Witryfikacja oocytów

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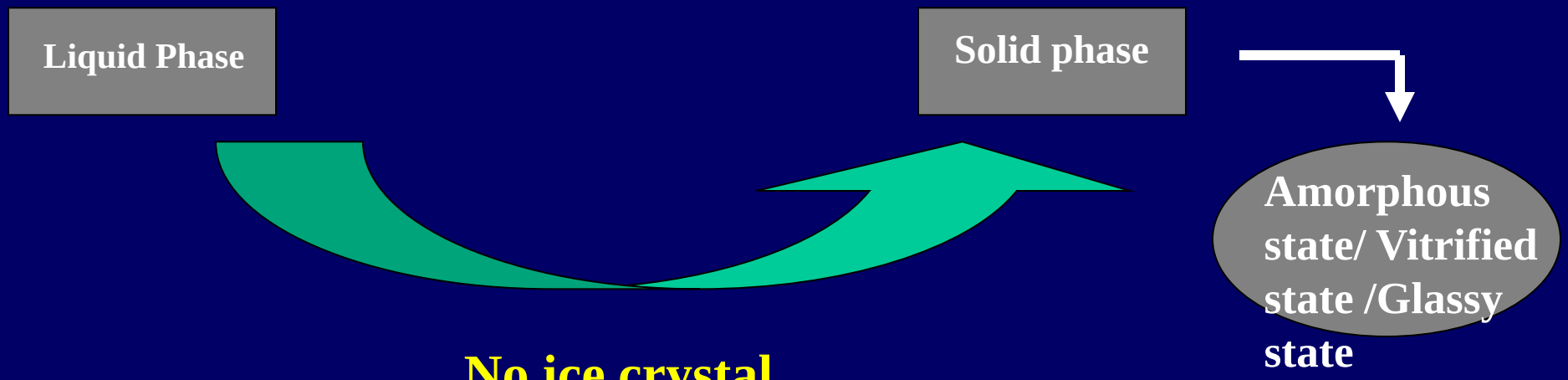
Zakład Rozrodu Zwierząt, Andrologii i Biotechnologii Rozrodu

VITRIFICATION

Vitrification is the solidification of a solution at low temperature without ice crystal formation



No ice crystal

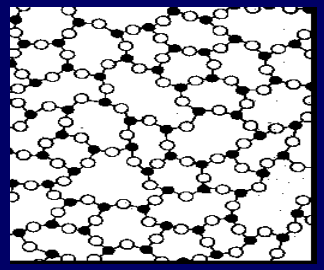
Possible osmotic stress

Absence of mechanical injuriy

CRYOPROTECTANT TOXICITY??

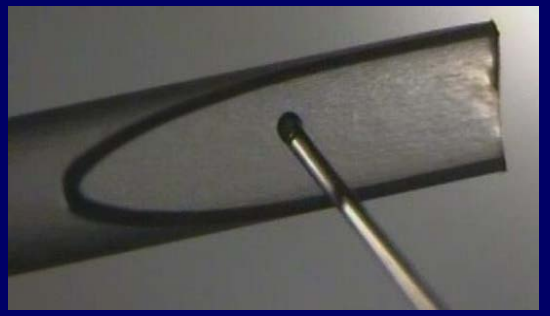
How to avoid ice crystal formation?

- **Use of high concentration of cryoprotectant provides:**
  - **liquid/solid transition temperature**
  - **high viscosity**
  - **impossibility for the molecules to re-arrange**



