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Extended search and cryopreservation of individual spermatozoa using SpermVD

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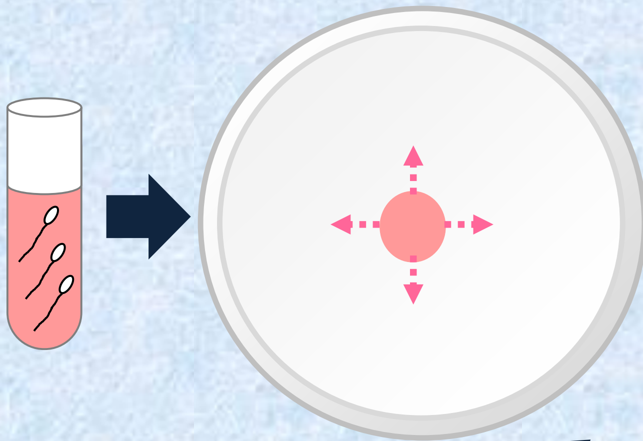
v5.2

Phase 1 – Evaluation of sample

1

Transfer a 10µl droplet of washed and concentrated sample onto a petri dish (flatten to achieve better spreading).

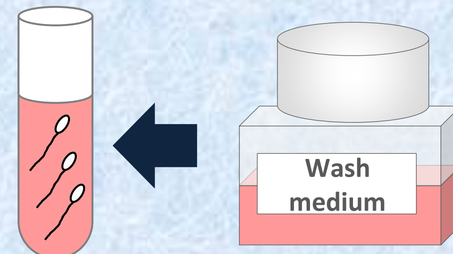


2

Observe under x200 magnification (may return the droplet back into the sample afterwards)

3

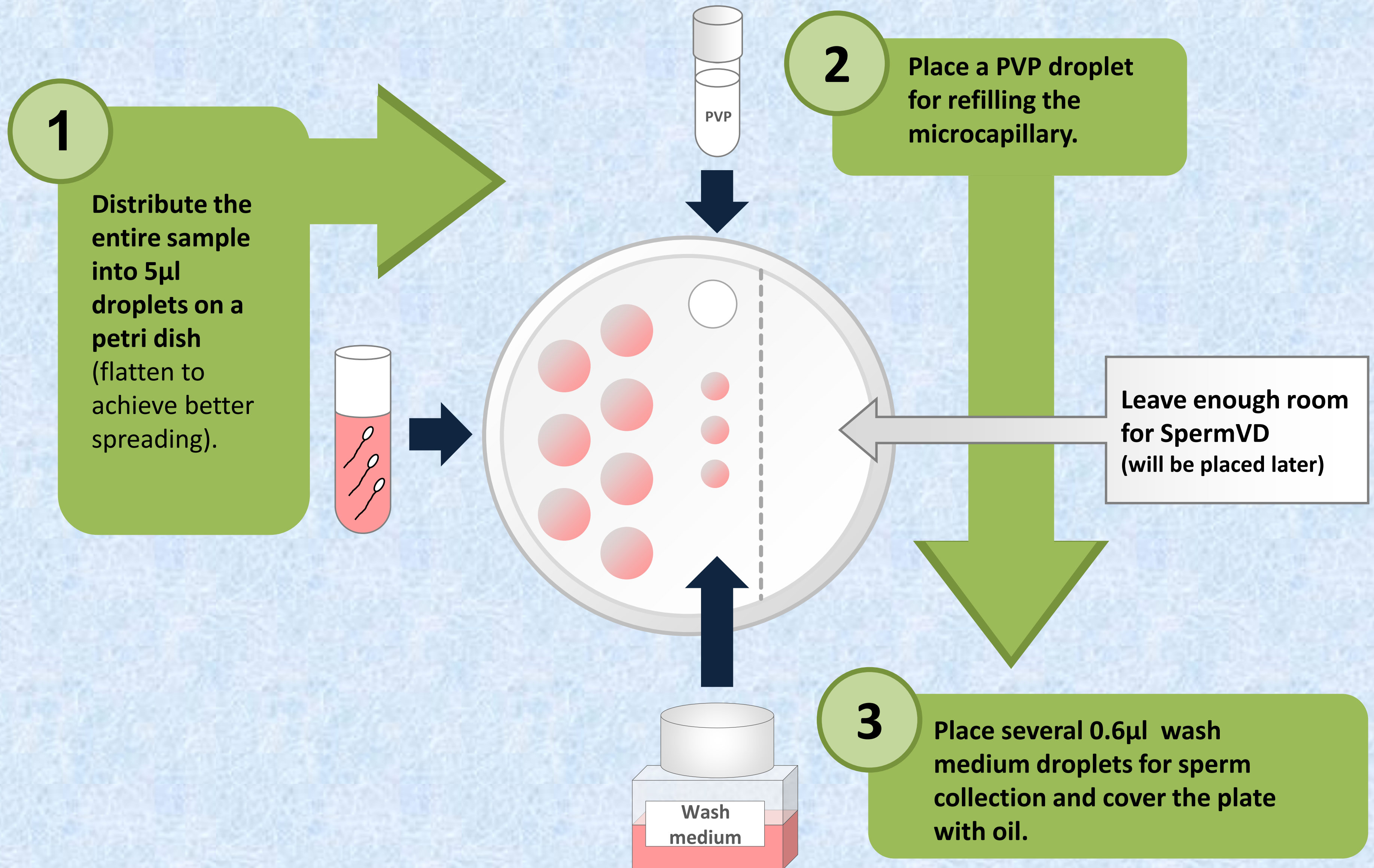
If sample is too clustered, dilute with wash medium to make the search convenient, then repeat Step 1.



