Extended search and cryopreservation of individual spermatozoa using SpermVD

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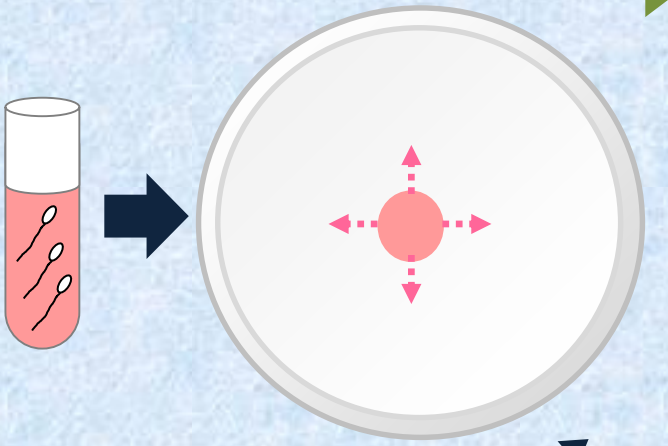
v6.1

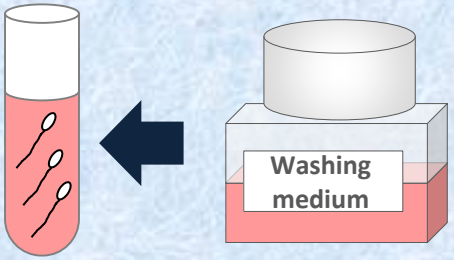
Phase 1 – Evaluation of sample

1 **Place a 5µl droplet of a 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)

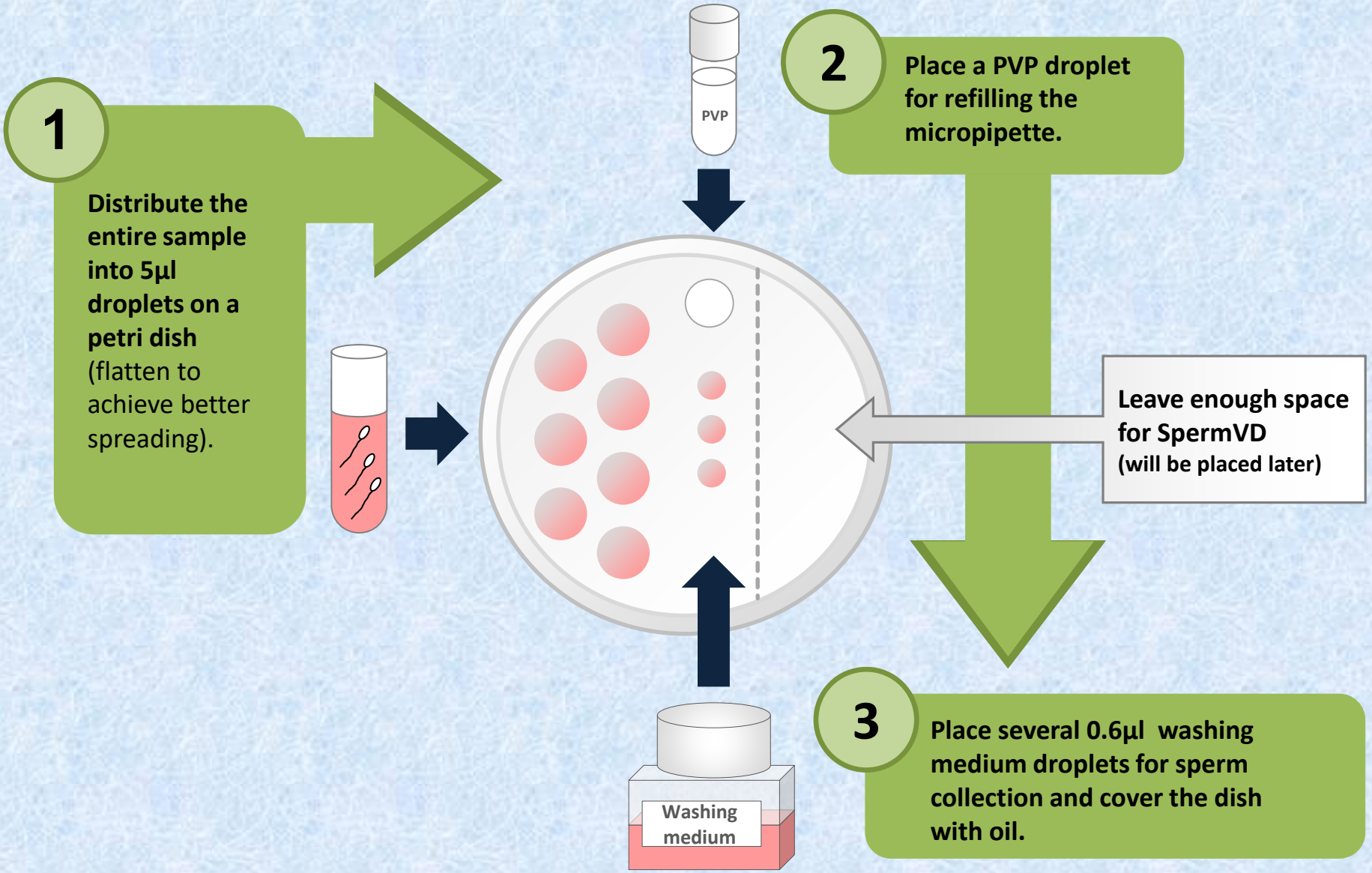
3 **If debris are too dense, dilute with washing medium to make the search convenient, then repeat Step 1.**



