TESTICULAR BIOPSY

-programmed freezing and cryostorage

- Step 1: immerse the biopsy in a 0.5 -0.8 ml cryomedium /examples: Sperm-Freeze, Medicult, Sperm Freeze Solution, Vitrolife/ (pre-conditioned to room temperature)
- Step 2: freeze up to -60° C within 5 min. /example of a freezing device: Nicoolbag 10/ (Air Liquide)
- Step 3: freeze exponentially to -120° C in the following 55 min.
- Step 4: immerse in liquid nitrogen
- Step 5: transfer cryotubes into the cryoshipper pre-filled with liquid nitrogen
- Step 6: store in a liquid nitrogen container /Testicular biopsies bank/

- *Ref.: Ježek D., Knuth U. A., Schulze W. Successful testicular spermatozoa extraction (TESE) in spite of high serum FSH and azoospermia: correlation between testicular morphology, TESE results, semen analysis and serum hormone values in 103 infertile men. Hum Reprod 1998; 13 (5): 1230-1234 (CC, SCI)*
- *Schulze W., Knuth U.A., Ježek D., Benson D.M., Fischer R., Naether O.G.J., Baukloh V., Ivell R. Intratesticular sperm extraction: basis for successful treatment of infertility in men with ejaculatory azoospermia. Adv. Exp. Med. Biol. 1997.; 424:81-88.*
- *Ježek D. (ed.): Atlas on the human testis: Normal morphology and pathology. Springer Verlag London, 285 pgs., London, 2013 (ISBN 987-1-4471-2763-5; DOI 10.1007/978-1-4471-2763-5)*

CRYOPRESERVATION OF HUMAN SPERM

- using cryovials or straws

- Freezing procedure:

1. Ensure that both liquified semen and Sperm Freeze Solution are at room temperature
2. Add Sperm Freeze Solution in ratio 1:1 slowly and dropwise to the semen and then carefully tilt after each drop added
3. Close the lid and turn the tube upside down couple of times
4. Leave in room temperature at least 10 min
5. Make sure that cryovials or straws are marked with patient ID
6. Load the semen mixture into cryovials or straws (do not fill cryovials completely to allow for expansion; ensure some air space in the lower part of the straw)
7. Place cryovials (upright) or straws (horizontally) on a 1-3 cm styrofoam board in a liquid nitrogen bath and leave for 30 min
8. Optional: step 7. can be performed using a slow-freeze machine programmed for sperm freezing:
    1) Start temperature: +20°C
    2) -5°C/min to -8°C
    3) Hold 1 min
    4) -10°C/min to -25°C
    5) -25°C/min to -150°C
9. Place cryovials or straws into the liquid nitrogen and store at -196 °C

CRYOPRESERVATION OF HUMAN SPERM

- using cryovials or straws

- Thawing procedure:

1. Remove cryovials or straws from liquid nitrogen (-196 °C) and place them in a water bath at:
   - 35 ± 2 °C/10 min for cryovials
   - 35 ± 2 °C/30 sec for straws

2. Wipe the cryovials or straws dry with a clean paper towel

3. Open cryovials or straws

4. Transfer semen mixture into clean test tubes and dilute with equal amount of HEPES buffered medium (medium should be added dropwise to the semen mixture and the solution carefully mixed after each addition)

5. Continue with gradient separation

– *Ref.: Stanić P, Sonicki Z, Suchanek E. Effect of pentoxifylline on motility and membrane integrity of cryopreserved human spermatozoa. International Journal of Andrology 2002;25(3):186-90.*

– *Stanić P, Tandara M, Sonicki Z, Šimunić V, Radaković B, Suchanek E. Comparison of protective media and freezing techniques for cryopreservation of human semen. European Journal of Obstetrics & Gynecology and Reproductive Biology 2000;91:65-70.*