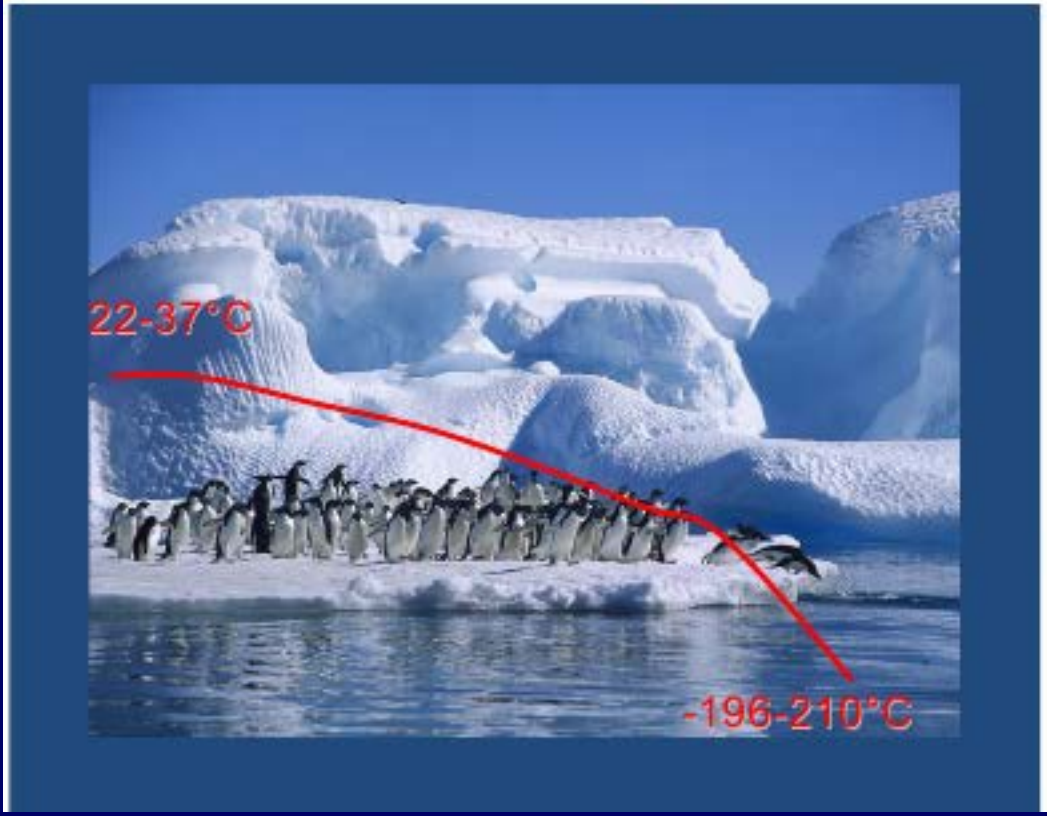
Amorphous state

- **High speed of cooling, which results from**
  - **low volume of cryoprotectant (~1-2µl)**
  - **type of carrier**



