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TOPIC OF DISCUSSION

"Post OPU procedures like denudation and Oocyte quality"

CHAT DISCUSSIONS COMPILED BY

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SUMMARY OF SURVEY RESULTS

Discussion on post OPU procedures like denudation and Oocyte quality:

By Team iHera

Introduction:

The Definition of Cumulus Cells (CCs) and the Differentiation of Granulosa Cells (GCs):

Cumulus cells (CCs) are the somatic cells surrounding the oocyte and play a crucial role in the growth, meiotic maturation, ovulation, and fertilization of mammalian oocytes. CCs originate from undifferentiated granulosa cells (GCs) found in primordial to preantral follicles. In the ovary, GCs are the primary cell type that provides the physical support and microenvironment necessary for the developing oocyte.

During the preantral stage, GCs can be categorized into two populations: mural granulosa cells (MGCs) and cumulus cells (CCs). MGCs line the wall of the follicle, while CCs are directly associated with the oocyte. The corona radiata is the outermost layer of CCs surrounding the oocyte [1].

CCs form a pseudostratified communicating epithelium and connect to the oocyte through membrane extensions that surround the corona radiata. As follicular development progresses, the structure of the cumulus oophorus is formed by undifferentiated GCs within the antral follicle. MGCs remain on the follicle wall, while CCs are in direct contact with the oocyte [2].

Cumulus cells (CCs) in contact with the oocyte form the cumulus-oocyte complex (COC). This complex facilitates communication between the oocyte and CCs by directly influencing gene expression and protein synthesis. This interaction leads to the differentiation and expansion of CCs, as well as the maturation of the oocyte [3]. CCs connect to the cytoplasm of the oocyte and penetrate the zona pellucida (ZP) through gap junctions. As the follicle develops from an antral stage to a pre-ovulatory stage, CCs undergo proliferation, and the gap junctions are gradually released from the ooplasm as the oocyte undergoes meiotic maturation [4].

How CCs impact oocyte development and protect the oocyte from harmful systemic diseases is crucial for the management of infertility:

Damage of the CCs under various pathogeneses can reduce the rates of fertilization and the chance of pregnancy. Current research has been focused on invasive techniques and involves the analysis of the somatic cells surrounding the oocyte, CCs, to determine the important factors of CCs that can predict the oocyte quality for high rates of maturation, fertilization, embryo development, and pregnancy [5].

Previous studies on transcriptomes in human CCs reported that, in 611 differentially expressed genes in CCs from early and non-early cleavage embryos, 24% were overexpressed in the early cleavage in CCs. These genes were involved in several signaling pathways including cell cycle, survival and death signaling, chemokine and cytokine signaling, angiogenesis, and lipid metabolism [6].

POLL RESULT:
After Oocyte Pick-Up (OPU), do you prefer to denude the oocytes and incubate them, or keep them with cumulus for a while before denudation?

Preference	Number of Votes	Percentage (%)
Keep with cumulus before denudation	274	91.3
Denude and incubate	3	1.0
Both methods tried, prefer keeping with cumulus	12	4.0
Both methods tried, prefer immediate denudation	11	3.7
Total	**300**	**100**

Discussion:

Poll results reveal a strong preference among embryologists for retaining cumulus cells on oocytes after Oocyte Pick-Up (OPU) before denudation.

Detailed Breakdown

1. Categories:

The graph categorizes responses into four groups based on preferences for oocyte handling:

- Keep with cumulus before denudation.
- Denude and incubate.
- Both methods tried, prefer keeping with cumulus.
- Both methods tried, prefer immediate denudation.

2. Votes and Percentages:

The poll involved 300 respondents, providing a comprehensive dataset for analysis

1. Keep with cumulus before denudation:

- Votes: 274
- Percentage: 91.3%
- The overwhelmingly positive response indicates a strong belief in the benefits of maintaining cumulus cells for oocyte quality.

2. Denude and incubate:

- Votes: 3
- Percentage: 1.0%
- The low percentage highlights a clear disfavor for this approach.

