

ABSTRACT

After fertilization of an oocyte, the occurrence of calcium oscillations becomes imperative for successful egg activation. These oscillations in calcium levels are mediated by ion channels situated in the plasma membrane in all mammals. Among these ion channels, the Transient Receptor Potential Melastatin (TRPM) family, particularly TRPM7, holds significant interest for this study. The TRPM7 protein is bifunctional as it acts as both an ion channel and a serine/threonine kinase. In the context of the mouse model, a TRPM7 knockout leads to embryonic lethality. To investigate the effects of TRPM7 knockout in oocytes, conditional knockout mice are used. Research indicates that mice with conditional knockout of TRPM7 exhibit subfertility, proving the essential role of TRPM7 in mouse fertility. This study aims to discern the expression levels and localization of TRPM7 in bovine oocytes, cumulus cells, and sperm. This is important as understanding these calcium channels can help us figure out ways to improve ART in the cattle industry. My results showed that TRPM7 is indeed present in bovine cells; however, further research is needed to expand on this finding.

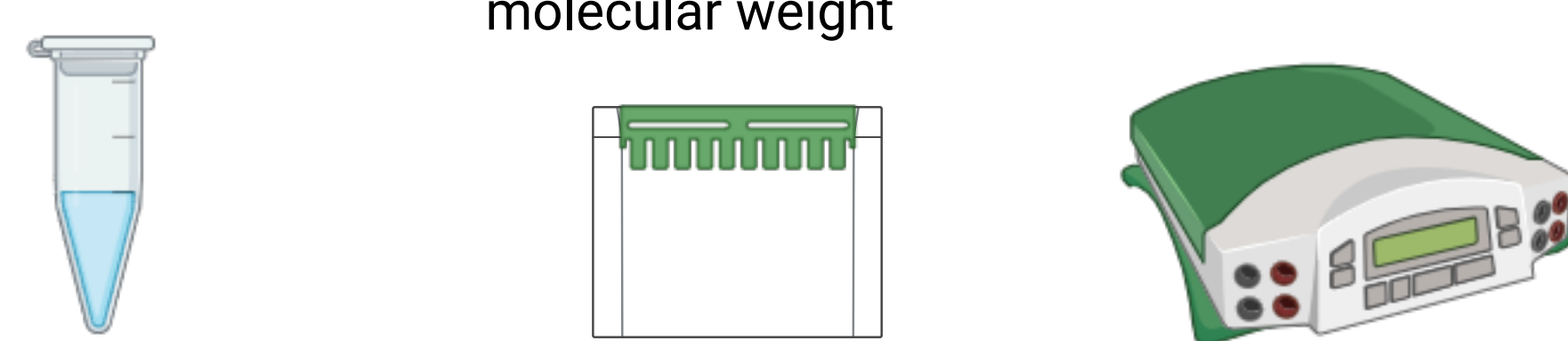
METHODS

- 1) Isolate RNA**
Isolates RNA from cell sample
- 2) Transform RNA to cDNA**
Reverse transcription transforms isolated RNA into cDNA
- 3) Analysis**
qPCR and mathematical analysis shows relative mRNA expression of target protein (TRPM7)