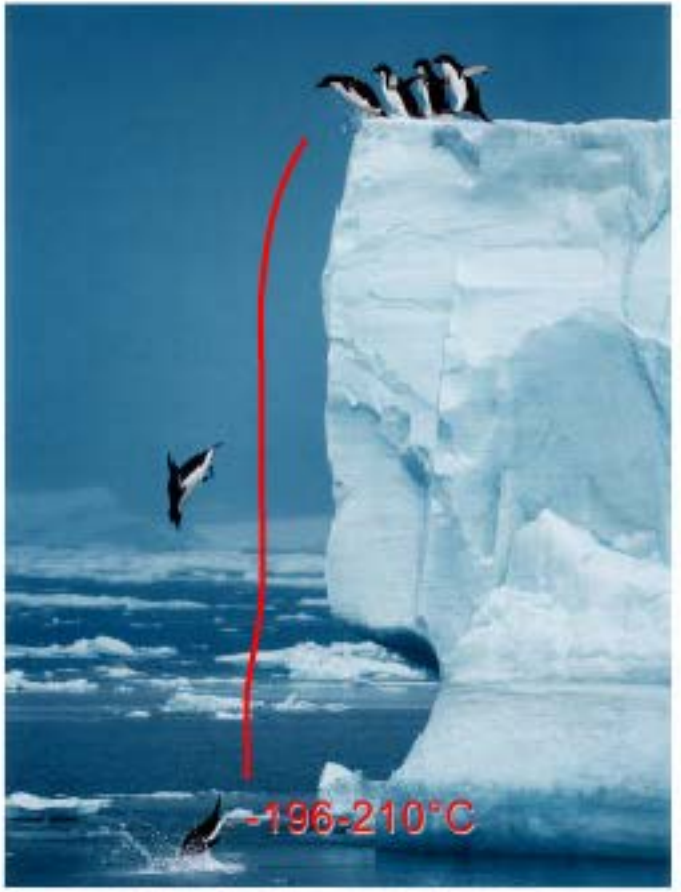
Techniques

Slow freezing

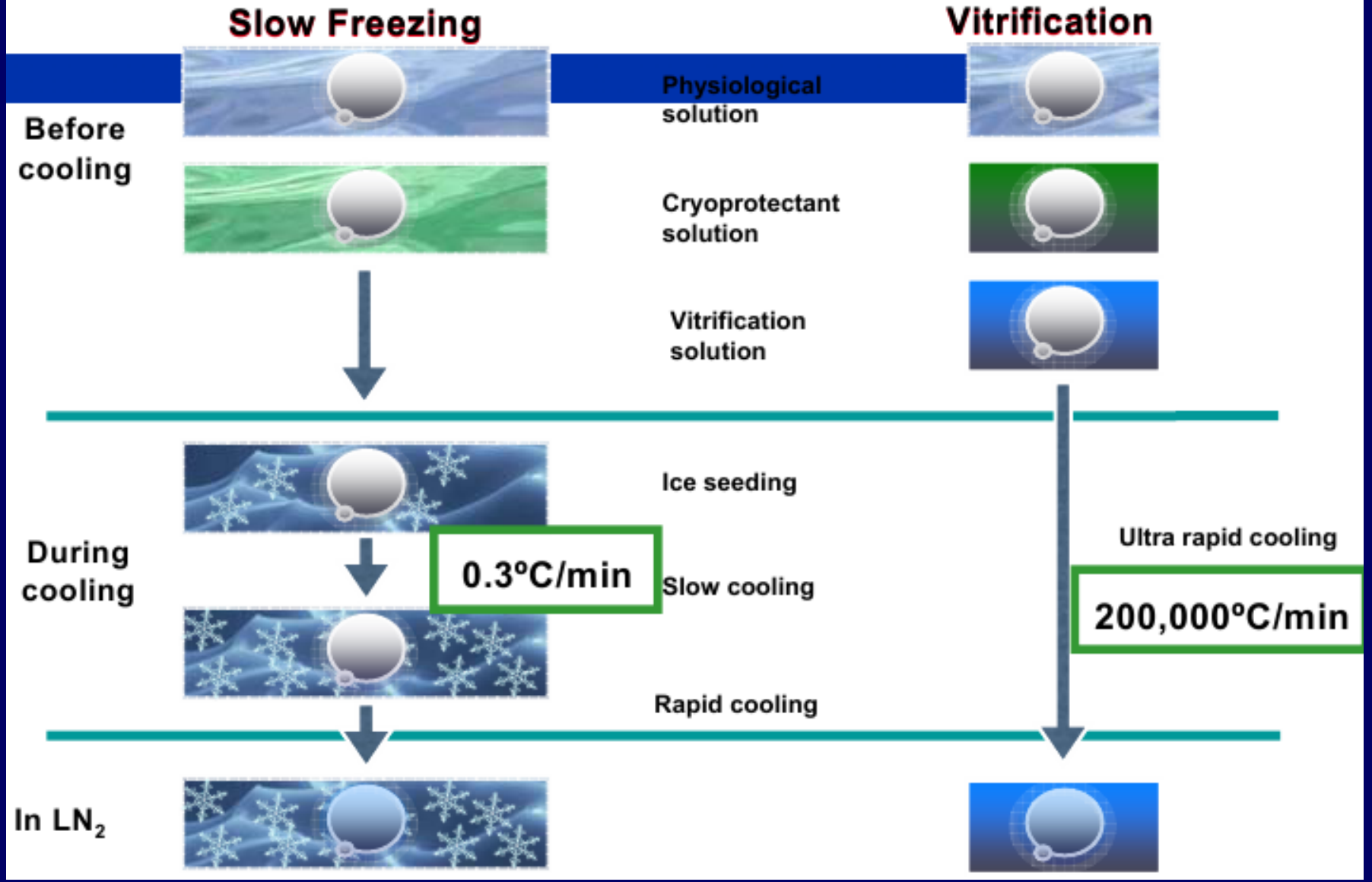


Vitrification

22-37°C



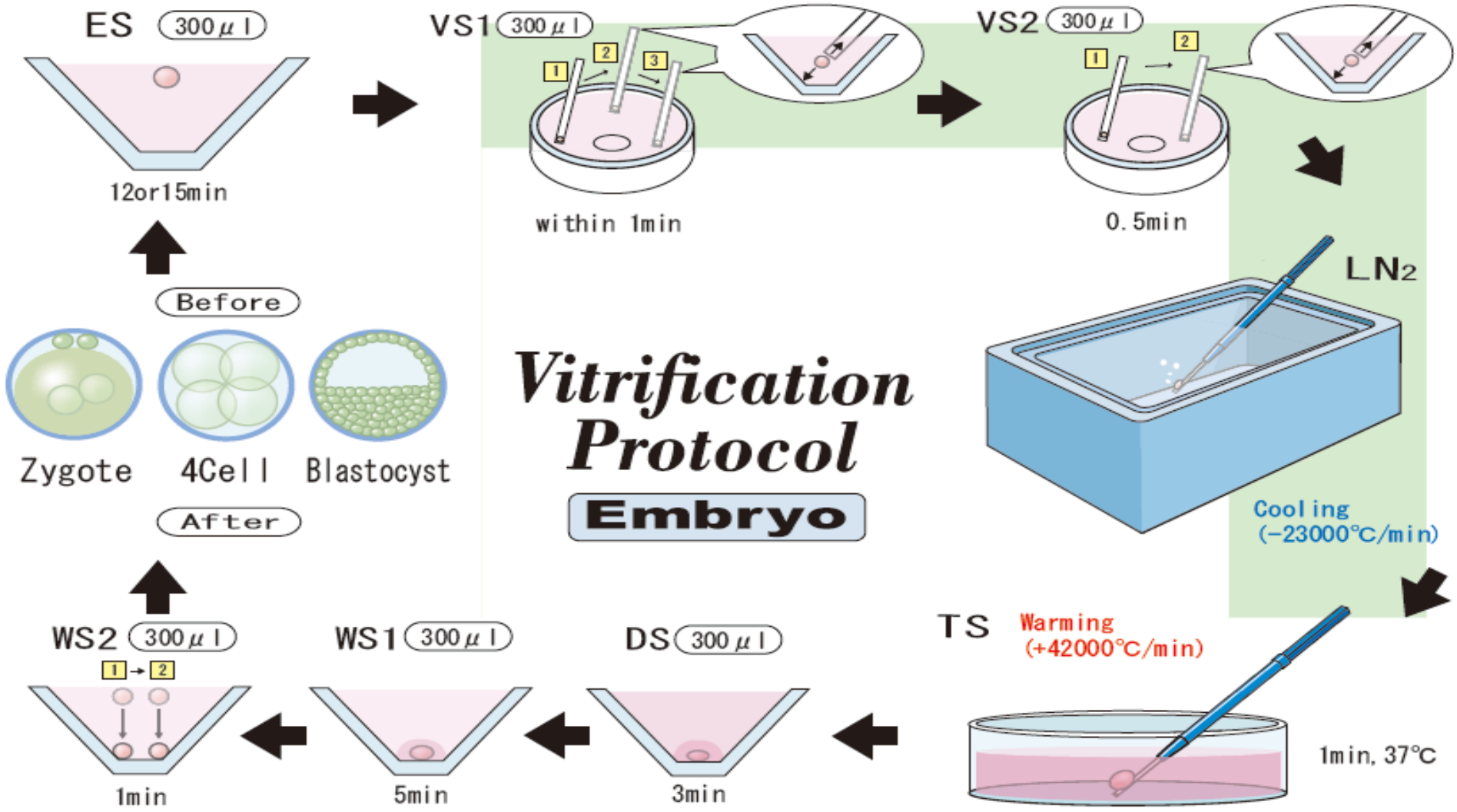
Techniques

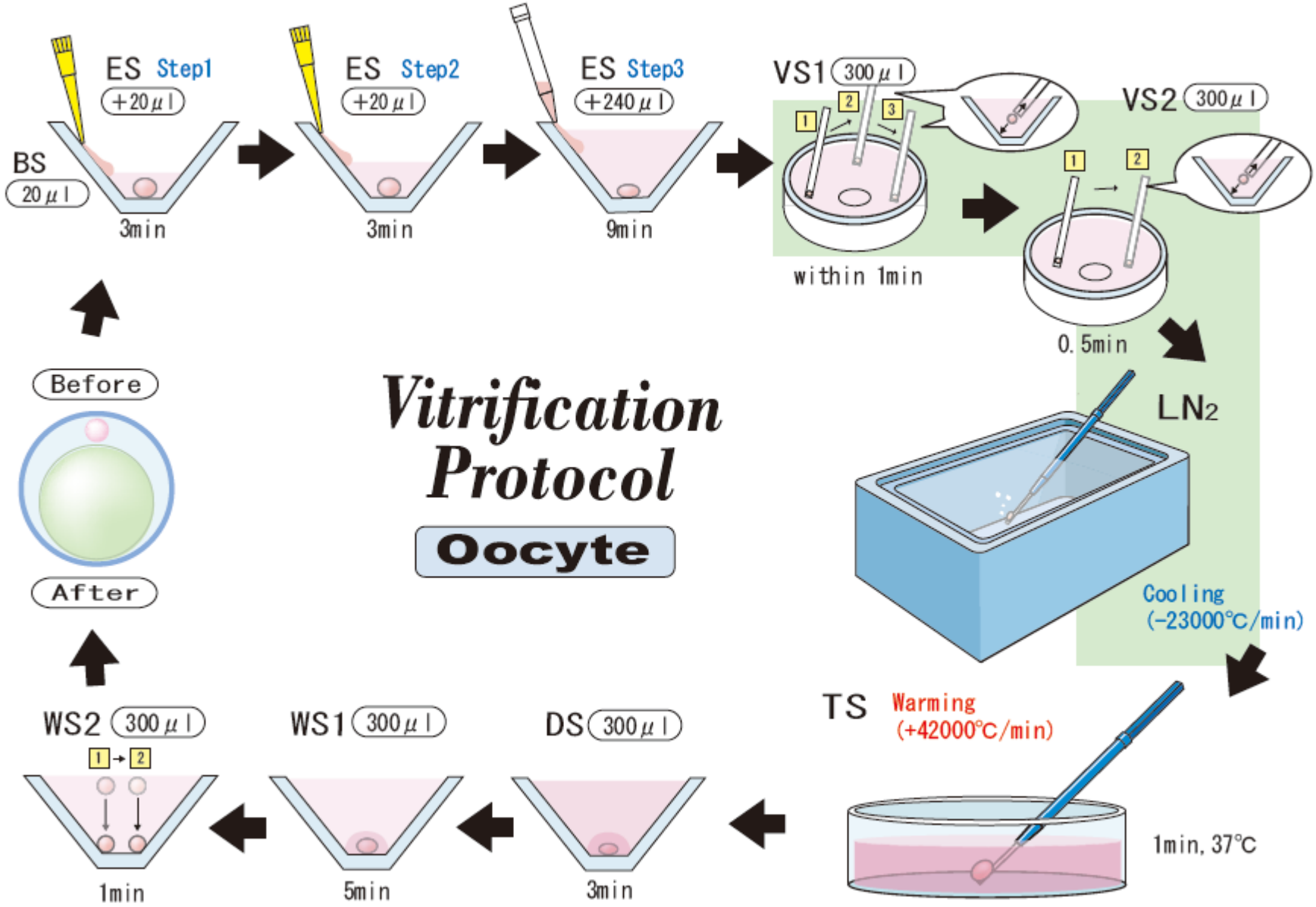

Slow Freezing
Vitrification
Physiological solution
Before cooling
Cryoprotectant solution
Vitrification solution
Ice seeding
During cooling
0.3ºC/min
Slow cooling
Ultra rapid cooling
200,000ºC/min
Rapid cooling
In LN_2


GV


M I


M II

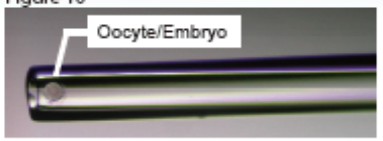

ES 300μl
12or15min
VS1 300μl
1
2
3
within 1min
VS2 300μl
1
2
0.5min
LN2
Cooling
(−23000℃/min)
TS
Warming
(+42000℃/min)
1min, 37℃
DS 300μl
3min
WS1 300μl
5min
WS2 300μl
1→2
1min
After
Zygote
4Cell
Blastocyst
Before
Vitrification
Protocol
Embryo


