Extended search and cryopreservation of individual spermatozoa using SpermVD

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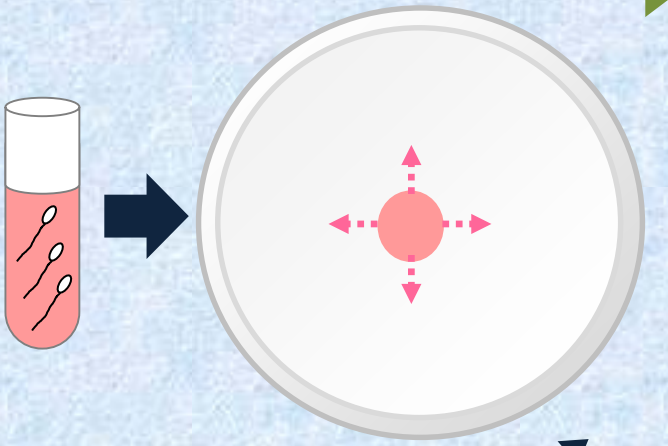
v5.2

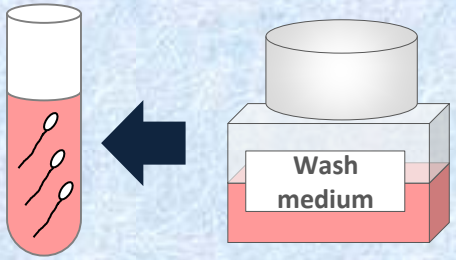
Phase 1 – Evaluation of sample

1 **Transfer a 5µl droplet of washed and concentrated sample onto a petri dish** (flatten to achieve better spreading).



2 **Observe under x200 magnification** (may return the droplet to the sample afterwards)

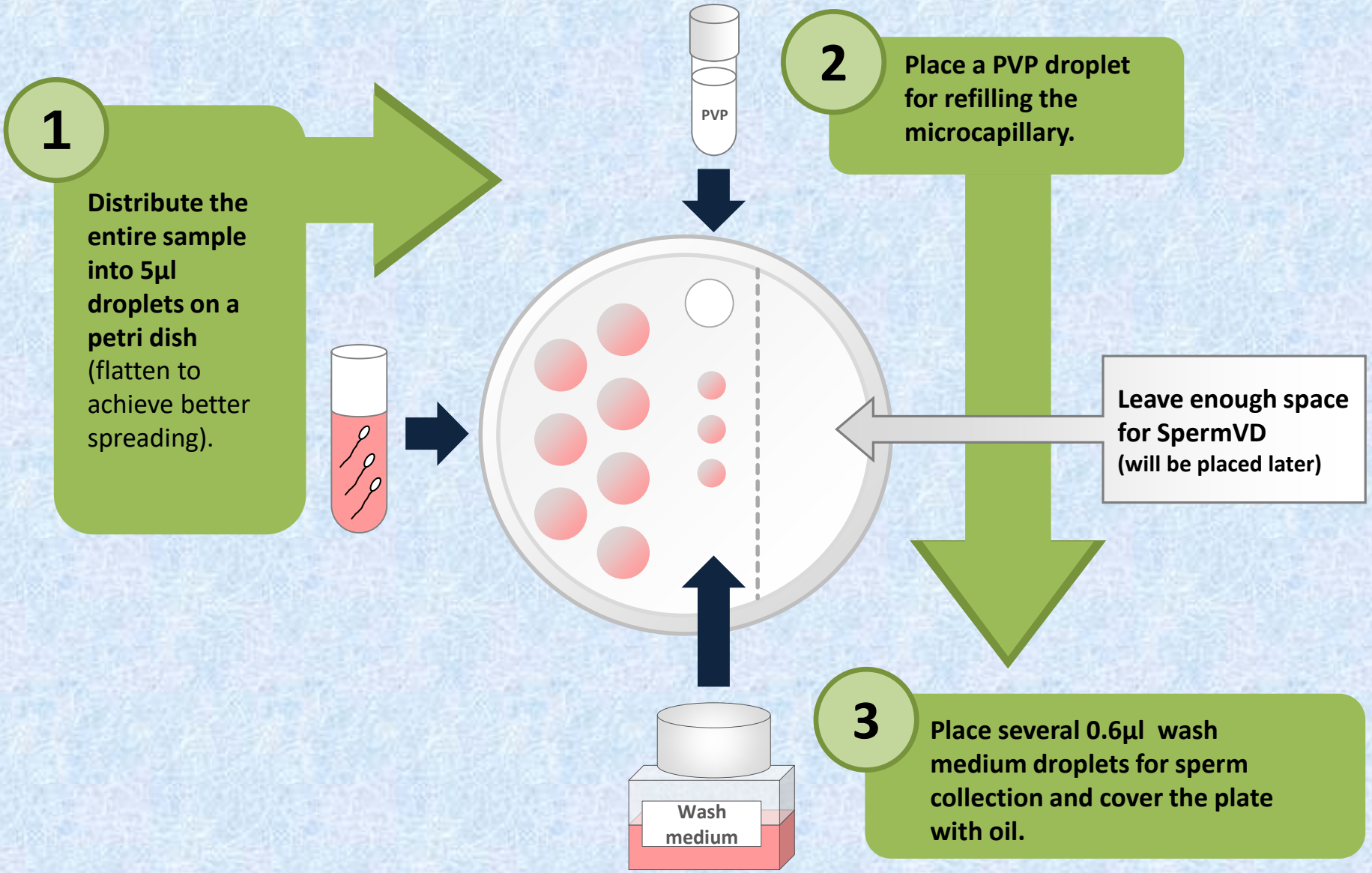
3 **If debris are too dense, dilute with wash medium to make the search convenient, then repeat Step 1.**