3. Both methods tried, prefer keeping with cumulus:

- Votes: 12
- Percentage: 4.0%
- This suggests that even among those familiar with both techniques, the preference for retaining cumulus cells remains strong.

4. Both methods tried, prefer immediate denudation:

- Votes: 11
- Percentage: 3.7%
- A small proportion of respondents favor immediate denudation, reinforcing the overall preference for the cumulus method.

Key Insights:

The findings reveal that the vast majority of embryologists (91.3%) favor keeping oocytes with cumulus cells before denudation, emphasizing the perceived importance of cumulus cells in supporting oocyte maturation and quality. This preference underscores the significance of strategies that enhance oocyte viability, critical for successful fertilization and embryo development. Conversely, the minimal support for immediate denudation reflects a widespread consensus against this method, aligning most professionals with the practice of maintaining cumulus cells.

DISCUSSION SUMMARY:

By Nibhash Kumar

In the embryo chat discussion group, a question was raised that after oocyte pickup (OPU), do you prefer to denude the oocytes immediately and incubate them, or keep them with cumulus cells for a while before denudation?

Why Not Denude and Then Incubate?

In oocyte handling, two main approaches are considered for denudation:

1. **Immediate Denudation**: The cumulus cells are removed promptly after oocyte retrieval.
2. **Late Denudation**: The cumulus cells are left intact for some time before being removed.

Some embryologists suggest that there are no significant differences in outcomes between these methods. However, it is important to consider the biological role of cumulus cells. Before fertilization, oocytes rely on cumulus cells for nutrient acquisition. After fertilization, oocytes can independently utilize these nutrients.

Denuded oocytes, however, are more vulnerable to fluctuations in pH levels due to the absence of the protective regulatory functions provided by cumulus cells. This underscores the importance of maintaining consistent protocols.

HYLURONIDASE Solution:

Some of the available hyaluronidase solution for chemical denudation include SynVitro™ Hyadase HYASE-10X, and V-HYADASE. These options differ in preparation and formulation:

- HYASE-10X: A ready-to-use solution designed for denudation.
- SynVitro™ Hyadase HYASE-10X: A concentrated product that requires dilution prior to use.
- V-HYADASE: A ready-to-use solution containing 80 U/mL of hyaluronidase.

Each product delivers the optimal hyaluronidase concentration for effective chemical denudation, offering slight formulation variations to cater to the specific requirements of ART laboratories.

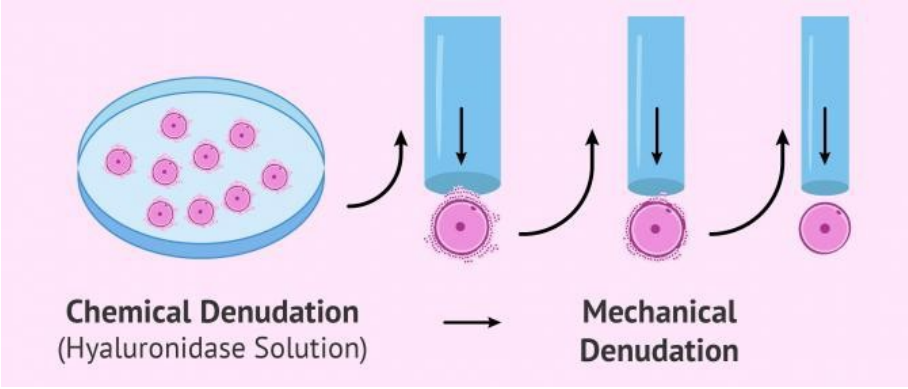

Figure1: process of Denudation (7)

Oocyte Denudation Procedure and Its Importance in ICSI

Oocyte denudation, the process of removing cumulus cells from oocytes, is essential for intracytoplasmic sperm injection (ICSI). This procedure ensures clear visualization of oocyte maturity and proper handling during ICSI. Denudation can be classified into two main types:

1. Chemical Denudation

This method involves immersing oocytes in hyaluronidase, an enzyme that breaks down hyaluronic acid, which holds cumulus cells together. The oocyte is exposed to hyaluronidase for less than 1 minute to ensure effective denudation without causing damage.