TIP

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

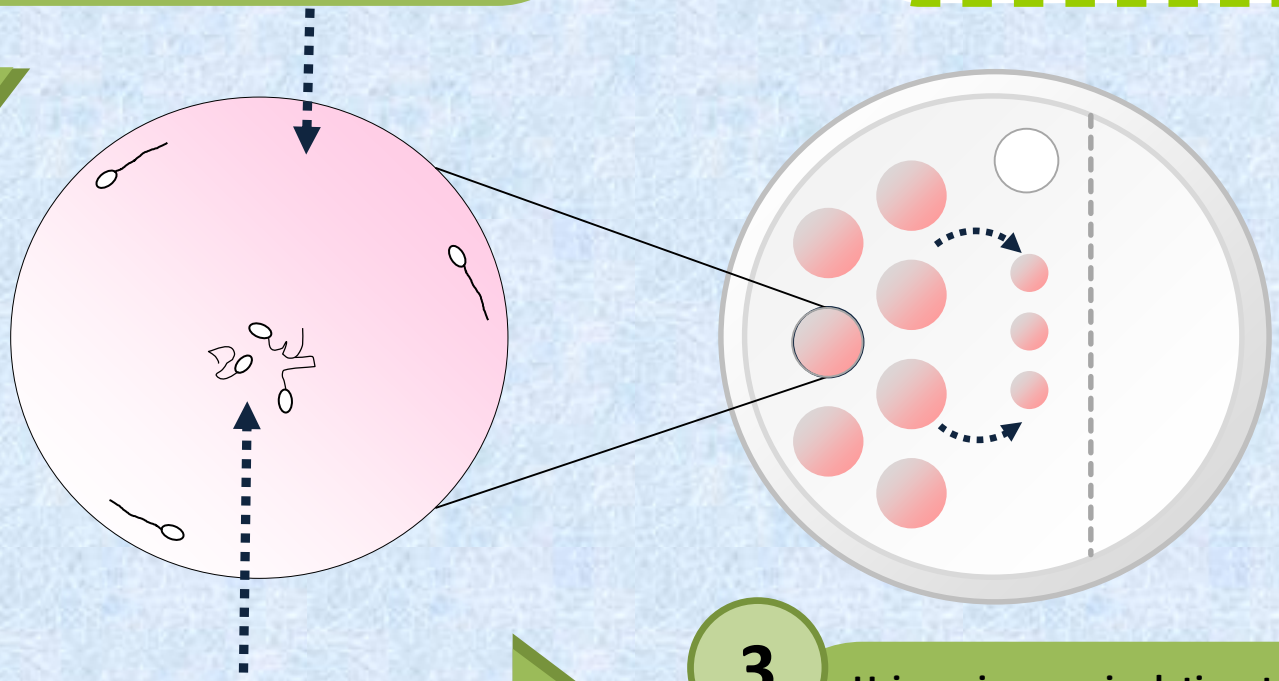
Phase 2 – Preparation of search plate



Phase 3 – Search

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

TIP If available, a micropipette with a larger diameter will allow to minimize contact with sperm membrane upon collecting, thus avoiding accidental immobilization. Also, a larger diameter helps to prevent clogging.