TIP

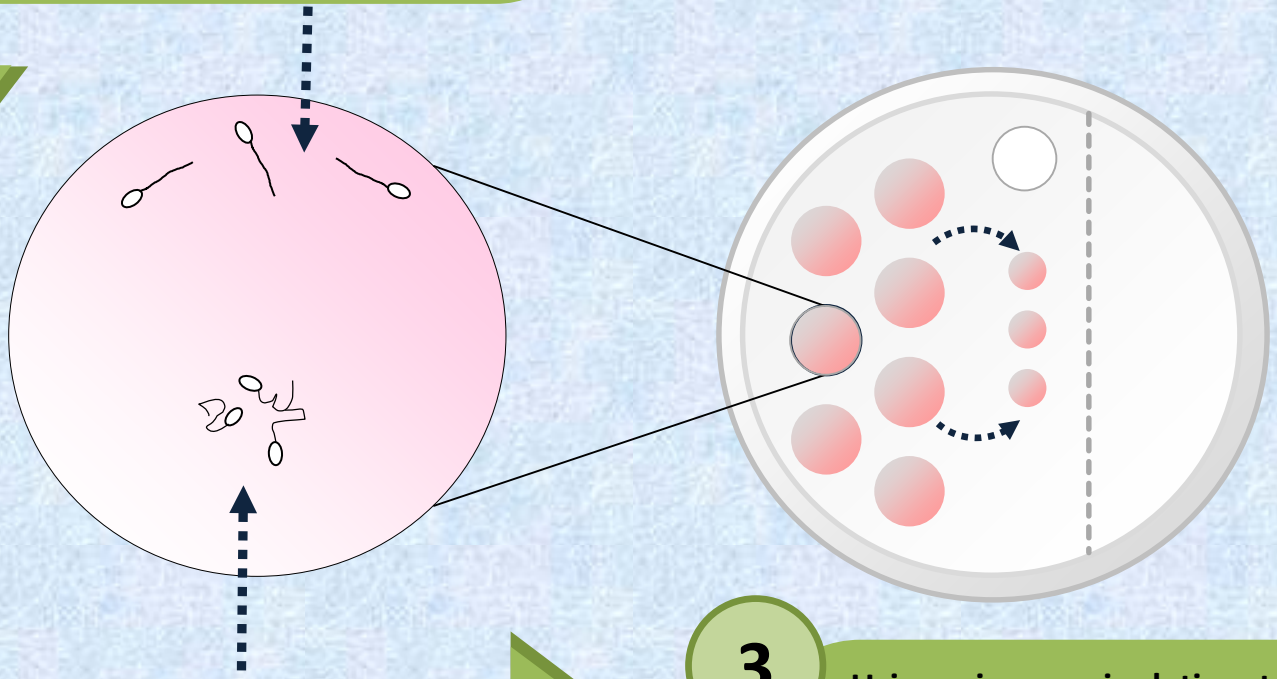
Flatten the droplet by gently tapping the plate on the work surface.

