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Title: Fertility Preservation Through Oocyte Vitrification: Clinical and Laboratory Perspectives

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**

If you require a microscope for your technique but can record movies/images through your microscope with your own camera, please indicate **Yes** here: **Y**

If yes, please capture and upload these files to your project page as soon as feasibly possible.

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Filmed by videographer

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **37**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Alberto Vaiarelli**: The oocyte vitrification and warming procedures are essential for fertility preservation. Following this protocol as demonstrated allows users to maximize the effectiveness of these procedures **[1]**.

1.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Laura Rienzi**: Fertility preservation through oocyte vitrification is the mostly established and ethically acceptable approach to achieve effective and consistent clinical outcomes and overcome the moral and legal limitations associated with embryo cryopreservation **[1]**.

1.2.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Roberta Maggiulli**: To achieve proficiency with the vitrification process, it is important to become familiar with all of the critical points of the protocol, paying particular attention to the exposure time of the samples to the cryoprotectants **[1]**.

1.3.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.4. Procedures involving human subjects have been approved by the Institutional Review Board (IRB) or equivalent body at Clinica Valle Giulia.

Protocol

2. Oocyte Vitrification

NOTE: Some lab media missing at the time of postshoot processing, authors promised to upload soon

2.1. Within 38 hours of retrieval and immediately after denudation, label all of the plastic supplies with the patient's name, ID, and type of solution **[1]** and ask a Witness to confirm the patient's information on the cryodevice **[2-TXT]**.

2.1.1. WIDE: Talent labeling supply

2.1.2. Other Talent checking cryodevice/nodding to confirm patient information
TEXT: Warm all solutions to 25 °C before use

2.2. When all of the supplies have been labeled, carefully invert each vial of oocytes several times to mix **[1]** and place a 30-microliter drop of HEPES (heepes)-buffer medium supplemented with human serum albumin and a 30-microliter adjacent drop of Equilibration solution on a Petri dish**[2-TXT]**.

2.2.1. Talent inverting vial, with scope, plate, and medium and ES containers visible in frame
2.2.2. Added shot: talent placing the drops on the plate

2.3. Use the stripper pipette to place the oocytes in the first drop and create a bridge of medium to a 30-microliter drop of equilibration solution to obtain a gradual increase in concentration of the cryoprotectants **[1]**.

2.3.1. Talent transferring the oocytes and brige being created
2.3.2. LAB MEDIA: To be provided by Authors: oocyte being transferred /Bridge being created / *Videographer: Important step*

2.4. After 3 minutes, add a second, 30-microliter drop of equilibration solution to the plate **[1]** and use the pipette to create a medium bridge between the drops of solution **[2]**.

2.4.1. Talent adding the drop and creating the bridge
2.4.2. LAB MEDIA: To be provided by Authors: Oocyte being transferred/bridge being created

2.5. While the oocytes are equilibrating, add one 30-microliter drop of equilibration solution for each cryodevice to be used onto the plate **[1]**. After 3 minutes, move the oocytes into the pure equilibration solution for 6-9 minutes to allow the oocytes to return to their initial size **[2]**.

2.5.1. Talent adding drops, with ES container visible in frame
2.5.2. LAB MEDIA: To be provided by Authors: Oocyte being moved

2.6. When the oocytes have recovered, transfer the oocytes into a central well IVF dish containing 1 milliliter of vitrification solution in as little medium as possible for 1 minute **[1]** carefully moving one oocyte from the bottom to the top of the dish to remove any excess equilibration solution **[2]**.

2.6.1. Talent placing oocyte into dish, with VS container visible in frame
2.6.2. LAB MEDIA: To be provided by Authors: Oocyte being moved from bottom to top of dish

2.7. Approximately 10 seconds before the end of the incubation, place a cryodevice labeled with the patient's information under the microscope **[1]** and adjust the focus on the tip of the cryodevice **[2]**.

2.7.1. Talent placing device under microscope
2.7.2. LAB MEDIA: To be provided by Authors: Tip coming into focus

2.8. Place the oocytes on the cryodevice beside the tip in the minimum amount of vitrification solution **[1]** and move the stripper pipette away from the oocytes to remove any excess vitrification solution, leaving the oocytes covered with a thin layer of medium **[2]**.