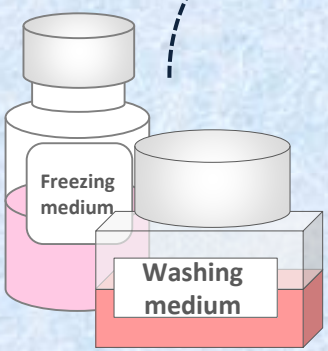


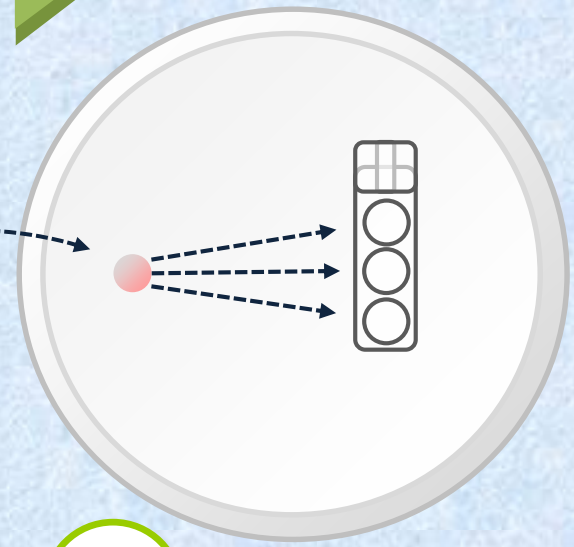
2 If no progressively motile spermatozoa are found, search for locally motile / immotile spermatozoa inside the droplets.

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 Create a cryoprotectant solution droplet on a new dish/dish cover by diluting freezing medium with sperm washing medium according to the freezing medium manufacturers protocol.





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

3 Place 0.8 – 1µl droplets of the cryoprotectant solution onto the circles on the SpermVD.

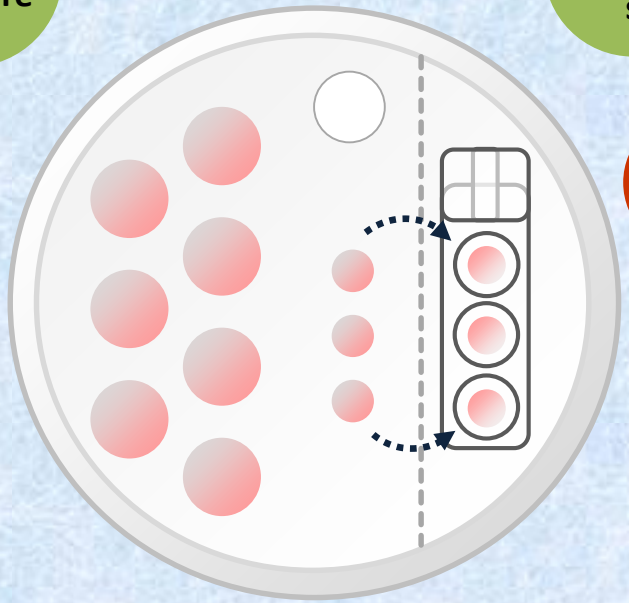
TIP Do not flatten the SpermVD droplets. Thicker droplets are more stable and allow better motility along the borders.

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

Phase 5 – Spermatozoa transfer

1 <u>Immediately</u> after preparation, gently submerge the SpermVD into the oil on the dish containing spermatozoa. Make sure that the cryoprotectant droplets are covered with oil.

2 Using micromanipulation, transfer the spermatozoa from the collection droplets to the SpermVD. It is possible to use one ore more cryoprotectant droplets. Use several devices if needed.



