Sperm Preparation for Intrauterine Insemination (IUI) by Swim-Up Method

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1 Introduction

Preparation of the sperm for IUI combines sperm washing with swim-up to remove seminal plasma and concentrate the most motile spermatozoa in a very small volume of sperm wash media (HTF) [1–2].

2 Specimen Collection

The patient is instructed on how to collect the specimen (see Semen Collection and Labeling Procedure). The patient collects the specimen into a sterile container and brings it to the laboratory. The patient or partner is told to return in ~1 h to pick up the washed specimen.

3 Equipment and Materials

A. Sperm washing media (modified HTF with 5 % human serum albumin and gentamicin
B. Disposable sterile 15 mL polystyrene conical centrifuge tube(s) with cap(s)
C. Centrifuge
D. 37 °C incubator
E. Sterile graduated serological pipettes (2 mL)
F. Computer-assisted semen analyzer (CASA)
G. Pipette tips (5 µL)
H. Disposable 20 µm sperm counting chamber
I. Makler counting chamber
J. Dilution cups 2-mL
K. Brown bags
L. Disposable sterile transfer pipettes
M. Viscosity treatment system (when applicable)—5 mg chymotrypsin

4 Quality Control

A. All IUI samples are checked periodically for sperm recovery and percent motility.
   Criteria: None defined.
B. Daily Precision:
   A patient specimen should be selected at random and run through the CASA. A manual count and motility reading should also be performed simultaneously.
   Criteria: All manual results should be within 20 % of the CASA value.
   Response: If results are not within the defined percentage difference, the sample must be repeated.
C. Technologists review patient results to check for technical errors prior to releasing the specimen for insemination.

5 Procedure

5.1 Prepare Reagents

Bring sperm wash media (HTF) to 37 °C for 20 min in the incubator.

5.2 Prepare Paperwork and Accept Specimen from Patient

1. After specimen collection, make sure the specimen container is labeled with two identifiers. Acceptable

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identifiers are the patient name and date of birth or patient name and medical record number.

Note: If all of the patient information on the specimen cup is not present, the container should be labeled in front of the patient.

2. Fill out all pertinent information on the "Sperm Wash Worksheet," "Artificial Insemination by Husband Form," "Specimen Drop-off/Pick-up Form," and on two IUI labels before accepting the specimen.
3. Have the person delivering the specimen sign the "Specimen Drop-off/Pick-up Form." They must present an acceptable ID card (e.g., driver's license, employee badge). The technologist accepting the specimen must record the type of identification presented and the appropriate ID number (e.g.. driver's license number, employee number) on the worksheet.
4. Label a 15 mL centrifuge tube with the patient's and partner's name, medical record numbers, and date. The tube should also be labeled with color-coded tape* as an extra identifier.
5. Label a 2 mL conical cup for the post-wash analysis. Remove a warm tube of sperm wash media (HTF with 5 % HSA) from the 37 °C incubator and label it with the patient's name and colored labeling tape* (same color as used above).

   ***Note**: Color-coded labeling tape should be used for all tubes, media, and paperwork and should be specific (of the same color) for each patient.

5.3 Analyze/Wash Specimen

Note: Sterile technique should be used throughout specimen processing.

1. Allow the specimen to liquefy completely for ~20 min in the 37 °C incubator before processing.
2. Measure volume using a sterile serological (2 or 10 mL) pipette.
3. Transfer specimen from a plastic cup into a sterile 15 mL conical centrifuge tube. If specimen is >4 mL, split into two tubes.
4. Gently mix the specimen with HTF in a ratio of 1:4 by using a sterile serological pipette.
5. Centrifuge the tubes at 1600 RPM for 10 min.

   Note: Occasionally samples do not liquefy properly and remain too viscous to pass through the gradient. In this case, add 5 mg of chymotrypsin to the tube (approximately 10 min before layering) to increase motile sperm yields.
6. While the specimen is in the centrifuge, perform pre-wash CASA analysis. Perform CASA analysis according to the "Routine Semen Analysis" protocol and record the results on the worksheet.