2.8.1. LAB MEDIA: To be provided by Authors: Oocytes being moved beside tip *Videographer: Important/difficult step*
2.8.2. LAB MEDIA: To be provided by Authors: Pipette being moved/VS being removed/oocytes being covered by medium *Videographer: Important/difficult step*

2.9. Quickly plunge the cryodevice into liquid nitrogen **[1]** and rapidly shake the device to remove any air bubbles from its surface **[2]**.

2.9.1. Cryodevice being plunged into LN2 *Videographer: Important step*
2.9.2. Device being shaken *Videographer: Important step*

2.10. Holding the protective cap of the cryodevice with tweezers, fill the cap with liquid

nitrogen **[1]** before inserting the device into the cap while keeping the propylene strips in liquid nitrogen **[2]**.

2.10.1. Cap being filled with liquid nitrogen
2.10.2. Device being inserted into cap

2.11. Then store the cryodevice in a visiotube labeled with the patient's information **[1]** and update the laboratory sheet **[2-TXT]**.

2.11.1. Talent placing device into tube
2.11.2. Talent updating lab sheet **TEXT: Witness should confirm all materials labeled with correct patient ID before use**

3. Oocyte Warming

3.1. For oocyte warming, carefully invert each vial of thawing, dilution, and washing solution warmed to room temperature **[1]** and add 1 milliliter of thawing solution to the central well of a Petri dish for an at least 1-hour incubation at 37 degrees Celsius **[2]**.

3.1.1. WIDE: Talent inverting vials, with other vials visible in frame
3.1.2. Talent adding solution to dish, with solution container visible in frame

3.2. Label all of the plastic supplies with the patient's name and ID and the type of solution **[1]** and ask a Witness to confirm the patient's information on the cryodevice **[2]**.

3.2.1. Talent labeling on material, with other labeled materials visible in frame
3.2.2. Witness checking information/confirming information with Talent

3.3. For each device to be warmed, add 200 microliters of dilution solution to the first well of a 6-well plate **[1]** and an equal volume of washing solution to the second and third wells of the plate **[2]**. Add PBS to the area on the outside of the wells to prevent solution evaporation **[3]**.

3.3.1. Talent adding solution to well, with solution container visible in frame
3.3.2. Talent adding solution to well(s), with solution container visible in frame
3.3.3. Talent adding PBS to area outside of well, with PBS container visible in frame

3.4. Place the dish of thawing solution under the stereomicroscope **[1]** and adjust the focus to the center of the dish **[2]**.

3.4.1. Talent placing dish under microscope
3.4.2. LAB MEDIA: To be provided by Authors: Focus being adjusted

3.5. Carefully twist to remove the protective cap of a cryodevice while keeping the propylene strips in liquid nitrogen **[1]** and transfer the cryodevice from the liquid nitrogen into the thawing solution as quickly as possible to avoid the risk of devitrification **[2]**.

3.5.1. Cap being twisted and removed *Videographer: Important/difficult step*
3.5.2. Device being transferring into thawing solution *Videographer: Important/difficult step*

3.6. After 1 minute, focus on the tip of the cryodevice to locate the oocytes **[1]** and eventually use a stripper pipette to gently remove medium from the device to release the oocytes from the device **[2-TXT]**.

3.6.1. LAB MEDIA: To be provided by Authors: Tip and/or oocytes coming into focus *Videographer: Important step*
3.6.2. LAB MEDIA: To be provided by Authors: Oocytes being released *Videographer: Important step*
TEXT: Do not aspirate oocytes from device

3.7. Use a 170-micron-diameter stripper pipette to transfer the oocytes and a small volume of thawing solution into the dilution solution in the first well of the 6-well plate **[1]**. After 3 minutes, move the oocytes into the first well of washing solution for 5 minutes **[2]**.

3.7.1. LAB MEDIA: To be provided by Authors: Oocytes being added to well *Videographer: Important step*
3.7.2. LAB MEDIA: To be provided by Authors: Oocytes being added to second well *Videographer: Important step*

3.8. At the end of the incubation, transfer the oocytes to the second well of washing solution for 1 minute **[1]** before transferring the oocytes into an appropriate volume of preequilibrated IVF culture medium for 1 hour **[2-TXT]** and updating the laboratory sheet **[3]**.

3.8.1. Talent adding oocytes to well
~~3.8.2.~~ Talent adding oocytes to medium
3.8.3. Talent updating laboratory sheet

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.3., 2.8., 2.9., 3.5.-3.7.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
2.8., 3.6.