TIP

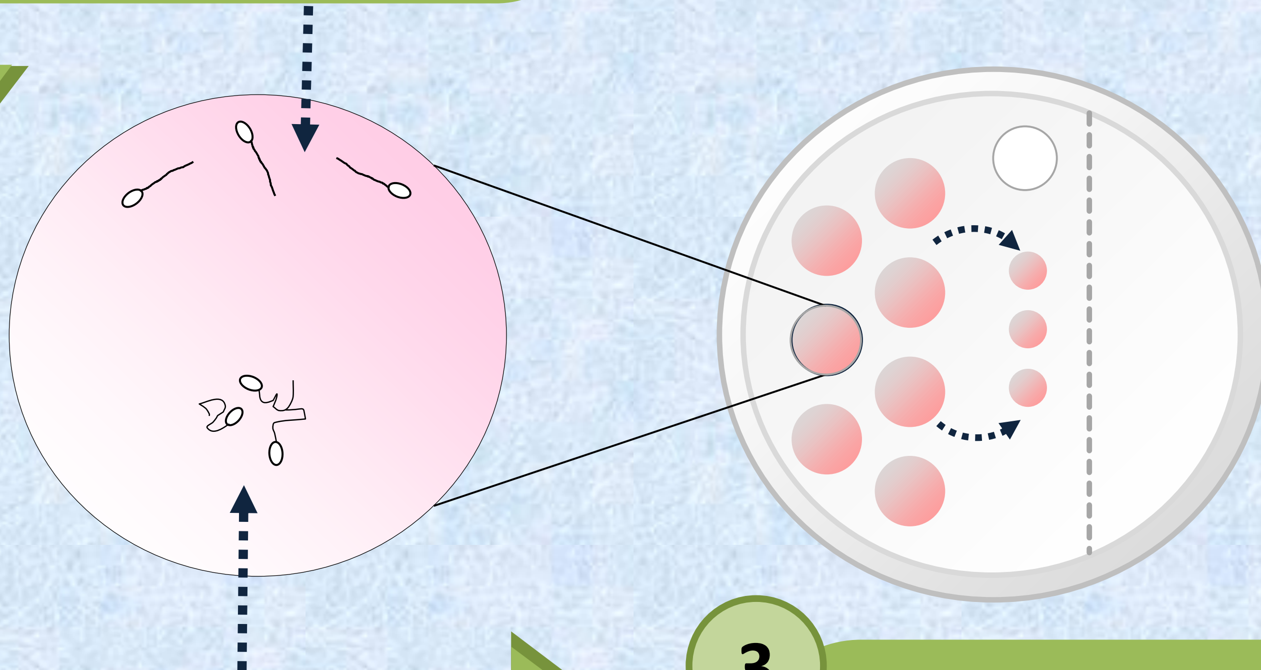
Flatten the droplet by gently tapping the plate on the work surface.

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

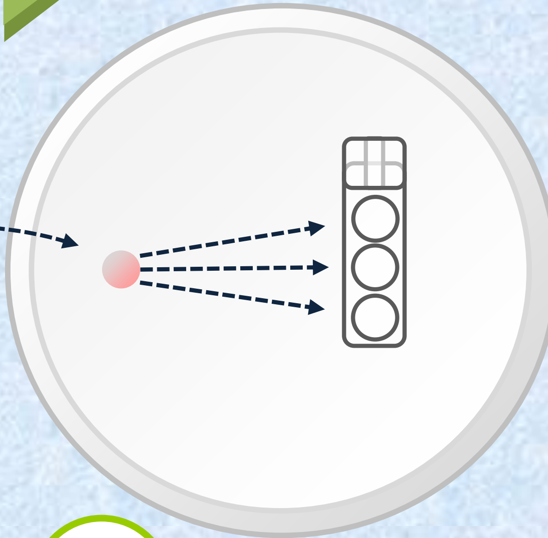
Place a droplet of 1:1 v/v wash medium and sperm freeze medium on a new plate and mix gently.

