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RNA Isolation from Mouse Oocytes at The Metaphase II (MII) Stage

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Abstract

RNA extraction from oocytes is a fundamental step in various fields of biological research, including developmental biology, reproductive biology, and transcriptomics. The unique molecular characteristics of oocytes make RNA analysis from these cells essential for understanding early developmental processes, reproductive health, evolutionary biology, and disease mechanisms. Below, we highlight the key areas where RNA extraction from oocytes plays a pivotal role, along with the challenges and advancements in this critical procedure.

Keywords: RNA extraction, Mouse oocytes, metaphase II

Background

1. Understanding Early Developmental Processes

Oocytes contain a diverse array of maternal RNAs that play essential roles in early embryonic development, particularly before the activation of the zygotic genome (1). These transcripts regulate processes such as cell division, differentiation, and the establishment of embryonic viability. Extracting and analyzing RNA from oocytes allows researchers to identify genes critical for early development and uncover the molecular pathways governing oocyte maturation. This analysis provides insights into how immature oocytes transition into fertilizable eggs, which is crucial for understanding reproductive success (1).

2. Reproductive Health and Fertility Studies

The quality of oocytes is a key determinant of fertility, and RNA analysis can reveal gene expression patterns associated with oocyte viability and developmental potential (2). These insights are essential for improving assisted reproductive technologies (ARTs), including in vitro fertilisation (IVF). By studying RNA extracted from oocytes, researchers can assess how environmental factors—such as diet, stress, and exposure to toxins—impact oocyte health and fertility (2). This information can help optimize ART protocols and improve fertility outcomes.

3. Comparative Genomics and Evolutionary Studies

RNA extraction from oocytes enables comparativestudies across different species, shedding light on the evolutionary conservation and divergence of genes involved in reproduction (3). Comparative analysis of RNA profiles can identify conserved regulatory pathways and reveal unique species-specific transcripts that influence reproductive strategies and mechanisms.

Such studies provide a deeper understanding of how reproductive systems have evolved and adapted in different organisms (3).

4. Epigenetics and Non-Coding RNA Studies

Oocytes are rich in non-coding RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs), which play critical roles in regulating gene expression during gametogenesis and early embryogenesis (4). Extracting RNA from oocytes enables the study of these non-coding RNA species and their impact on gene regulation, transcript stability, and chromatin structure. Additionally, RNA extraction facilitates the study of epigenetic marks and their influence on oocyte development and early embryonic processes (4).

5. Disease Modeling and Therapeutics

RNA extracted from oocytes can reveal aberrant gene expression patterns and mutations associated with infertility and developmental disorders (5).

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This information is vital for understanding the molecular basis of reproductive disorders, such as polycystic ovary syndrome (PCOS) and premature ovarian failure. RNA analysis can also help identify potential therapeutic targets and guide the development of treatments to improve oocyte quality and reproductive outcomes (6).

Challenges in RNA Extraction from Oocytes

Extracting high-quality RNA from oocytes, particularly at the metaphase II (MII) stage, presents several significant challenges due to the unique biological characteristics of oocytes and the limitations of current RNA extraction methods (7).

Oocytes contain relatively low amounts of RNA, making it difficult to obtain sufficient quantities for downstream applications such as reverse transcription-polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR) (8). Standard commercial RNA extraction kits often yield insufficient RNA for these applications, compromising the accuracy and reliability of gene expression analysis. The integrity of the extracted RNA is crucial for accurate results, and RNA degradation during extraction remains a critical challenge (9).

MII oocytes are particularly sensitive to temperature fluctuations and the cryoprotective agents used during vitrification and thawing procedures. These factors can negatively affect spindle integrity and overall cellular function, ultimately compromising RNA quality (10). Additionally, the maturation process itself alters mRNA profiles, adding further complexity to the RNA extraction process (9).

Due to the low RNA yield from individual oocytes, researchers often resort to pooling multiple oocytes—sometimes up to 300—for RNA extraction (8). While this approach increases the RNA yield, it introduces variability and potential biases in the data, as pooled samples may not accurately represent the transcriptomic profile of a single oocyte. This limitation is a significant concern in studies requiring high-resolution gene expression analysis.

Current methods for RNA extraction often focus on polyadenylated (polyA+) RNAs, which may overlook other important RNA species present in oocytes(7). Furthermore, traditional lysis techniques can result in significant loss of RNA due to the presence of cellular debris and lipids that complicate the extraction process. while extracting high-quality RNA from mouse oocytes at the MII phase remains a complex task fraught with challenges, ongoing research into improved methodologies continues to enhance our ability to study gene expression in these critical cells.

In this study we modified RNA manual extraction using RNA extraction solution like TRIzol reagent or RNX-Plus (CinnaGen Co.Iran) from mouse oocytes at the metaphase II (MII) stage.

Description of protocol

Materials and reagents

- Oocytes (fresh or frozen)
- RNA extraction Solutions (e.g., TRIzol reagent or RNX-Plus (CinnaGen Co.Iran)
- Sterile RNase-free water
- RNase-free pipette tips and tubes
- Chloroform (CAS 67-66-3/ 102445)
- Isopropanol (CAS 67-63-0 /109634)
- Ethanol (70%, RNase-free)
- Centrifuge with cooling capabilities
- Phase lock gel tubes (optional)
- Dry ice (if working with frozen oocytes)
- Heating block set at 55°C (optional for aiding dissolution)
- RNA storage buffer (e.g., RNAlater or TE buffer or DEPC Water)

Procedure

1. Sample Preparation

- At least 10 oocytes are needed to achieve optimal RNA yield.
- Thaw frozen oocytes on ice, or collect fresh oocytes in an RNase-free tube.
- Count the oocytes and transfer them to a clean, RNase-free microcentrifuge tube.
- Remove as much surrounding medium as possible using an RNase-free pipette.