2. Mechanical Denudation

Here, pipetting is used to physically strip the cumulus cells. The process begins with a pipette of 275 µm and progresses to smaller sizes—175/170 µm and eventually 135/140 µm. For partial denudation, a 150 µm pipette can be used to minimize mechanical stress while removing the cells.

Step-by-Step Denudation Procedure

The denudation dish can be set by preparing drops of hyaluronidase solution as per the protocol and the enzyme should be warmed at 37 C before the oocytes are exposed to it.

1. **Prepare the Enzyme Solution:**
   - Mix hyaluronidase to the appropriate concentration for oocyte treatment.
2. **Chemical Denudation:**
   - Immerse the oocyte in hyaluronidase solution for less than 1 minute, ensuring even exposure.
   - Monitor closely to confirm detachment of cumulus cells.
   - Remove the oocyte promptly from the enzyme solution after the digestion period.
3. **Mechanical Denudation:**
   - Begin with a pipette tip size of 175 µm to gently aspirate the oocyte.
   - Progressively use pipettes of 150 µm and 135 µm to dislodge remaining cumulus cells through gentle squeezing.
4. **Final Observation:**
   - Confirm sufficient removal of cumulus cells under a microscope.
5. **Documentation:**
   - Record observations about oocyte morphology and the effectiveness of denudation.

The Role of Incubation Before ICSI

Post-OPU incubation is critical, typically lasting about 3 hours. This period allows the oocyte's cytoplasm to achieve maturity, complementing its nuclear maturity, both of which are essential for successful fertilization and embryo development.

Timing of Denudation and ICSI

Denudation should occur 30 minutes to 1 hour before ICSI, starting immediately after retrieval. Immersing oocytes in a prepared hyaluronidase solution and agitating the dish ensures uniform exposure to the enzyme.

Optimal Timing for OPU and ICSI

Denudation and ICSI should be done within 36-40 hours of trigger.

OOCYTE MORPHOLOGY

A mature M2 oocyte is a spherical cell characterized by the presence of a well-formed polar body. moderately granular cytoplasm and diameter ranging from 110 to 120 microns. It is surrounded by a multilayered, fluffy Cumulus (COCs), identifiable by the distinct radiating arrangement of corona cells, indicating maturity. The perivitelline space (PVS) appears normal. The zona pellucida (ZP), which is clear and colorless, has a thickness of approximately 20 microns. The single polar body represents less than 5% of the total cell volume, with one-third of the egg in contact with the inner membrane of the ZP and two-thirds floating in the PVS.

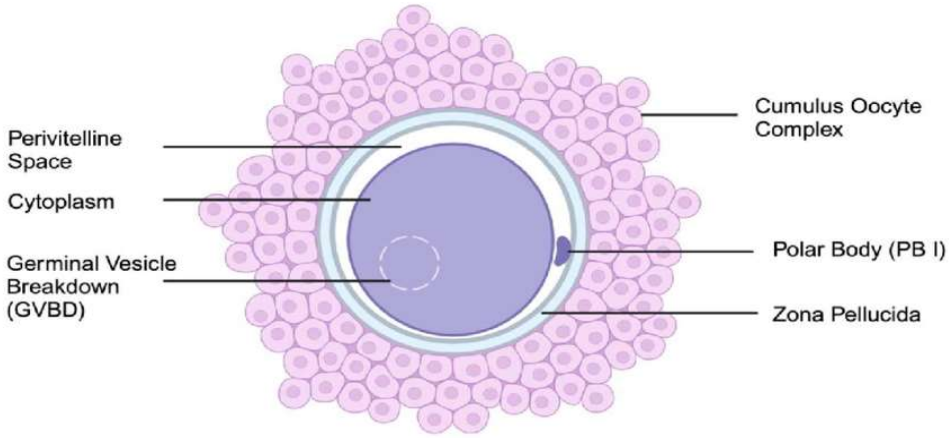

Figure 2: Morphology of Oocyte (8)