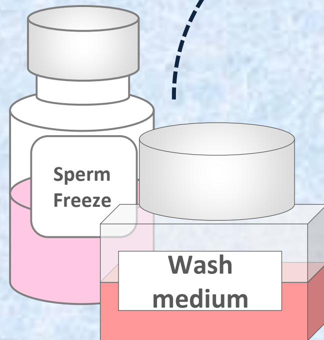
2

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.

!

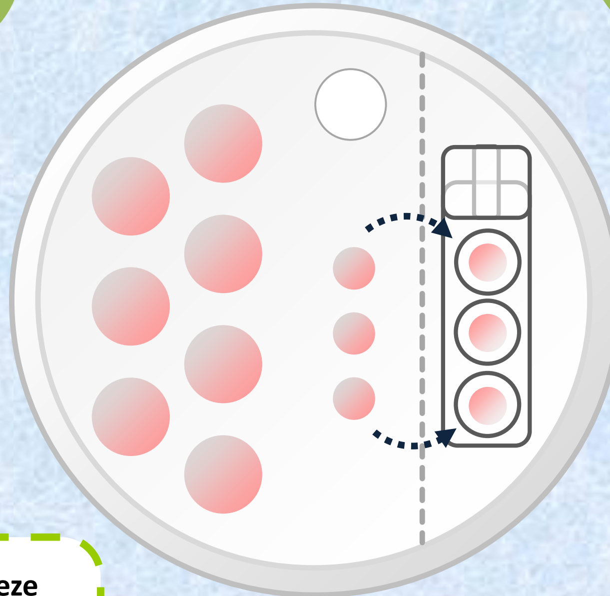
Attention!
Due to quick wash medium evaporation, create a new mixture droplet for each SpermVD!

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.



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

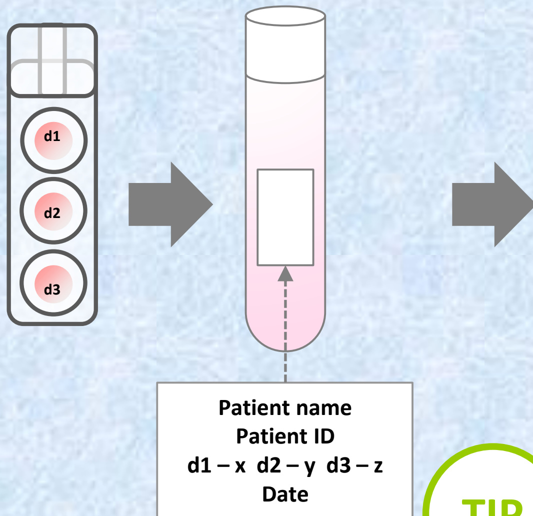
! Attention!
The SpermVD containing spermatozoa needs to be frozen within a time limit of ~10 minutes, beginning with the placement of the first cell. It is quite similar to bulk freezing, where a 10-minute equilibration at room temperature is needed prior to freezing.

Phase 6 – Cryopreservation

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

2 Place the cryovial on an aluminum holder and plunge it into LN2. No slow cooling required.

! **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.



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

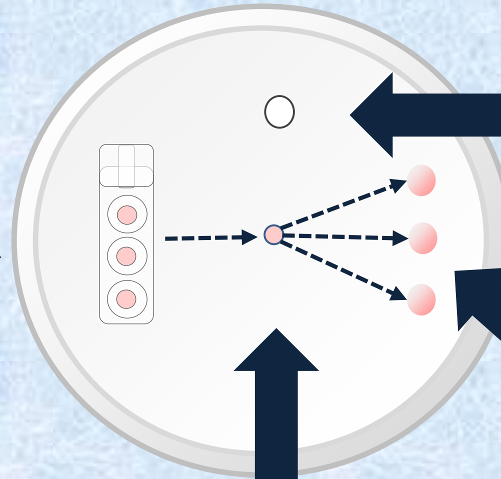
TIP Leave any excess oil on the SpermVD. It serves as an additional protection layer for the droplets.

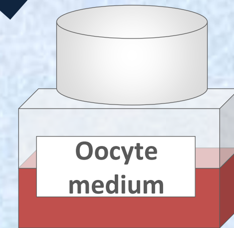
Phase 7 – Thawing and retrieval

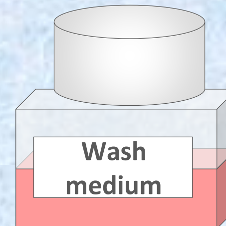
1 Remove the cryovial from LN2, unscrew the cap and let it thaw at room temperature until the oil covering the droplets liquifies completely (3-5 minutes).

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, transfer to washing medium droplets to wash off the remains of the cryoprotectant and proceed with ICSI.







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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