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

! **Attention!**
SpermVD containing spermatozoa must be frozen within a time limit of ~8 minutes, <u>beginning with the placement of the first cell</u>.

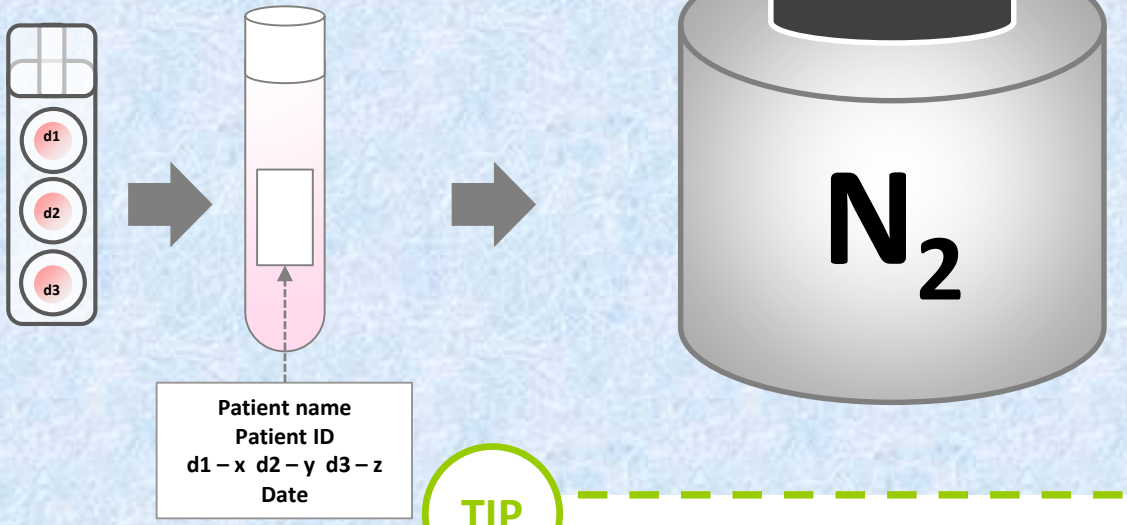
TIP It is recommended to freeze no more than 15-20 cells per device, to minimize the excess not used for ICSI.

Phase 6 – Cryopreservation

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

2 Place the cryovial into LN2 (no slow cooling required) and transfer it to storage in LN2 tank OR place it on a cryorack and lower directly into LN2 tank.

! **Attention!**
This is the most delicate phase. Take caution when placing the cryovial on the rack or lowering the rack into the LN2 tank. The droplets are stable but may be dislodged by an abrupt shake.



