Cryopreservation of Client Depositor Testicular Tissue

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1 Introduction

Therapeutic bank patients, referred to as "client depositors," can address the risks of infertility inevitable in certain medical treatments and surgeries by taking advantage of the controlled practice of cryopreservation.

2 Principle

Motile spermatozoa recovered from a testicular biopsy can be preserved in TEST-yolk buffer and stored in liquid nitrogen at extremely low temperature (−196 °C). The sample can be used for procreation utilizing assisted reproductive technique such as intracytoplasmic sperm injection [1, 2].

3 Equipment

A. Aliquot mixer
B. −20 °C freezer
C. LN_2 Dewar 11″ canisters
D. Sterile serological pipettes, 1 mL
E. Sterile American cryovials, 2 mL
F. Colored cryomarkers
G. Cryovial racks
H. Stainless steel canes for cryovials
I. Plastic cryosleeves
J. Cryogloves
K. Protective goggles
L. Latex gloves
M. 37 °C incubator
N. LN_2
O. Sperm washing media
P. Freezing medium
Q. Sterile mineral oil
R. Sterile tubes 60 × 15 mm
S. Inverted microscope (400×)

4 Reagents

A. Freezing medium (TEST-yolk buffer with glycerol) packaged as sterile and for one-time use.
B. Parameters for acceptable reagent performance:
   1. Quality control consists of a freeze and post-thaw done on each new lot of reagent using semen specimen from a normal donor meeting the criteria of 50 % survival calculated by the following formula:

$$\frac{\%\text{post thaw motility}}{\%\text{pre freeze motility}}$$

   2. Results are recorded in the Reagent Quality Control Book. Any unacceptable results are addressed in a quality assurance report with supervisory review.
   3. Storage requirements: Stored frozen at a temperature below −10 °C until time of use.
   4. The lot number and vial expiration date are checked and recorded on the Client Depositor Cryopreservation Worksheet.

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C. Quinn's Sperm Washing Media (Modified HTF containing 5.0 mg/mL HSA):
   1. The reagent is stored at 2–8 °C; the media must be warmed at 37 °C prior to use.
   2. Quality control is performed on all new lots of media using a normal donor specimen meeting the following criteria: >50 % recovery of motility.
   3. The results are recorded in the appropriate Quality Control Book.

D. Mineral oil sterile. Store at room temperature.

5 Specimen

A. Client depositor testicular tissue will be obtained by the surgeon performing the testicular biopsy. The specimen must be collected and placed in a clean sterile 1 mL clear microcentrifuge tube containing 0.5–1 mL of sperm washing media. The specimen(s) must be labeled with the patient's name and medical record number (MRN#). Often times there will be two or more specimens obtained from different sites. These must be treated as separate specimens. The lab technologist must report to surgery where the procedure is taking place to obtain and identify the specimen(s).
B. All tissues obtained are considered acceptable.
C. Client depositor confers with the Lab Director of Andrology and/or his referring physician(s) as to the acceptability of the banked specimen and its future use.

6 Calibration

No calibration standards exist for this procedure.

7 Quality Control/Quality Assurance

Quality assurance is maintained by the quality control of the freeze media and the testing equipment, which is documented in the Reagent Quality Control Book. Cryosurvival on a processed normal donor specimen is evaluated on the new lot numbers of TYB media.

8 Procedure

Note: Sterile techniques should be used throughout specimen processing. Gloves are mandatory for all procedures dealing with body fluids. Latex, however, can be toxic to sperm. Therefore, care should be taken to prevent contamination of the specimen with latex or talc. Vinyl gloves are an available alternative.