   Note: While examining the specimen, pay particular attention to any extraneous round cells, debris, and bacteria that are present. If round cells are greater than or equal to 1.00×10^6/mL, perform an Endtz test immediately. A positive Endtz test should be reported to the appropriate medical personnel as soon as possible. The Endtz test result should also be written on the tube's outer label at the end of the wash procedure before handing the specimen to the patient.
7. Carefully aspirate the supernatant without disturbing the pellet and resuspend the pellet in 3 mL of fresh HTF. Transfer the resuspended sample into two 15-mL sterile round bottom tubes using a serological pipette (1.5 mL in each).
8. Centrifuge the tubes at 500 RPM in the centrifuge for 5 min.
9. Position the tubes in a rack at a 45° angle and incubate at 37 °C for 1 h for swim-up (Fig. 15.1).
10. After the incubation period, aspirate the entire supernatant from the round bottom tube using a Pasteur pipette with the tip placed just above the pellet surface.
11. Pool supernatant from the two round bottom tubes into a single 15-mL conical centrifuge tube. Centrifuge the tube at 1600 RPM for 7 min.
12. Aspirate the supernatant from the top of the meniscus using a Pasteur pipette being careful not to disturb the pellet.
13. Resuspend the pellet in a volume of 0.5-mL HTF media using a 2-mL sterile pipette. Note the final volume on the worksheet.
14. Remove a small, well-mixed aliquot (~50 μL) and place it into a labeled conical beaker for the post-wash analysis.
15. Seal the tube using tamper-evident tape, label it with the premade label, and place it in the incubator until the patient arrives. Show the color-coded tube to the patient to verify the patient's and partner's names, medical record numbers, and date. Place the sample in a brown bag and hand it to the patient. Be sure the patient signs the specimen pick-up form and presents appropriate identification. The patient should carry the specimen directly to the gynecologist department for the insemination. The gynecologist determines whether the sperm count and

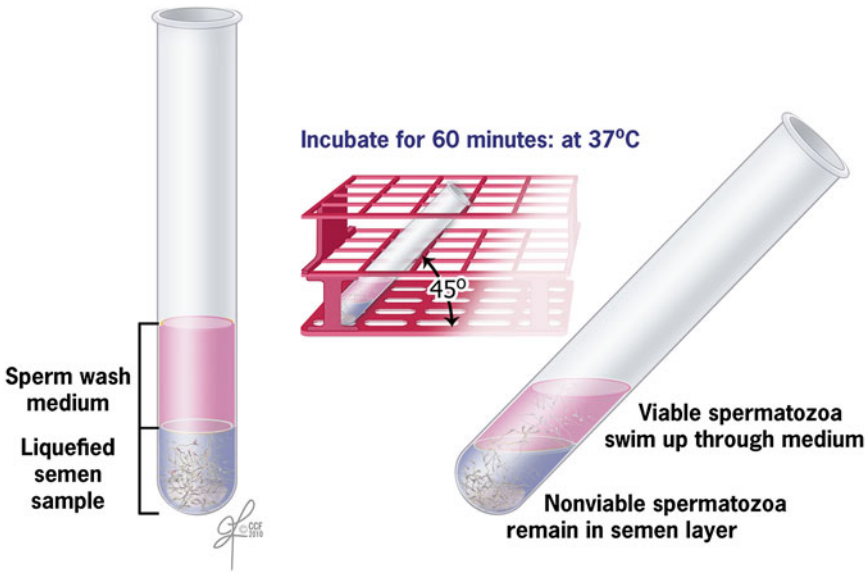

Fig. 15.1 Swim-up procedure [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

motility are sufficient for insemination. If no motile sperm are found on the post-wash specimen, call the gynecology department prior to releasing the specimen.

Note: The technologist must always look at the post-wash specimen on a sperm counting chamber before releasing it to the patient and notify the inseminator immediately if bacteria are present. If there is a positive Endtz test, it should be reported to the gynecologist and written on the outside label of the tube before handing it to the patient. The gynecologist should be paged in the event of a positive Endtz test result. If the prewash Endtz is positive and the post-wash round cell count is less than 1.0 M/mL, note the round cell count on the outside label of the tube.

16. Perform post-wash CASA analysis according to the "Routine Semen Analysis" protocol and record the results on the worksheet.
17. Record appropriate information (below) on the "Artificial Insemination by Husband" form:
    (a) MRN
    (b) Date of insemination
    (c) Total motile post-wash sperm
    (d) Wash type (e.g., swim-up)
    (e) Insemination performed by (Gyn nurse)
    (f) Comments (e.g., positive Endtz test, agglutination)
    (g) Tech initials
18. After checking for technical and clerical errors, enter the results and print the final report. Check the final report to ensure the accuracy of the final results.

