Processing of human IVF/IVM oocytes for single cell RNA sequencing

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Method Article

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Abstract

The protocol describes the step-by-step procedure of isolating and preparing human oocytes 1) oocytes donated in connection with IVF/ICSI treatment and 2) *in vitro* matured (IVM) oocytes from small antral follicles donated in connection to fertility preservation. It details how cumulus cells and the zona pellucida are enzymatically removed to ensure that only the oocyte is analyzed. We have successfully used this protocol in connection with SMART-Seq2 (Takara).

This oocyte preparation has recently been used in Sankar et al. 2020 (Sankar *et al.* 2020).

Introduction

Reagents

Phosphate-buffered-saline (PBS)

10X

AM9625, Invitrogen™, Thermo Fisher Scientific™, Denmark

Sterile, distilled water

Polyvinyl alcohol (PVA)

25 grams

341584, Sigma-Aldrich, Denmark

Hyaluronidase

5 × 1 ml

15115001, SynVitro™ Hyadase, Origio, Denmark

Tyrode's solution

5× 0.2 ml

10605000A, Origio, Denmark

Oil

100 ml

10029, VitroLife, Denmark

Equipment

Blue cap bottle

100 ml

e.g. 9072331, Buch & Holm A/S, Denmark

Collection tube

0.2 ml

AB0620, Thermo Fisher Scientific™, USA

Syringe needles

18G

e.g. Z118044-100EA, Merck, Denmark

IVF petri dish

Diameter: 50 mm

351006, BD Falcon™, Corning Inc., USA

Denudation pipette

145 µm

7-72-3145/20, RI Consumables, United Kingdom

Pipettes (e.g. **Eppendorf Research plus)**

20 µl and 200 µl

e.g. 3124000040 and 3124000083, Eppendorf, Merck, Denmark

Pipette tips

2-200 µl

0030 075.021, Eppendorf, Merck, Denmark

IVF workstation

UV-light

e.g. WM1500RE, Origio, Denmark

Procedure

Prepare the washing solution

10 ml	10× PBS (final concentration 1× v/v)
90 ml	Sterile water
0.1 g	PVA (final concentration: 1% w/v)

All reagents are mixed in a hot water bath or in a pot adding boiling water to dissolve the PVA. Shake the bottle frequently to avoid the PVA stick to the bottom of the bottle. Repeat the process until the PVA is dissolved. The washing solution can be stored at room temperature for several months in the dark.

The day before oocyte retrieval:

1. Prepare the label for the collection tube.

2. If possible, UV-light treat the collection tubes, pipettes, box of 20 µl tips and *washing solution* overnight to decrease risk of contamination.

At the day of oocyte retrieval:

3. If cumulus cells are also being collected, first remove the cumulus cells from the cumulus-oocyte-complex (COC) mechanically with two syringe needles (size: 18G) as if they were a knife and fork and keep the oocyte in the incubator until step 5.

4. Prepare a petri dish with one drop of 50 µl hyaluronidase and three drops of *washing solution*. Cover the drops with oil.

5. Transfer the COC to the drop of hyaluronidase to remove the cumulus cells. Use the pipette with a denudation pipette (size: 145 µm) to 'mix' the COC with the hyaluronidase. Wash the oocyte in the three sequential *washing solution* drops and assess stage (GV, MI, MII) and morphology under the ICSI microscope (or light microscope with 400× magnification).

6. Prepare a petri dish with one drop of 25 µl Tyrode's solution and two drops of *washing solution*.

7. Transfer the oocyte to the Tyrode's to remove zona pellucida. Use the denudation pipette (size: 145 µm) to speed up the process by pipetting the oocyte up and down.

8. The zona-free oocyte is washed twice by pipetting up and down in the *washing solution*. The oocyte is transferred with 2 µl *washing solution* in a denudation pipette (size: 145 µm) to the bottom of the UV irradiated 0.2 ml collection tube.

9. Flash freeze the collection tube in liquid nitrogen and store at -80 °C until analysis. The oocyte is lysed by the flash freezing and can be used for omics research, but not for biopsies.

Troubleshooting

All steps: Use gloves, mask and hat to avoid contamination with your own DNA. All steps are performed at the IVF workstation under the microscope except when using the ICSI microscope in step 3. The tabletop in the IVF workbench are heated to 37 °C.

Washing solution: This protocol has also been used for *in vitro* matured oocytes from small- and large-antral follicles. For these oocytes a simpler wash solution of 1× PBS alone was used.

Step 2: If a UV source is not available, make sure to use sterile tubes and tips.

Prior to step 4: The pipettes are rinsed with *washing solution* before handling the oocyte to avoid the oocyte sticking to the inside of the pipette.

Step 5: Use the Atlas of Human Embryology provided by ESHRE (http://atlas.eshre.eu/) to determine the stage and morphology of the oocyte. MII oocytes are characterized with a visible polar body I.

Step 6: The zona will slough off and breakdown within the Tyrode's solution. Once the zona is removed, pipette the oocyte in Tyrode's for an additional few seconds before moving to wash solution. Leaving the oocyte in Tyrode's for too long can lead to bursting; however, removing the oocyte too soon may leave behind a thin layer or the zona and potentially influence reactions during the scRNA-seq processing.

Step 8: Minimize transfer of the *washing solution*. By transfer the oocyte to the bottom of the tube it facilitates further processing.

Time Taken

Preparation: 30 mins + overnight UV treatment

Working time: 30 mins

Anticipated Results

Single zona-free human oocyte to use for future single cell RNA-seq analysis.

References

Sankar A, Lerdrup M, Manaf A, Johansen JV, Gonzalez JM, Borup R, Blanshard R, Klungland A, Hansen K, Andersen CY*, et al.* KDM4A regulates the maternal-to-zygotic transition by protecting broad H3K4me3 domains from H3K9me3 invasion in oocytes. *Nature Cell Biology* 2020; **4**: 380-388.

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