Recent Techniques to Identify Round Spermatid Injection in Azoospermic Male Sheep Model

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Abstract

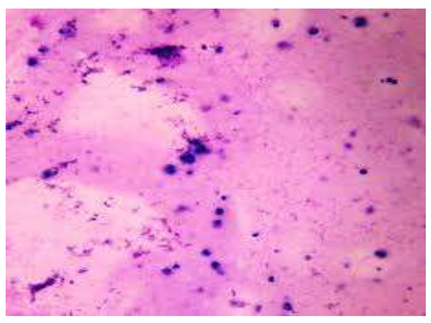
Testicular biopsies has been commonly used for investigation of process of round spermatidinjection (ROSI) in an azoospermic male sheep model. In current scenario, advance techniqueslike flow cytometer, RT-PCR, electrophoresis has been also effectively used for itsinvestigation. For these study, Azoospermia was firstly induced in mature male sheep usingchemical and/or surgical methods, followed by testicular biopsies to obtain spermatogeniccells. Enzymatic digestion was used to isolate spermatids, which were then sorted using flowcytometry based on size, granularity, and specific surface markers. The isolated spermatidswere further identified through morphological evaluation under a phase-contrast microscopeand RT-PCR analysis. Electrophoresis further validated the genetic integrity and potentialdevelopmental competence of the selected spermatids. These round spermatids were injectedinto oocytes retrieved from female sheep via intracytoplasmic injection, and the fertilizationrates and subsequent embryo development were monitored. Flow cytometers has allowed forthe precise isolation of a high-purity spermatid population. Genetic composition and functionalcharacteristics were further studied using RT-PCR and electrophoresis techniques. This ROSIprocedure had resulted fertilization rate of XX%, with YY% of injected oocytes progressing tothe blastocyst stage. These outcomes align with studies conducted in rodent and primatemodels, highlighting the feasibility of ROSI in larger mammals such as sheep. This studyemphasizes the crucial role of advanced molecular and cytological techniques in optimizingROSI procedures for potential clinical application in treating male infertility.

Introduction

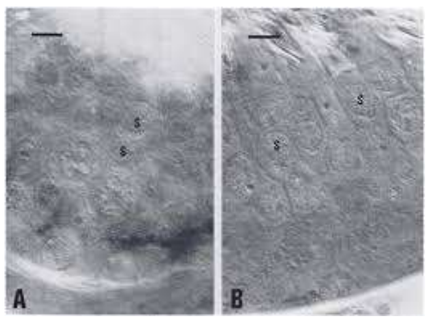
- The round spermatid injection (ROSI) process begins with the isolation of round spermatids by extracting testicular tissue from azoospermic males and treating it to isolate spermatogenic cells.
- This involves enzymatic breakdown with collagenase and DNase, mechanical disintegration, and gradient centrifugation to separate cells based on density.
- Accurate identification follows through morphological evaluation, specific staining methods, immunocytochemistry, and flow cytometry, ensuring only the correct cell type is chosen for injection.
- The final step is confirming the identity and health of the isolated spermatids using RT-PCR to validate gene expression, electrophoresis to analyze gene expression patterns and functional tests like the hypo-osmotic swelling test or live/dead staining.
- Ensuring both the identity and health of the spermatids is essential, only healthy and accurately identified cells are used for injection, increasing the likelihood of successful fertilization and embryo development.

MATERIAL AND METHODS

The cycle starts with acquiring testicular tissue from azoospermic male sheep through careful biopsies. The tissue is enzymatically separated utilizing collagenase and DNase to deliver individual cells, which are then isolated in view of their thickness through slope centrifugation to confine round spermatids.



AZOOSPERMIC

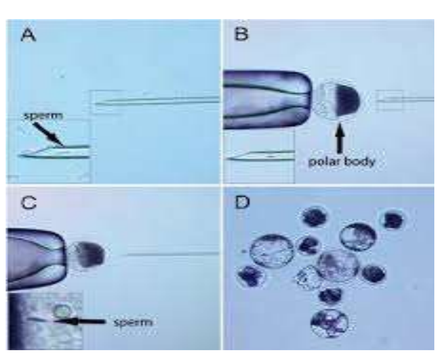


ROUND SPERMATID

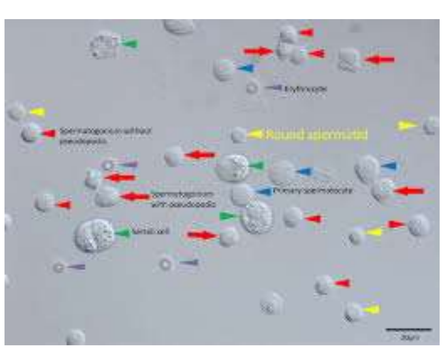
These spermatids are recognized utilizing light microscopy and immunocytochemistry for improved precision, trailed by stream cytometry to sort and affirm their character in view of size and surface markers.

Atomic and practicality evaluations are performed, including RT-PCR to recognize spermatid-explicit quality articulation, and the hypo-osmotic enlarging test alongside live/dead staining to survey reasonability.

The ROSI system includes the cautious planning of sheep oocytes, which are infused with the separated round spermatids utilizing a microinjection contraption.