Note: Immediately correct any inaccurate results.

6 Procedural Notes

A. In the case of a highly viscous specimen, add 5 mg of viscosity treatment system (VTS) to the tube and let the sample incubate at 37 °C for 10 min or until the specimen is completely liquefied. If sample viscosity is not reduced, use more VTS and divide into two tubes before centrifugation.

Note: Be sure to record the lot number and expiration date of the VTS on the patient worksheet.

B. It is important that all plasticware and glassware used in the sperm washing procedure are sterile.
C. Each patient specimen should be kept in a separate specimen rack during the time of processing.
D. A single technologist should process the patient specimen(s) from beginning to end. In rare cases when a second technologist is called to help during the processing, a reverification of the patient's paperwork should be made by the assisting technologist.

References

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2. Agarwal A, Allamaneni S. Artificial insemination. Section 6: infertility and recurrent pregnancy loss. Clinical reproductive medicine and surgery: Tommaso Falcone and William Hurd. New York: Elsevier.

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IUI Sperm Preparation by Swim Up

Procedure

A highly motile sperm preparation for intrauterine insemination by swim up involves the following steps:

A. Prepare Reagents:

1. Bring sperm wash media (HTF) to 37°C for 20 minutes in the incubator. Have the person delivering the specimen sign the "Specimen Drop-off/Pick-up Form." The technologist accepting the specimen must record the type of identification presented and the appropriate ID number (ex. driver's license #, employee #) on the worksheet.

2. Label a 15 mL centrifuge tube with the patient's and partner's name, medical record numbers and date. The tube should also be labeled with color-coded tape as an extra identifier.

3. Label a 2 mL conical cup for the post-wash analysis. Remove a warm tube of sperm wash media (HTF w/ 5% HSA) from the 37°C incubator and label it with the patient's name and colored labeling tape (same color as used above).

B. Analyze/Wash Specimen:

Note: Sterile technique should be used throughout specimen processing.

1. Allow the specimen to liquefy completely for ~20 minutes in the 37°C incubator before processing.

2. Measure volume semen using a sterile serological (2 mL) pipette.

3. Transfer s semen pecimen from a plastic cup into a sterile 15 mL conical centrifuge tube. If specimen is >4 mL, split into two tubes.

4. Gently mix the specimen with HTF in a ratio of 1:4 by using a sterile serological pipette.

5. Centrifuge the tubes at 1600 rpm for 10 minutes.

Note: Occasionally samples do not liquefy properly and remain too viscous to pass through the gradient. In this case, add 5 mg of chymotrypsin to the tube (approximately 10 minutes before layering) to increase motile sperm yields.

6. While the specimen is in the centrifuge, perform pre-wash CASA analysis. Perform computer-assisted semen analysis according to the "Routine Semen Analysis" protocol and record the results on the worksheet.

7. Carefully aspirate the supernatant without disturbing the pellet and resuspend the pellet in 3 mL of fresh HTF. Transfer the resuspended sample into two 15mL sterile round bottom tubes using a serological pipette (1.5 mL in each).

8. Centrifuge the tubes at 500 rpm for 5 minutes.

9. Position the tubes in a rack at a 45° angle and incubate at 37°C for 1 hour for swim-up **(Figure 1).**

Figure 1. Swim-up procedure

10. After the incubation period, aspirate the entire supernatant from the round bottom tube using a Pasteur pipette with the tip placed just above the pellet surface.

11. Pool supernatant from the 2 round bottom tubes into a single 15-mL conical centrifuge tube. Centrifuge the tube at 1600 rpm for 7 minutes.

12. Aspirate the supernatant from the top of the meniscus using a Pasteur pipette being careful not to disturb the pellet.

13. Resuspend the pellet in a volume of 0.5 mL HTF media using a 2-mL sterile pipette. Note the final volume on the worksheet.

14. Remove a small, well-mixed aliquot (~50 μL) and place it into a labeled conical beaker for the post swim up analysis.

15. Seal the tube using tamper-evident tape, label it with the pre-made label, and place it in the incubator until the patient arrives. Show the color-coded tube to the patient to verify the patient's and partner's names, ID numbers, and date.