ml) for seminal fluid
    - 1 Falcon tube (1 ml) for sperm pellet
    - 2 Falcon tubes (15 ml) for urine
    - 2 Falcon tubes (2ml) for serum
  - **Instruments and medical sheets**
    - Orchidometer
    - Special gift for the conscript (Swiss knife, iTunes card)
    - Printed questionnaire for urological assessment
    - Excel sheet with list of conscripts

When the conscript arrives

- Ask for his study number
- Provide the doctor with the appropriate kit and questionnaire for the urological assessment

Before the conscript leaves

- Indicate to the conscript that his results will be sent within 3-month only if he explicitly stated in the consent form that he is interested in receiving the results of the semen analysis
- Hand him the chosen gift

Handling the biological samples

- **Urine**: Aliquot in 2 tubes and store urine at -20°C
- **Blood**:
  - Centrifuge (program 3, 3000rpm, 10min, 25°C)
  - Divide the serum in 2 aliquots
  - Store at -20°C

- **Sperm**:
    - Weigh the sampler with the sperm sample
    - Incubate the sperm sample at 37°C for 20-40 min (max)
    - Mix the sample carefully by pipetting in-out through a Pasteur pipette
    - Measure the pH using the pH paper (Merck)
    - Verify the liquefaction and measure the viscosity (drop method)
    - Measure the concentration using a Makler chamber
    - Prepare an appropriate dilution to reach a final concentration of 15-20 millions/ml. Write down the dilution used (1:1, 1:2, …., 1:8).
    - Prepare this dilution carefully using Gilson pipettes.
    - Mount a pre-heated (37°C) Leja chamber by depositing 5 µL of diluted sperm
    - Analyse the sperm sample with the CASA system (see below)
    - Prepare two smears for morphology and let the dry (label with study number, date)
    - Centrifuge the sperm sample (program 8, 3000 rpm, 10 min, 30°C)
        - Remove and store the supernatant into two labelled tubes
        - Store at -20°C

Analytical procedures

Use of the CASA system (SCA System, Microptic SL)

- **Quality control using QC-beads**
    - Mix carefully the two QC-beads tubes using a vortex
    - Mount the lower (Lo) and higher (Hi) in two separate chambers of a Leja counting chamber
    - For each concentration, perform three measurements using the CASA
    - Note the measured concentrations in the Excel sheet on Dropbox
    - Requirements:
        - **Lo** should be between 15 - 21 millions/mL
        - **Hi** should be between 30 - 40 millions/mL
- **Serm concentration and motility determinations**
    - Deposit 5 µL of diluted sperm in a Leja counting chamber
    - Observe a central region of the chamber
    - Capture at least 3 fields, in order to include at least 200 spermatozoa
    - Clean each capture as follows:
        - Eliminate the round cells that may have been recognized as spermatozoa or clumps of spermatozoa
        - Eliminate thedebris that may have mistakenly been recognized as immotile spermatozoa

        - Eliminate drifting immotile spermatozoa if necessary. In case of a persistent drift, redo the measurements once drift has stopped
    - Verify that all captures provide results that are within the accepted range
    - Save the measurements as a .mot and .xls files using the study number as the filename (for example: 123456.mot; 123456.xls)
    - These files are also saved on a pendrive within a folder named by the date of measurements, as well as in the central database (by the data manager)
    - Caveat: the settings for the VAP classification must be set as 8 ≤ 15 ≤ 25
- **Sperm morphology**
    - Air-dried smears are stained using the Papanicolaou staining procedure (WHO) and mounted in a Eukitt medium.
    - On regular basis, the morphometric determinations are performed using the SCA system.
    - 100-200 spermatozoa are analysed. The corresponding captured file (.mrf) and result file (.xls) are saved using the study number as the filename (for example: 123456.mrf; 123456_MORPH.xls)
    - These files are also saved on the central database for later use.

Post-analytical procedures

- **Technical validations**
    - Once the .mot and .xls files are saved, the results are considered as technically validated
    - The Excel Result sheet is filled out and sent to the medical supervisor
    - All frozen specimen are stored in boxes and transported every two months to Lausanne, where the samples are stored at -80 °C.

- **Final validation**
    - The biologist in charge of the final validation reviews the .mot sequences and performs if necessary some final corrections of the captured sequences
    - The .xls file which contains the exported data are imported into the Filemaker database (database “fertiletude”)
    - A form containing all relevant data is printed as a pdf file and deposited in a dropbox folder. This form is printed by the medical supervisor of the study and labelled with the name and address of the conscript. The results are then sent to the volunteers by postal mail.

- **Data management**
    - Import of data from the .xls files was performed once a month by the data manager
    - Data were again validated at this stage before being liberated for printing

- Statistics on the progress of the study are regularly published internally by the data manager
- Entering of the questionnaires (conscript, mother) into the database is performed in batches by students or secretaries who are familiar with the corresponding language (french, german, italian).
- Data cleaning is performed by batches on a regular basis, and on a larger scale on exported data by the epidemiologist in charge of the statistical analysis. Any correction is then back-corrected in the central database.

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