Discussion on Oocyte Morphology Assessment and Quality

The discussion shifted to the evaluation of oocyte morphology, maturity, and the factors influencing oocyte quality. Key questions and insights discussed are summarized below:

1. Is Observing a Polar Body Sufficient to Confirm MII Maturity?

No, the presence of a polar body does not unequivocally confirm MII maturity. While the polar body indicates nuclear maturity, cytoplasmic maturity plays a critical role in oocyte quality and developmental potential. Some oocytes with an expelled polar body may fertilize to form 2PN but exhibit poor cleavage or developmental competence, emphasizing the need to consider both nuclear and cytoplasmic maturity.

2. Is There a Reliable Way to Assess Cytoplasmic Maturity?

Currently, there is no definitive method to assess cytoplasmic maturity. Unlike nuclear maturity, which can be identified by the extrusion of the first polar body, cytoplasmic maturity lacks clear markers.

- Observational indicators like the appearance of the polar body or cytoplasmic characteristics during ICSI offer partial guidance.
- Research into cytoplasmic markers, may improve our ability to assess this aspect in the future.

3. How Do Cytoplasmic Characteristics Impact Oocyte Quality?

Cytoplasmic traits, including texture and homogeneity, provide insights into oocyte health:

- Watery or dense cytoplasm often correlates with poor oocyte quality or immaturity.
- Hyper-mature oocytes may exhibit fragile cytoplasm, which can negatively affect fertilization and developmental outcomes.
- These observations, while valuable, are largely based on empirical experience and lack robust scientific evidence.

4. What Role Does Cytoplasmic Volume Play in Degeneration and Maturity?

Cytoplasmic volume influences the likelihood of oocyte degeneration, but its relationship with maturity is multifaceted:

- Adequate cytoplasmic volume supports physiological maturity, ensuring sufficient organelles, such as mitochondria, are present.
- Research into cytoplasmic organelle quality may inform methods to optimize cytoplasmic maturity and reduce degeneration.

5.Why Does Post-ICSI Degeneration Occur, and What Contributes to It?

Post-ICSI degeneration arises from multiple factors, including:

- Technical Errors: Mistakes during denudation or ICSI, such as over-manipulation or excessive force, can damage the oocyte.
- Morphological Defects:
  - The presence of vacuoles or granulation in the cytoplasm can compromise developmental outcomes.
  - Wide perivitelline space (PVS) or low cytoplasmic volume is linked to increased degeneration rates.
- Cytoplasmic Immaturity:
  - Common in polycystic ovary (PCO) patients due to high oocyte retrieval numbers, which can compromise cytoplasmic quality.
  - Immature cytoplasm may not support the necessary physiological processes for successful fertilization and development.

Conclusion

This discussion highlighted the critical importance of meticulous monitoring and technique during oocyte handling, denudation, and ICSI. The interplay between nuclear and cytoplasmic maturity is fundamental to optimizing outcomes, and future research into the role of cytoplasmic organelles, particularly mitochondria, could lead to more reliable assessment methods and improved ART success rates.

The report also revealed a strong consensus among embryologists regarding the handling of oocytes post-Oocyte Pick-Up (OPU). A significant 91.3% of respondents prefer to maintain oocytes with their cumulus cells before denudation, emphasizing the potential benefits for oocyte maturation and quality.

Cumulus cells (CCs) are vital for oocyte health and meiotic maturation, underscoring their role in preserving the oocyte's microenvironment and enhancing fertilization outcomes. While a minority supports immediate denudation, the prevailing preference for retaining cumulus cells reflects a broader inclination toward leveraging natural biological associations in assisted reproductive technologies.

Overall, these findings reinforce the importance of cumulus cells in promoting oocyte quality. They also highlight the need for further research into their interactions, offering promising avenues for advancing practices in reproductive medicine.

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