Phase 2 – Preparation of search plate



Phase 3 – Search

1 **Search for progressively motile spermatozoa along the borders of the droplet using phase contrast under x200 magnification.**

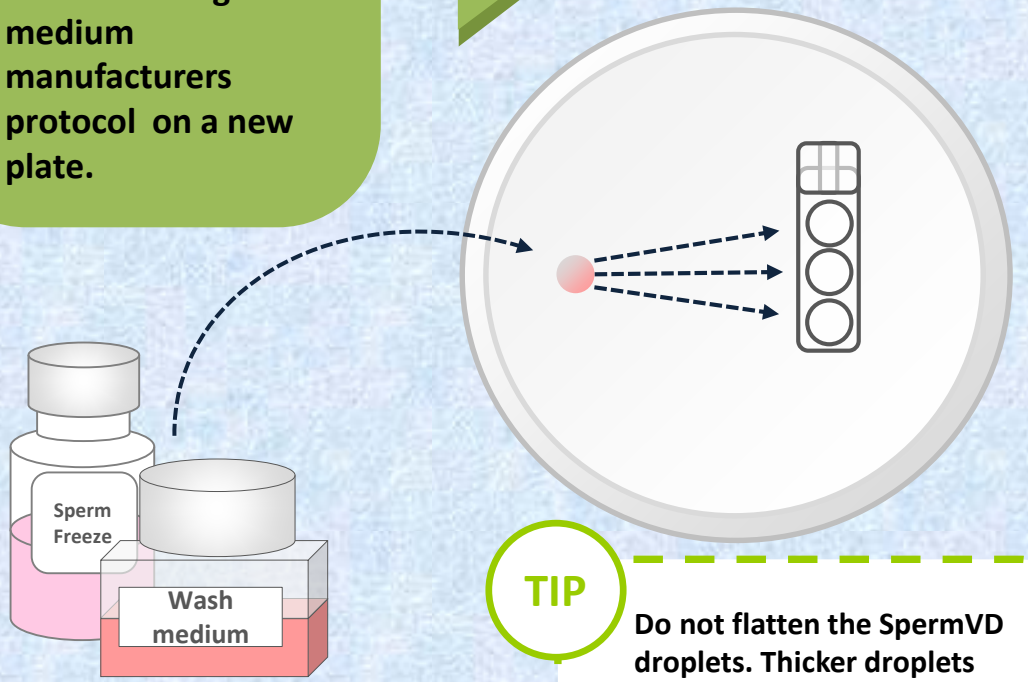


2 **If no progressively motile spermatozoa are found, search for locally motile / immotile spermatozoa inside the droplets.**

3 **Using micromanipulation, transfer the spermatozoa into the collection droplets.**
(e.g. 1st for progressive,
2nd for locally motile,
3rd for immotile sperm).

Phase 4 – SpermVD Preparation

1 Mix wash medium and sperm freeze medium according to the freezing medium manufacturers protocol on a new plate.



2 Using sterile forceps, remove the SpermVD from its package and place it on the plate. Do NOT cover with oil.

3 Place 0.8 – 1µl droplets of the mixture onto the wells.

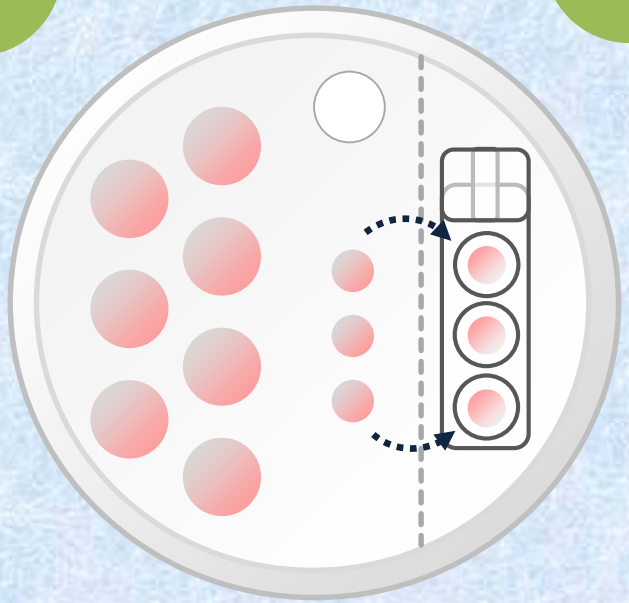
TIP Do not flatten the SpermVD droplets. Thicker droplets are more stable and allow better motility along the borders.