Results

4. Results: Single Oocyte Vitrification and Warming Analysis

4.1. Over a 12-year period, 285 women underwent at least one oocyte retrieval entailing the vitrification of the whole cohort of mature eggs collected **[1]**.

4.1.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize text boxes at top center of Figure*

4.2. Most of these women underwent a single retrieval **[1]**. Thirty-five underwent multiple retrievals **[2]**.

4.2.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize 250 women line*
4.2.2. LAB MEDIA: Figure 3 yellow/gold table *Video Editor: please emphasize 28, 4, 1, 1, and 1 women lines*

4.3. The reasons for undergoing oocyte retrieval for egg vitrification **[1]** were generally characterized as medical other than cancer **[2]**, cancer **[3]**, non-medical **[4]**, and other **[5]**.

4.3.1. LAB MEDIA: Figure 3
4.3.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize 8% and 9% data bars*
4.3.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize 6% and 16% data bars*
4.3.4. LAB MEDIA: Figure 3 *Video Editor: please emphasize 80% and 53% data bars*
4.3.5. LAB MEDIA: Figure 3 *Video Editor: please emphasize 3% and 23% data bars*

4.4. Patients with a medical reason for egg vitrification **[1]** and patients undergoing fertility preservation because of cancer were younger **[2]** and showed higher Antral Follicular Counts **[3]** than patients with non-medical or other reasons **[4]**.

4.4.1. LAB MEDIA: Table 1 top of table
4.4.2. LAB MEDIA: Table 1 top of table *Video Editor: please emphasize 33.6+6.4 and 34.6+5.9 data cells*
4.4.3. LAB MEDIA: Table 1 top of table *Video Editor: please emphasize 12.7+5 and 13.3+7 data cells*
4.4.4. LAB MEDIA: Table 1 top of table *Video Editor: please emphasize 37.0+3.4, 38.2+4.3, 11.6+6.1, and 10.7+6.7 data cells*

4.5. However, as the mean maturation rates were slightly lower in the "medical reasons" patients **[1]**, the number of oocytes vitrified on average was similar between the groups **[2]**.

4.5.1. LAB MEDIA: Table 1 top of table *Video Editor: please emphasize 72.4+13.6 and 72.1+19.6 data cells*
4.5.2. LAB MEDIA: Table 1 top of table *Video Editor: please emphasize 8.8+3.3., 10.0+7.2, 8.7+17.5, and 8.0+6.7 data cells*

4.6. Approximately half of the patients **[1]** with medical reasons other than cancer **[2]** and the majority of patients with other reasons for egg vitrification actually returned for warming **[3]**.

4.6.1. LAB MEDIA: Table 1 bottom of table
4.6.2. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize 10/19 52.6% data cell*
4.6.3. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize 44/58 75.9% data cell*

4.7. Conversely, very few patients who underwent fertility preservation for cancer **[1]** or non-medical reasons used their vitrified oocytes for IVF **[2]**.

4.7.1. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize 7/40 17.5% data cell*
4.7.2. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize 17/133 12.8% data cell*

4.8. Despite differences in the time elapsed between vitrification and warming **[1]**, the survival rate of the oocytes was similar between patient groups, confirming the efficacy and safety of the oocyte vitrification and warming protocols **[2]**.

4.8.1. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize Days between vitrification and first warming row*
4.8.2. LAB MEDIA: Table 1 bottom of table *Video Editor: please emphasize Survival rate data row*

4.9. Moreover, the survival rate **[1]** was independent of the vitrification **[2]** and warming operators' experience **[3]**.

4.9.1. LAB MEDIA: Figure 4
4.9.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize Vitrification graph*
4.9.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize Warming graph*

Conclusion

5. Conclusion Interview Statements

5.1. **Laura Rienzi**: The most crucial steps that may affect the consistency of the results are the ones related with warming. Therefore, it is important to carefully control the volume and temperature of the thawing solution. **[1]**.

5.1.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1., 3.2.)

5.2. **Alberto Vaiarelli**: Ovarian tissue cryopreservation is the only strategy currently under investigation as an option for prepuberal patients. Although promising, this approach has important limitations which limited its application.

5.3. **[1]**.

5.3.1. LAB MEDIA: Named talent says the statement above in an interview-style shot, looking slightly off-camera