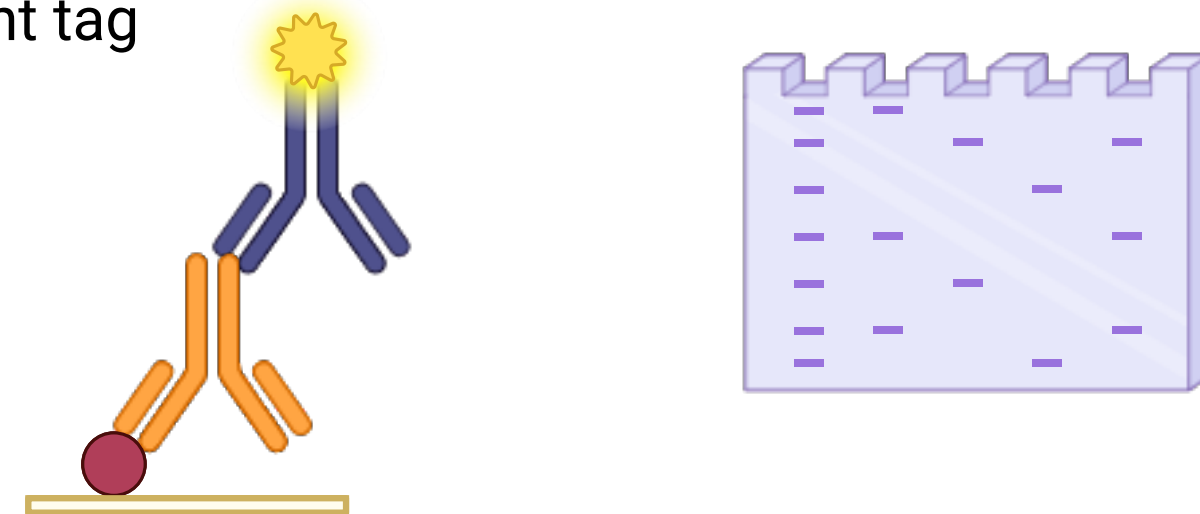


qPCR protocol was the same for all cell types.

- 1) Extraction of Protein**
separates protein from the cell sample
- 2) Protein separation by size**
arranges protein by molecular weight
- 3) Electrotransfer**
transfers protein from gel to membrane

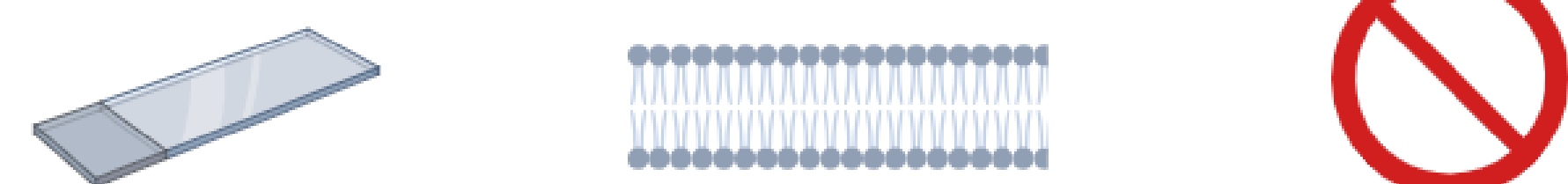


- 4) Primary and Secondary Antibody**
marks target protein with fluorescent tag
- 5) pr**

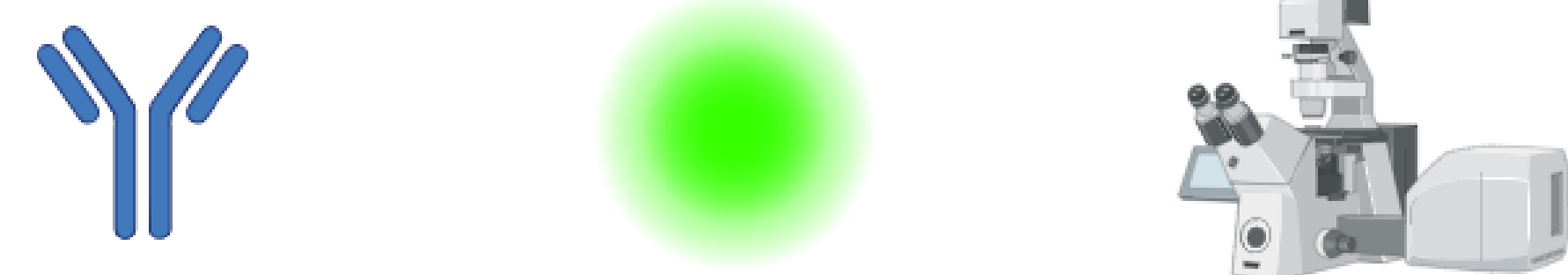


Western Blot extraction protocol was being analyzed.