! **Attention!**
Due to quick wash medium evaporation, create a new mixture droplet for each SpermVD!

Phase 5 – Spermatozoa transfer

1 Immediately after preparation, gently submerge the SpermVD into the oil on the plate containing spermatozoa. Make sure the wells are covered with oil.

2 Using micromanipulation, transfer the spermatozoa from the collection droplets to the wells. Use several devices if needed.



TIP For better control of progressively motile sperm, it is possible to transfer them into PVP droplet first and then into SpermVD droplets. Note that post-thaw motility might be affected.

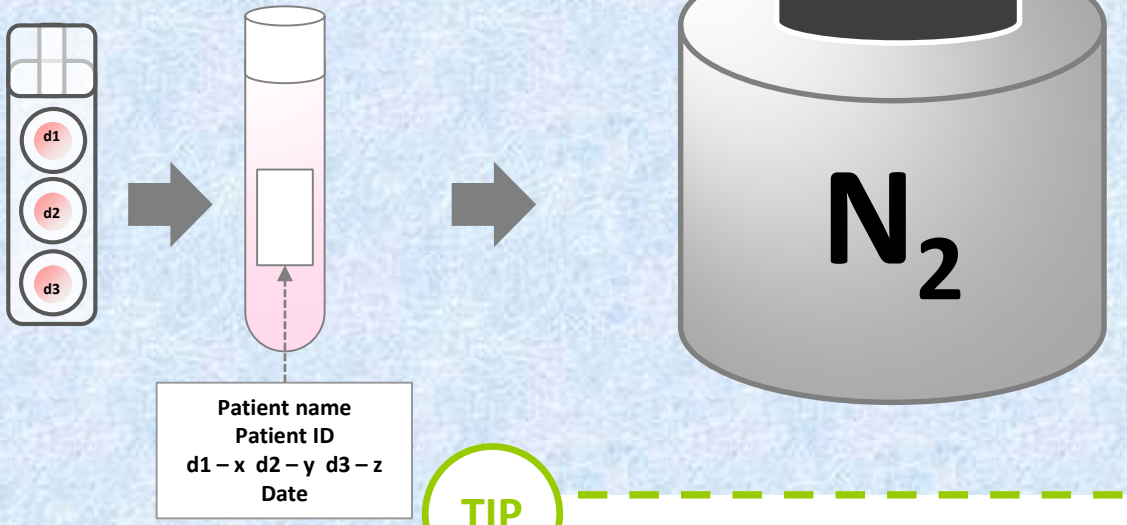
TIP It is recommended to freeze ~15-20 cells per device, to minimize the excess not used for ICSI.

! **Attention!**
The SpermVD containing spermatozoa needs to be frozen within a time limit of ~10 minutes, beginning with the placement of the first cell. It is quite similar to bulk freezing, where a 10-minute equilibration at room temperature is needed prior to freezing.

Phase 6 – Cryopreservation

1 Gently pick the SpermVD from the plate, and transfer it into a labeled 1.8mL or 3.6mL cryovial.

2 Place the cryovial directly into LN2 and transfer it to storage in LN2 tank.



! **Caution!**
This is the most delicate phase. Take care while placing the cryovial into the holder or lowering the holder into the LN2 tank. The droplets are stable but may be dislodged by an abrupt shake.

TIP Leave any excess oil on the SpermVD. It serves as an additional protection layer for the droplets.