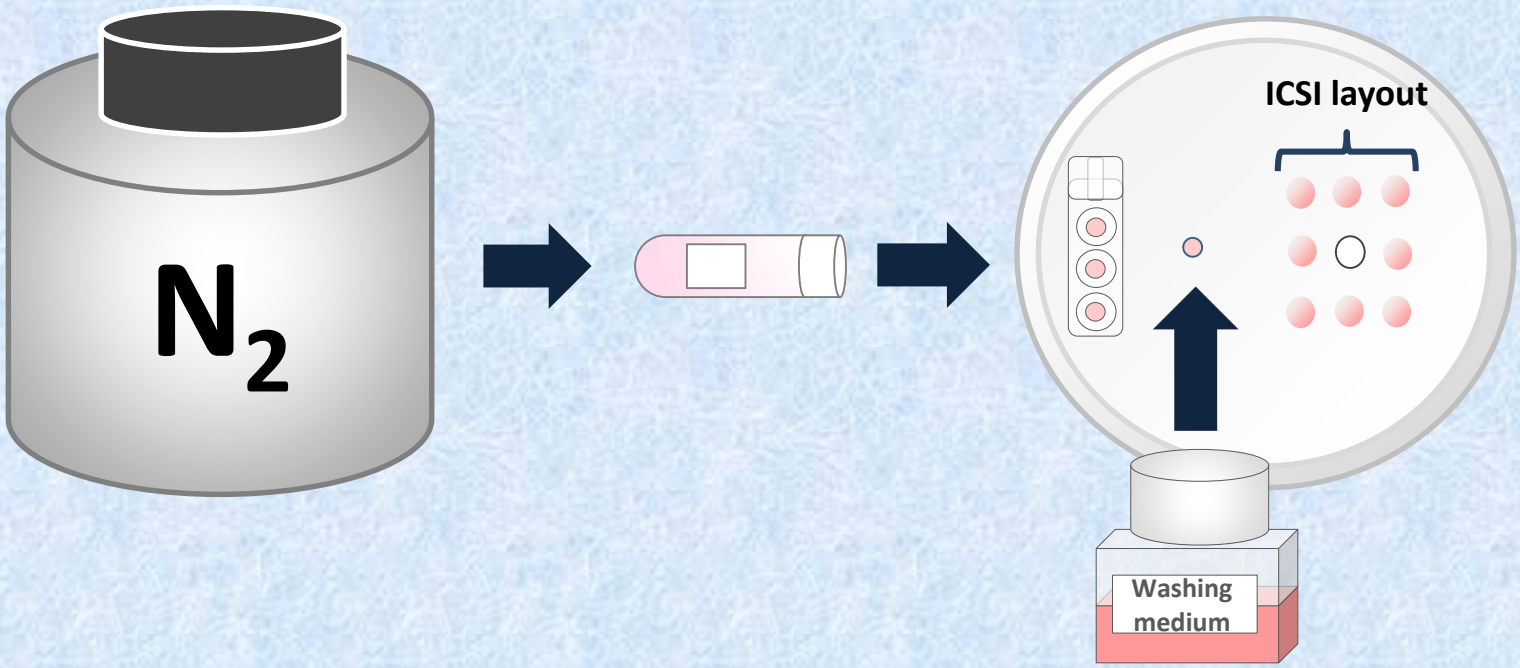
TIP Leave any excess oil on the SpermVD. It serves as an additional protection layer for the droplets.

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

Phase 7 – Warming

1. Remove the cryovial from LN2, carefully unscrew the cap and let it thaw in a horizontal position at room temperature or on a heated surface until the oil covering the droplets liquifies completely.

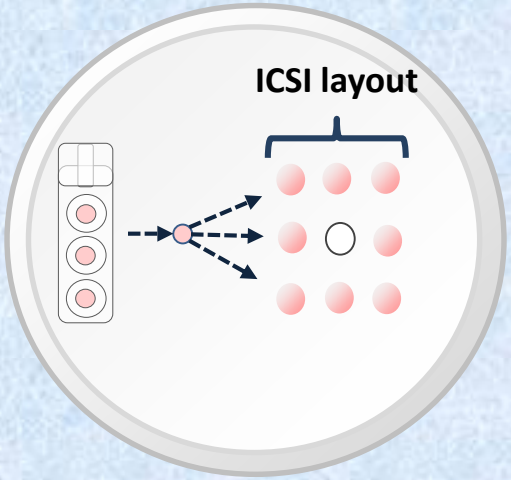
2. Remove the SpermVD from the cryovial and place it on an oil-covered ICSI dish with one or more extra sperm washing medium droplets in addition to your standard ICSI layout.



Phase 8 – Sperm retrieval

1 Search for spermatozoa in SpermVD droplets and transfer them to a washing medium droplet. Motile sperm should be prioritized for transfer. Once all (or enough) cells have been transferred, it is possible to perform ICSI on the same dish.

! **Attention!**
Sperm retrieval should be performed <u>immediately</u> after placing the SpermVD under oil.



! **Attention!**
Retrieved cells should be injected immediately. When not possible (such as in cases with frozen oocytes), sperm should be kept in a washing medium droplet until injection.

TIP If only motile cells were frozen, they may be safely used for ICSI even after loss of motility. However, in cases of low post-thaw motility, it is possible to use motility inducers, such as pentoxifylline or theofylline, perform flagellum flexibility test, or HOST (less recommended, as it adds stress to the already stressed cells).

Thank you!
Enjoy using SpermVD

Link to video demonstration: https://www.youtube.com/watch?v=nDyvr2Z6jKs&t=2s

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

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