- 1) Fixing Sample**
preserves the sample and maintains morphology
- 2) Permeabilization**
allows antibody access through the cell membrane
- 3) Blocking**
reduces non-specific binding of antibody

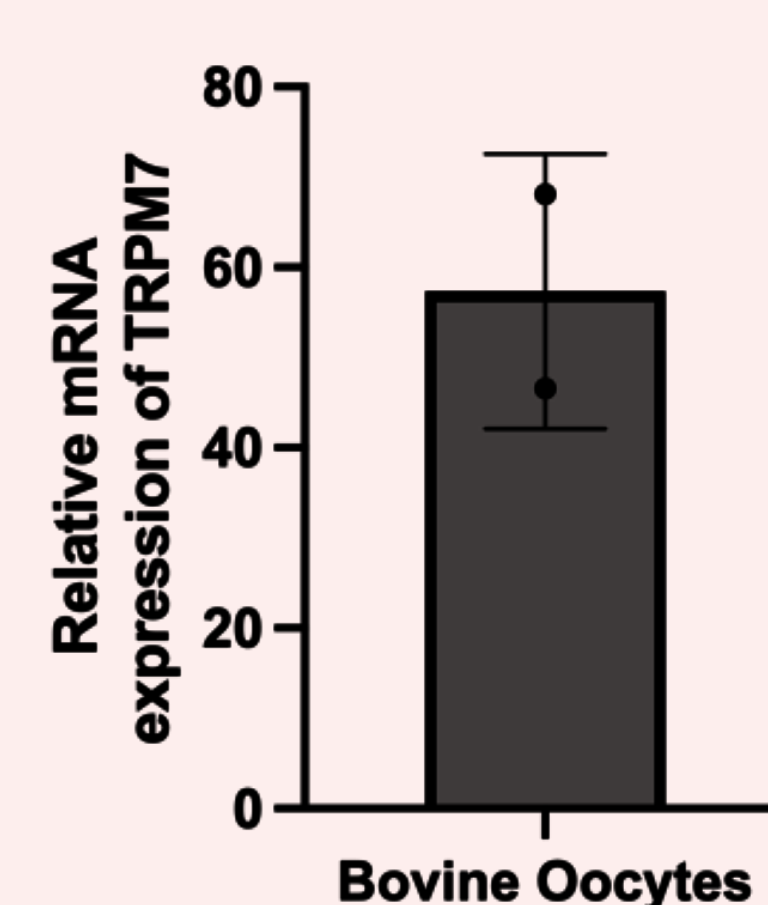


- 4) Primary Antibody**
binds to the target protein (TRPM7)
- 5) Secondary Antibody**
binds to the primary antibody; source of fluorescence
- 6) Confocal Microscopy**
check the localization of target protein (TRPM7), image processed using ImageJ