2. Lysis

- Add 500 µL of TRIzol reagent or the RNX-Plus to the oocytes.
- Homogenize the oocytes using a RNase-free pestle or by repeatedly pipetting with a fine-tip pipette until no visible debris remains.

3. Phase Separation

- Add 0.1 mL of chloroform to the samples. (The ratio of chloroform should be 1:5 of the RNA extraction solutions)
- Shake the tube vigorously for 15 seconds, then incubate on ice for 2–3 minutes.

- Centrifuge at 12,000 rpm for 15 minutes at 4°C.
- Transfer the upper aqueous phase (contains RNA) to a new RNase-free tube without disturbing the interphase.

4. RNA Precipitation

- Add an equal volume of isopropanol to the aqueous phase.
- Mix gently by inversion and incubate at -20°C for at least 45 minutes.
- Centrifuge at 12,000 × g for 15 minutes at 4°C to pellet the RNA.

5. Washing the RNA Pellet

- Remove the supernatant carefully and wash the RNA pellet with 1 mL of 75% ethanol.
- Centrifuge at 7,500 × g for 8 minutes at 4°C.
- Discard the ethanol and air-dry the pellet for 5–10 minutes (do not over-dry).

6. Resuspension

- Re-suspend the RNA pellet in 35–50 µL of RNase-free water or an RNA storage buffer or DEPC-treated water.
- If the pellet is difficult to dissolve, incubate at 55°C for 10 minutes.

7. RNA Quality and Quantity Check

- Measure RNA concentration using a spectrophotometer (e.g., NanoDrop) or fluorometer.
- Assess RNA integrity using agarose gel electrophoresis or a bioanalyzer.

8. StorageStore the RNA at -80°C for long-term use.

Notes and Tips

- Always work in an RNase-free environment to prevent RNA degradation.
- Use gloves and RNase inhibitor reagents when necessary.
- If dealing with low RNA yields, consider pooling multiple oocytes or using carrier RNA during the precipitation step.
- For small RNA species, ensure the protocol and kit support their extraction.

Limitations

If the oocytes are not collected properly and the cold chain is not maintained from the time of sample collection to the time of transfer to the -80 freezer, the quantity and quality of extracted RNA will be significantly reduced.

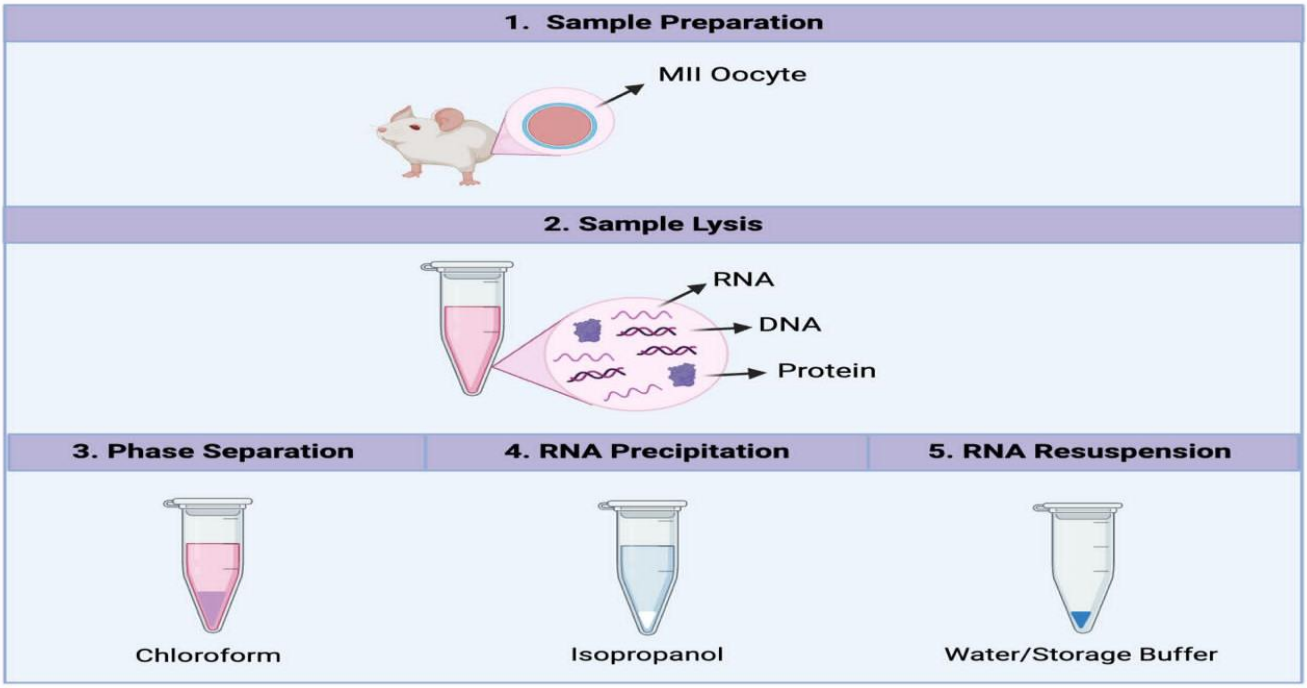

Figure 1. The steps of RNA Isolation from Mouse Oocytes at The Metaphase II (MII) Stage

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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