ES Step1
+20μl
BS
20μl
3min
ES Step2
+20μl
3min
ES Step3
+240μl
9min
VS1 300μl
1
2
3
within 1min
VS2 300μl
1
2
0.5min
LN2
Cooling
(-23000℃/min)
TS
Warming
(+42000℃/min)
1min, 37℃
DS 300μl
3min
WS1 300μl
5min
WS2 300μl
1→2
1min
Before
After
Vitrification
Protocol
Oocyte

Vitrification 5

Aspirate the shrunk Oocyte (Embryo) in **VS2** at the tip of the pasteur pipette (See Figure 10). Place the Oocyte (Embryo) by the black part of Cryotop sheet with minimal volume (less than 0.1 μ l) of **VS2** (See Figure 11a and 11b). For more than 2 Oocytes (Embryos), make 1 droplet for each (See Figure 12a and 12b).

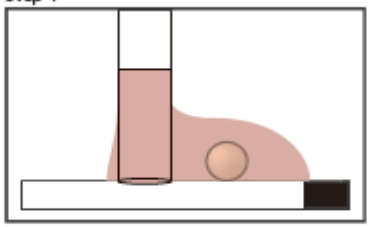
Figure 10



Removal of the excess VS on the sheet

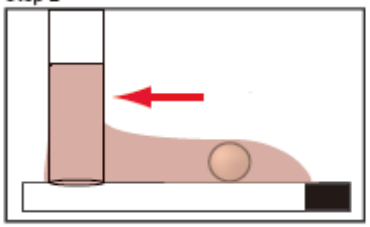
After putting oocytes on the Cryotop sheet, the excess VS should be removed by aspirating using pipette.

Step 1



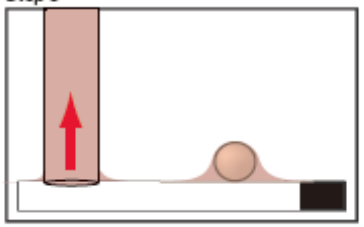
Put the top of the pipette on the bottom end of the big VS drop.

Step 2



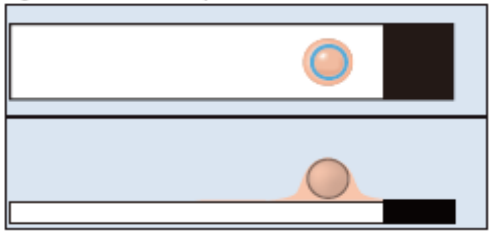
Slide the pipette horizontally to outside, and make the VS drop lower.

Step 3



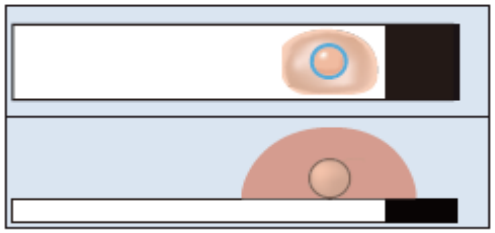
Aspirate the excess VS, and minimize the VS drop (not aspirating oocyte).

Figure 11a: Good example



Make a planar droplet by the black mark of Cryotop sheet.

Figure 11b: Bad example



Make a steric droplet by the black mark of Cryotop sheet. The volume of VS2 is too much.

Figure 12a: Good example

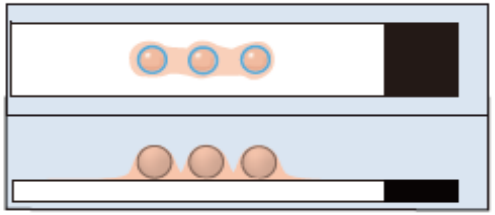
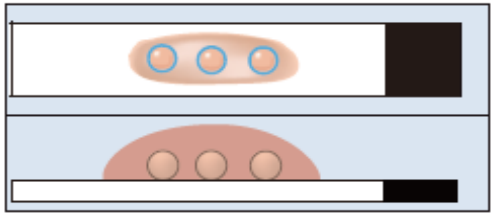


Figure 12b: Bad example



Vitrification 6

Check if the Oocyte (Embryo) is on the sheet with minimal volume of **VS2** under the microscope.
Plunge the Cryotop into fresh liquid nitrogen quickly.