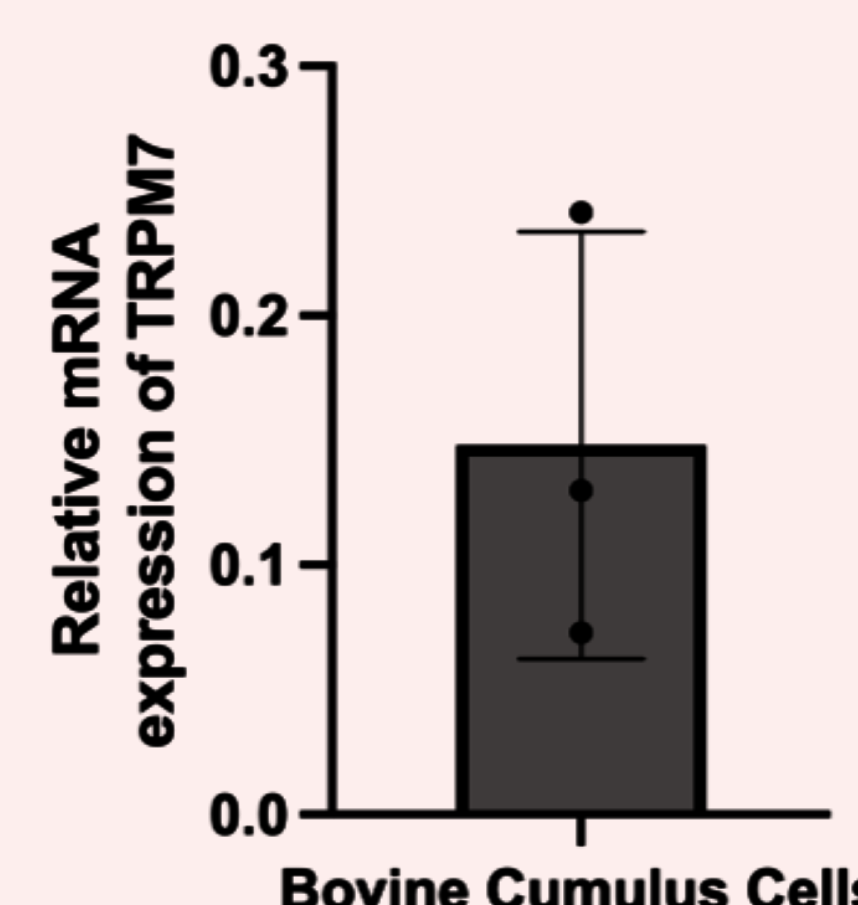


IF protocol was altered depending on

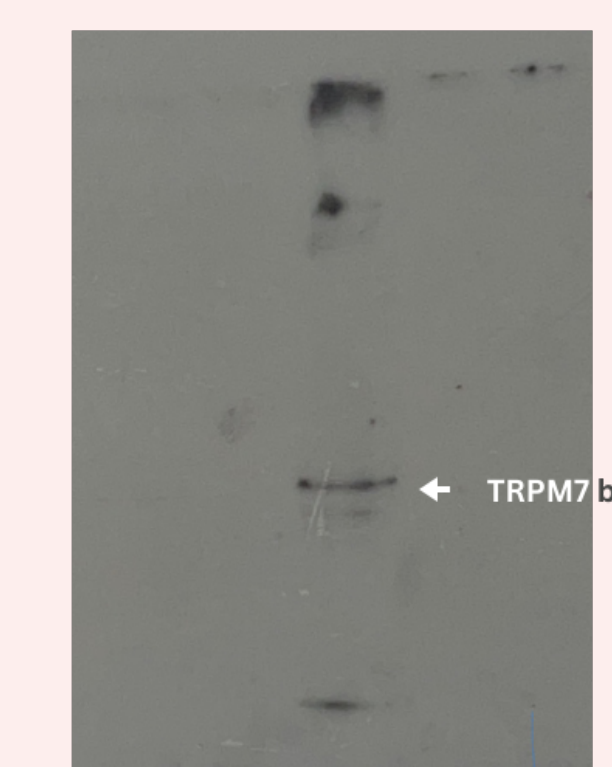
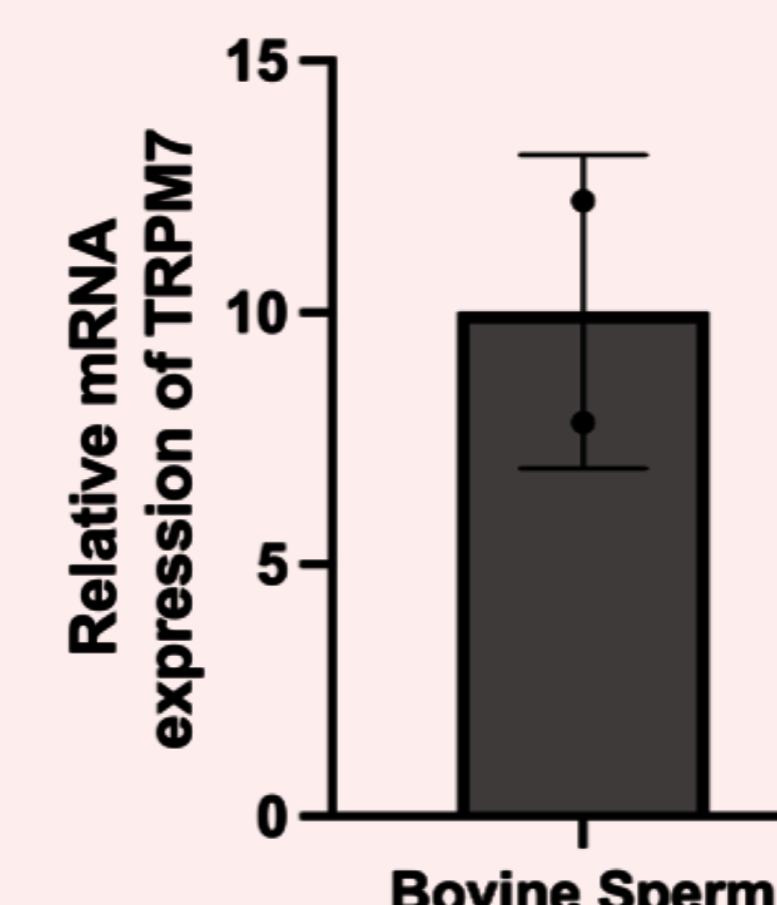
RESULTS



