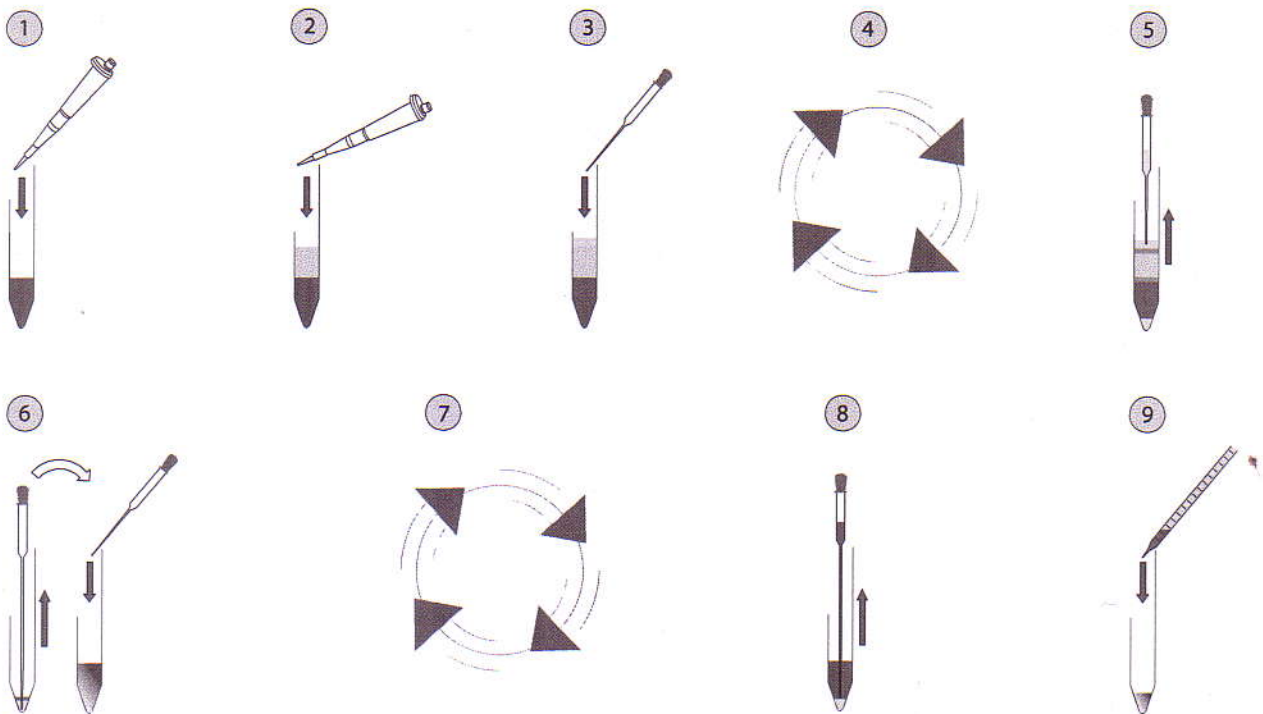


Procedure for washing sperm with density gradient

Bring all solutions to room temperature.

1. Use a pipette with a sterile tip to add 2mL of PureSperm®80 to a conical centrifuge tube
2. Use a new sterile pipette tip to carefully layer 2mL PureSperm®40 on top of the PureSperm®80
3. Use a sterile Pasteur pipette to carefully layer liquefied semen (up to 1.5mL) onto the PureSperm®
4. Centrifuge at 300 x g for 20 minutes. Do not use the brake
5. Use a new sterile Pasteur pipette to aspirate, in a circular movement from the surface, everything except the pellet and 4-6mm of PureSperm®80. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5mL



6. Use a new sterile Pasteur pipette to aspirate the pellet (or the lowest 0.5mL liquid). Transfer sperm pellet to new tube and resuspend pellet in 5mL PureSperm®Wash. Combine sperm pellets if double procedure has been used
7. Centrifuge at 500 x g for 10 minutes. Do not use the brake
8. Aspirate PureSperm®Wash supernatant leaving as little liquid as possible above pellet. If no pellet is seen, leave the bottom 0.25mL fluid
9. Resuspend the sperm pellet in a suitable volume of culture medium to obtain the required sperm concentration. The sperm sample is now ready for analysis or use

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