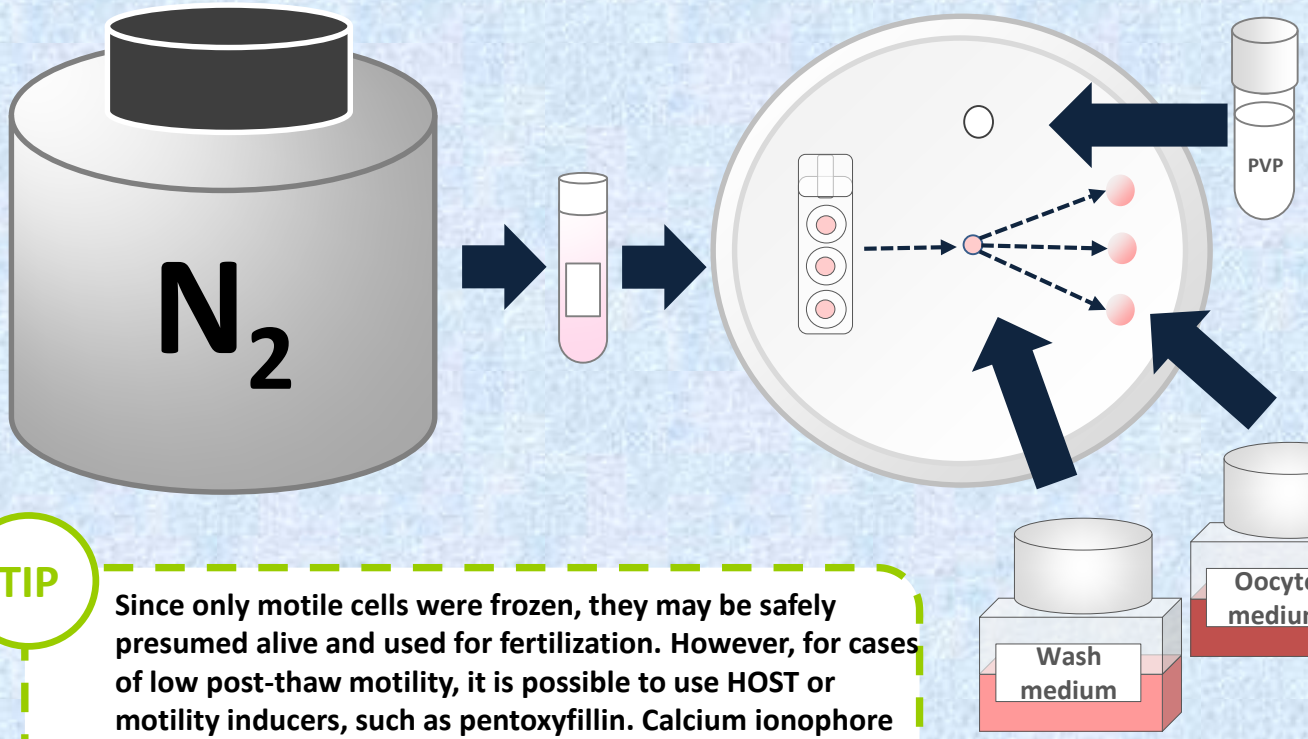
TIP Do not tighten the cryovial cap completely. LN2 vapors that will enter the cryovial will prevent thawing if exposed to ambient temperature.

Phase 7 – Thawing and retrieval

1 Remove the cryovial from LN2, unscrew the cap and let it thaw at room temperature until the oil covering the droplets liquifies completely (~3 minutes).

2 Remove the SpermVD from the cryovial and place it on an oil-covered plate containing droplets of PVP, wash medium and oocyte medium as described.

3 Search and locate the spermatozoa inside the droplets and transfer them to a washing medium droplet. Once all cells have been transferred, it is possible to proceed with ICSI .



! **Attention!**
The transfer to washing medium droplets should be performed immediately after placing the SpermVD under oil.

TIP Since only motile cells were frozen, they may be safely presumed alive and used for fertilization. However, for cases of low post-thaw motility, it is possible to use HOST or motility inducers, such as pentoxyfillin. Calcium ionophore may be used to facilitate fertilization for immotile cells.

Thank you!
Enjoy using SpermVD

Link to video demonstration: www.youtube.com/watch?v=7vno9ReEbh0&feature=youtu.be

Link to article on PubMed: www.ncbi.nlm.nih.gov/pubmed/30285105

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