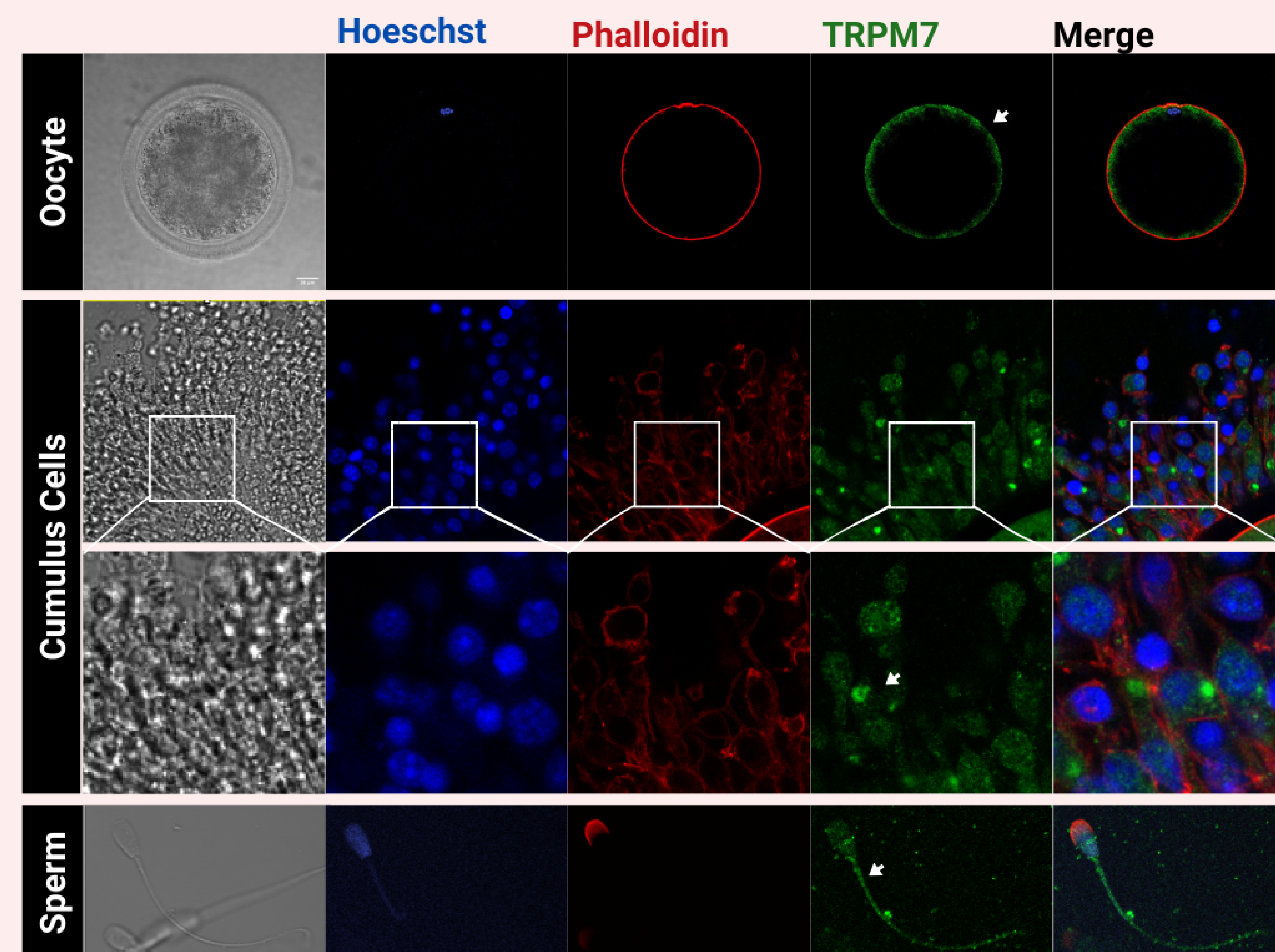
qPCR was used to obtain relative mRNA expression of TRPM7 in bovine oocytes. 2 biological replicates with 2 technical replicates each were performed.