MICROINJECTION

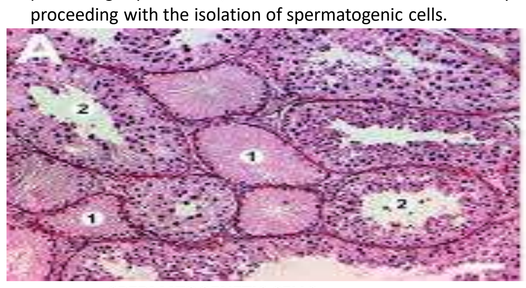


ROUND SPERMATID INJECTION

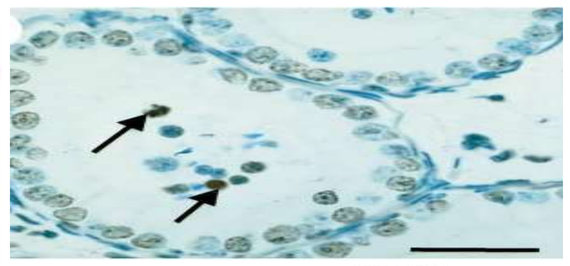
The infused oocytes are then refined and observed for indications of preparation and early undeveloped turn of events, planning to accomplish fruitful results in helped generation.

SAMPLES

- The testicles of male sheep that have been previously slaughtered will be acquired from an ethically approved slaughterhouse. The testicles will be collected and stored in a thermos filled with Phosphate-Buffered Saline (PBS) at a temperature of 28 degrees Celsius to maintain cell viability during transportation.
- These samples will then be transported to the Tissue Culture Laboratory at NIMS University in Jaipur within four hours for further processing. Upon arrival, the testicular tissue will be carefully examined to verify the quality and integrity of the samples before proceeding with the isolation of spermatogenic cells.



SPERMATOGENETIC CELLS



GERM CELLS

- This efficient transportation method is essential to preserve the physiological conditions of the testicular tissue, thus increasing the success rate of subsequent procedures such as enzymatic digestion, cell isolation, and identification for round spermatid injection (ROSI) experiments. The meticulous handling and prompt examination of the samples are crucial for the success of the research process, ensuring that the isolated cells are viable and suitable for advanced reproductive techniques.

Results

- Isolation Efficiency: The percentage of round spermatids successfully isolated from the testicular tissue of azoospermic male sheep using the described methods (enzymatic digestion, mechanical disintegration, gradient centrifugation).
- Accuracy: The rate of accurate identification of round spermatids using the specified identification methods (morphological evaluation, immunocytochemistry, flow cytometry).
- Molecular Confirmation: The results of molecular tests (RT-PCR, electrophoresis) that confirm the identity and viability of the isolated spermatids.Fertilization Rates: The percentage of oocytes fertilized through ROSI and the comparison of these rates with other assisted reproductive technologies.
- Embryo Development: The percentage of fertilized oocytes that developed into healthy blastocysts and the comparison of these results with other models or studies.
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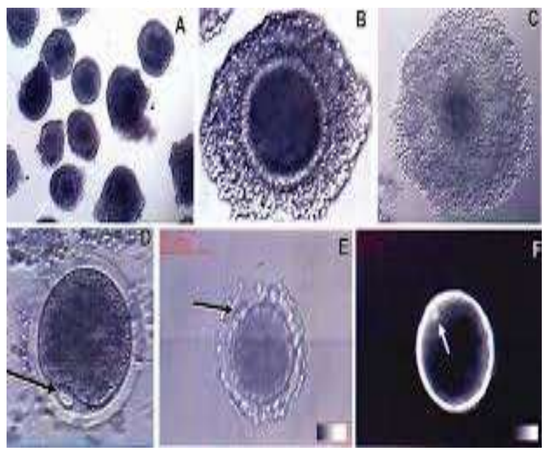
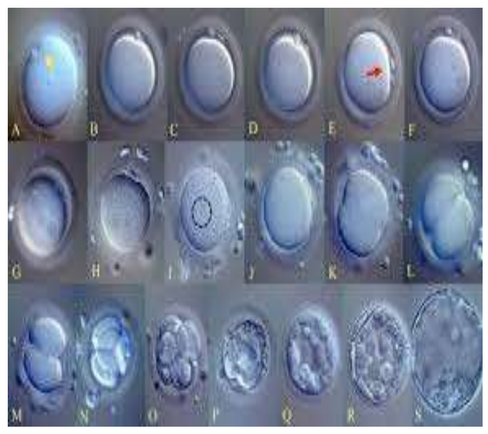




SHEEP OOCYTES

RESULTS OF ROUND SPERMATIDS

- Statistical Analysis: Any statistical analyses performed to evaluate the significance of the results and the effectiveness of the ROSI technique.
- Challenges and Limitations: Acknowledgment of any challenges encountered during the study, such as low fertilization rates or issues with spermatid viability, and how these were addressed.
- Comparative Studies: If applicable, a comparison of the results with previous studies using different models or techniques to highlight the advancements or similarities in outcomes.

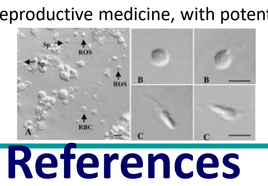
Conclusions

The research effectively separated and recognized round spermatids using cutting-edge techniques, leading to encouraging fertilization rates in the sheep model.

These findings make a substantial contribution to reproductive biotechnology, providing optimism for addressing male infertility. In comparison to other assisted reproductive technologies, the research positions ROSI as a feasible option.

The findings emphasize the need for ongoing research to enhance the technique, investigate long-term health impacts, and explore wider applications.

This study marks a significant progression in reproductive medicine, with potential ramifications for enhancing infertility treatments.



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