

Sperm Freezing, Thawing and Preparation

Before freezing:

In case the sperm count is very low, it is advisable to concentrate the sperms before freezing. This may increase sperm quality after thawing and will reduce the number of freezing vials to be frozen.

After thawing:

Use sperm preparation/concentration techniques after thawing the semen to eliminate debris and dead sperm cells. Dilute the concentration sperm pellet with a suitable semen washing solution (e.g. Hi-Tech HTF/Ham-f10 solutions)

Post wash freezing procedure

Allow the semen to liquefy at 37° C temperatures in Semen Warmer for 30 minutes after collection.

Separates spermatozoa can be prepared with density gradient solutions (single layer/double layer density gradient technique) and washed concentrated sperm pellet with washing solutions (Modified HTF/Modified HAM-F10). Resuspend the final pellet with washing solutions (Modified HTF/Modified HAM-F10) and dilute with equal volume (1:1) of SpermPlus+freeze and washing solutions.

Caution: make sure freezing solution is at room temperature to avoiding cold shock

(To avoiding osmotic shock) add SpermPlus+freeze slowly drop by drop and mixture carefully tilted after each drop added. Close the cap tightly and turn the tube upside down 10 times, being careful not to create bubbles.

Leave the mixture for 10 minutes at room temperature for equilibration.

Load the mixture sample into the freezing vials.

Close the vials cap, coding identity and Place in refrigerator at 4° C for 10 minutes.

Hang and Freeze the vials just above the Liquid Nitrogen label (without contacting with LNO₂) for 20 minutes.

Completely dip & store in liquid nitrogen.

Thawing procedure

Remove as required vials from liquid nitrogen container.

Place the vials in semen warmer at 37° C for 20 minutes.

Gently mix the semen and take a drop on the sperm counting chamber and again put the slide back to the semen warmer at 37° C for 10 minutes. Close the vials cap and place in semen warmer.

Now take the sperm counting chamber, examine under 20 x objective for post thaw parameters and calculate the yield.

Pre wash freezing procedure

Allow the semen to liquefy at room temperature for 30 minutes.

Add 0.7 ml SpermPlus+freeze with 1 ml liquefied mix semen

(To avoiding osmotic shock) add SpermPlus+freeze slowly drop by drop and mixture carefully tilted after each drop added. Close the cap tightly and turn the tube upside down 10 times, being careful not to create bubbles.

Caution: make sure freezing solution is at room temperature to avoiding cold shock.

Leave the mixture for 10 minutes at room temperature for equilibration.

Load the mixture sample into the freezing vials.

Close the vials cap, coding identity and Place in refrigerator at 4° C for 10 minutes.

Hang and Freeze the vials just above the Liquid Nitrogen label (without contacting with LNO₂) for 20 minutes.

Completely dip & store in liquid nitrogen.

Thawing procedure

Remove as required vials from liquid nitrogen container.

Place the vials and other required material in semen warmer at 37° C for 15 minutes.

Layer the semen over a suitable density gradient processing solution in a conical centrifuge tube (at least 2 ml per 1 ml semen) without mixing.

Centrifuge for 15 minutes at 1500 RPM. (After following centrifugation pellet will be visible)

Remove seminal fluid, dead cells and debris (without disturbing the concentrated sperm pellet).

Dilute the concentrated sperm pellet in a suitable sperm washing solution 0.5 ml (e.g. Hi-Tech modified HTF/Ham-f10) Mix slightly and transfer to new centrifuge tube. Add 3-4 ml same washing solution mix well.

Centrifuge at 1000 RPM for 5 minutes. Remove supernatant (without disturbing the pellet) and re-suspend the sperm pellet in 0.5 ml suitable washing solutions (e.g. Hi-Tech modified HTF/Ham-f10). Examine a drop of suspended prepared semen sample for post thaw processing parameters and calculate the yield.