qPCR was used to obtain relative mRNA expression of TRPM7 in bovine cumulus cells. 3 biological replicates with 2 technical replicates each were performed.



Western blot analysis of bovine cumulus cells



IF images of bovine oocyte, cumulus cells, and sperm. Localization of TRPM7 in oocyte is around the cell membrane. In cumulus cells, TRPM7 appears to be scattered. In sperm, TRPM7 appears to be mainly focused in the equatorial region of the head and the midpiece.

FUTURE DIRECTION

We attempted to perform a Western blot analysis on bovine sperm, oocytes, and cumulus cells but encountered difficulties in obtaining meaningful results. Several potential reasons may have contributed to our unsuccessful outcomes.

Firstly, bovine oocytes are known to possess a high lipid content, which could have posed challenges during the protein extraction process. The abundance of lipids in the oocytes may have hindered the efficient extraction of proteins, potentially leading to the lack of detectable bands on the Western blot.

Additionally, for the Western blot analysis of oocytes, we used approximately 40 oocytes in our samples. It is conceivable that using a larger number of oocytes may yield more reliable results. Increasing the sample size could help ensure that a sufficient amount of the target protein is present for detection.

Regarding the sperm Western blot analysis, we employed varying amounts of sperm—6 million, 10 million, and 15 million—but none of these samples exhibited bands corresponding to TRPM7 or alpha tubulin. This suggests that the issue may lie within the protein extraction process. Notably, we utilized cryopreserved sperm for our experiments, and it is plausible that using fresh sperm could yield better results. Fresh sperm may have a higher protein quality and integrity, making it more suitable for Western blot analysis.

Remarkably, the only cell type that produced detectable results in our Western blot analysis was the cumulus cells. This observation underscores the importance of considering cell-specific factors in the protein extraction process and Western blot protocol.

Another potential explanation for the absence of TRPM7 protein expression on the Western blot could be related to phosphorylation of the protein. When TRPM7 undergoes phosphorylation, it can become less accessible for binding by the primary antibody. To address this issue in future investigations, we propose considering the addition of a phosphatase enzyme during the protein extraction process. This enzymatic treatment could help dephosphorylate TRPM7 and facilitate the binding of the primary antibody, potentially leading to more reliable Western blot results.

In summary, for future research in this project, we plan to increase the sample size for all cell types, optimize the protein extraction process for oocytes, explore the use of fresh sperm, and consider the inclusion of a phosphatase enzyme to enhance the detection of TRPM7 in Western blot analyses. These adjustments should help us overcome the challenges we faced and improve the reliability of our results.

ACKNOWLEDGEMENTS

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