A. The client depositor is registered, if not already a patient. MRN is necessary for identification of client vial.
B. When the laboratory receives the call from surgery that the testicular tissue is ready, take out one bottle of frozen TEST-yolk media and place it in the 37 °C incubator to thaw.
C. A technologist will go to the operating room to bring the specimen(s). The labeled specimen(s) is identified by the surgeon, nurse, and technologist before it leaves the operating room.
D. Place the labeled tissue specimen vial(s) into a biohazard bag and keep it warm during the transit to the lab.
E. Label appropriate number of cryovials. The 1st testicular tissue cryovial is labeled with the red cryomarker.
   Note: Examine each cryovial while labeling for any evidence of defects (crack/damage); discard if defect is found.
   1. Label a set of cryovials with the orange cap to the left:
      a. Client depositor name
      b. MRN#
      c. Freeze number, i.e., F15-009A
      d. Date
      e. Word "Testis Tissue"
      f. Tech Initials
   2. If there is a second tissue, the cryovial is labeled using a blue cryomarker (a different color) with the same as in the 1st specimen except for the freeze number which is labeled as F15-009B.
F. Label an extra cryovial for each tissue for a post-thaw analysis.
G. Label a Kontes pellet tube for each specimen.
H. Pipet 100 µL of sterile sperm washing medium into each Kontes pellet tube.
I. Carefully transfer each testis tissue into the corresponding labeled Kontes pellet tube.
J. Add 100–150 µL of fresh sperm washing medium to each Kontes pellet tube. Each testis tissue is now suspended in 200–250 µL of sperm washing medium.
K. Remove a sterile pestle and manually grind each tissue.
L. Using sterile technique, pipet 5 µL of each tissue homogenate onto a petri plate.
M. Overlay the droplets with mineral oil (approximately 5–10 mL).
N. Observe each droplet for motile sperm using the inverted microscope at 400×.
O. Record the number of motile sperm per field. Record the average progression. Page the surgeon with the results.
P. Transfer each tissue solution into its corresponding prelabeled cryovial.

Q. Add equal amounts of TEST-yolk buffer to each cryovial, e.g., if tissue is suspended in 250 μL HTF, then add 250 μL of TEST-yolk buffer.
R. Mix the specimens and transfer 250 μL to the second labeled tube of the same bank. Transfer 50 μL to one tube marked post-thaw for that particular bank.
Note: Freeze two cryovials for each bank plus a post-thaw tube to check for cryosurvival.
S. Label a cryocane in red for the 1st specimen with the patient's last name and freeze number. If a second specimen was frozen, label another cryocane in blue with the patient's last name and freeze number.
T. Place the appropriately labeled vials into the correspondingly labeled cryocane. Place the cryovial at the end of the cryocane (away from the labeled end). Insert the cryosleeve and place it in the −20 °C freezer for 30 min; set a timer.
Note: Exposure to freezing conditions should occur within 1.5 h of specimen collection.
U. Remove the cryocanes and put into a liquid nitrogen vapor tank. Insert the cryocanes into one of the canister with the vials facing toward the top of the tank. Set a timer for 30 min.
V. Record the location on the Semen Cryopreservation Worksheet.
W. Flip the cryocanes, submerging the specimens into liquid nitrogen until transfer to long-term storage.
X. Cryosurvival should be determined no sooner than 24 h after freezing and recorded on the Cryopreservation Worksheet.
Note: The Cryopreservation Worksheet will indicate the patient name, MRN number, freeze number, color, date of collection, volume of specimen (0.25 mL), volume of freeze media (0.25 mL), lot number, and expiration date of freeze media, technologist name, time in vapors, time in LN_2, and location of the specimen.
Y. Record patient's demographic information and the pre-freeze and post-thaw results into the patient folder.

9 Patient Interview

Note: The patient meets the Director the day after the surgery to discuss the future usefulness of his frozen specimen (s).

A. A final step for positive identification at a later time is made by taking the client depositor's picture. Once the picture is taken, the client's name, MRN number, and freeze number are written at the bottom and are then stored in the client depositor's cryobank folder.

10 Post-thaw Analysis

Note: Use cryoglove and goggles.

A. Remove the tubes labeled post-thaw for each testis tissue frozen from the LN_2 tank and place in −37 °C incubator for 20 min.
B. Vortex the specimens.
C. Transfer 5 μL of each post-thaw to a sterile petri dish and overlay with 5–10 μL of mineral oil.
D. Using the inverted microscope at 400×, observe for motile sperm.
E. Record the results on the Cryopreservation Worksheet.
F. The results are expressed as number of sperm/HPF (high power field), and the motility is calculated as a percent motile with a note of the degree of progression.

References

1. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 5th ed. Geneva, World Health Organization, Switzerland; 2010.
2. Allen JA, Cotman AS. A new method for freezing testicular biopsy sperm: three pregnancies with extracted from the cryopreserved section of seminiferous tubule. Fertil Steril. 1997;68:741–4.