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Tips for Handling Abnormal Sperm

Preparing sperm for IUI, IVF, or ICSI can encounter a variety of complications. FUJIFILM Irvine Scientific has compiled a series of tips from Dr. Jayant G. Mehta from the Queen's Hospital in Romford Essex to help deal with abnormal sperm samples. For general sperm separation and processing, please refer to our product inserts or andrology protocols.

FUJIFILM Irvine Scientific has not validated these procedures and each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

The following tips feature the use of ISolate (Catalog # 99264) for gradient sperm separation and Multipurpose Handling Medium (MHM, Catalog # 90163) for sperm washing and handling. For different gradient percentages of ISolate, please dilute with MHM following the directions found in the product insert. Dr. Mehta uses a 40% / 80% ISolate gradient for normal sperm processing.

SPECIMEN ISSUE:

Low sperm concentration and/or reduced progressive motility of <30%

- Use 1 mL layers of 30% and 60% ISolate gradient.
- Resuspend the resulting pellet in 0.5 mL of MHM.
- Follow with swim up by layering 0.5 mL of MHM.
- Incubate for 30–45 minutes in a heating block (not CO_2 incubator).
- Transfer the top 40% of media to a new tube and count motile sperm.
- Remove a further 20% if few or no sperm are seen.

SPECIMEN ISSUE:

High cellular debris

- To prevent debris from clogging the gradient interfaces, load ISolate with small volumes of semen sample at <0.5 mL.
- Instead of layering the semen sample, gently mix the semen sample directly into the top 10% of the ISolate upper layer.
- Due to the smaller volumes, this will require multiple gradient tubes.

SPECIMEN ISSUE:

Retrograde ejaculated urine specimens

- Centrifuge urine at 1600 RPM for 10 minutes.
- Remove supernatant and dilute the pellet by adding 3 mL of MHM.
- Layer the resuspended pellet on 40% ISolate gradient.

SPECIMEN ISSUE:

High Viscosity

METHOD 1:

- Mix the semen sample with equal volume of MHM prior to gradient layering.
- If sample fails to disperse in 2–3 minutes, place it in 37°C for 10–15 minutes.
- Samples displaying high viscosity may require gentle repeated pipetting or passage through a blunt 18 gauge needle as per the WHO manual. Use of blunt needle avoids damaging sperm during pipetting. Alternatively, you can use a sterile glass pipette to do the same. Pipetting 8–10 times normally breaks down the viscous sample.

METHOD 2:

- Use 0.2% solution of α-amylase for 5 minutes.
- Wash the enzyme on liquefaction: after 5 minutes, add 5 mL of MHM and centrifuge at 1600 RPM for 5 minutes.
- Remove the supernatant and resuspend the pellet in 1 mL of MHM.
- Layer the resuspended pellet on 40% ISolate.

SPECIMEN ISSUE:

Cryopreserved semen sample

- Since osmolality of semen cryoprotectant is high, the spermatozoa will swell greatly upon entering the 40% ISolate layer and becoming too buoyant.
- Slowly dilute the thawed cryoprotectant semen with 5X volume of MHM, adding drop-wise with constant gentle mixing over a period of 15 minutes.
- Layer on 40% ISolate gradient.

*Dr. Jayant G. Mehta is one of the editors of Male Infertility – Sperm Diagnosis, Management and Delivery.

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