הכינוס השנתי ה-66 של
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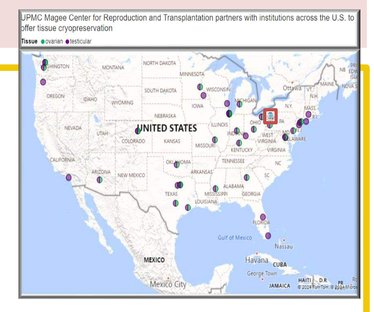
THE ISRAEL FERTILITY ASSOCIATION (IFA)
الجمعية الإسرائيلية لأبحاث الخصوبة

From the Operating Room to Cryopreservation: A Comparative Analysis of Different Media for Testicular Tissue Cryopreservation

Myriam Safrai (1,2), Amanda Colvin Zielen (), Meena Sukhwani (1), Livianna Knisely Myklebust (1,3), Kyle E. Orwig (1)

1- Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh,, USA
2- Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 52621, Israel
3- Molecular Genetics and Developmental Biology Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, USA



INTRODUCTION

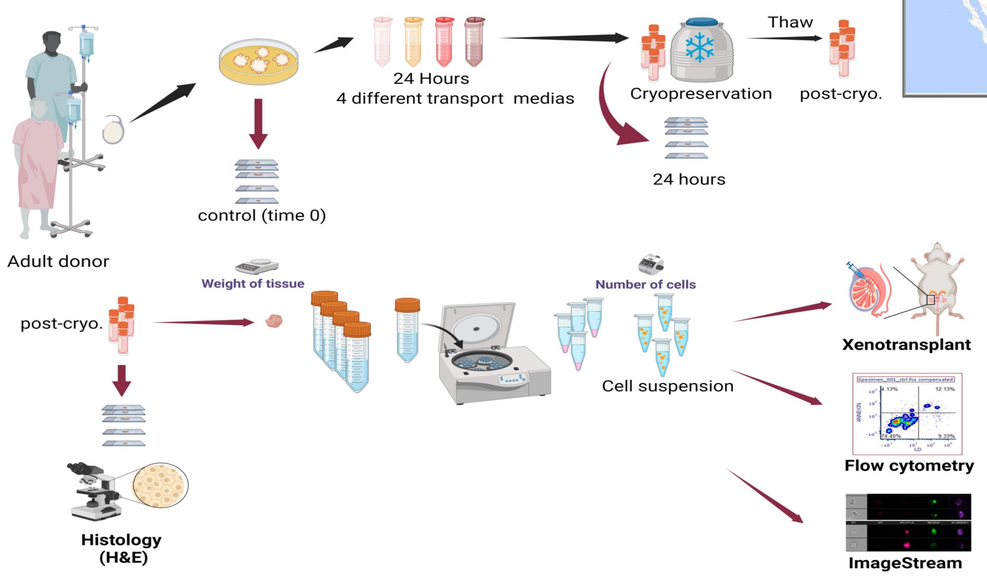
Testicular tissues are shipped for cryopreservation and storage, but limited data exist on optimal transport conditions.

Aim: To assess the impact of different transport media on the quality of testicular tissue and provide subsequent recommendations for the transport of testicular tissue.

Characteristics of testicular transport media

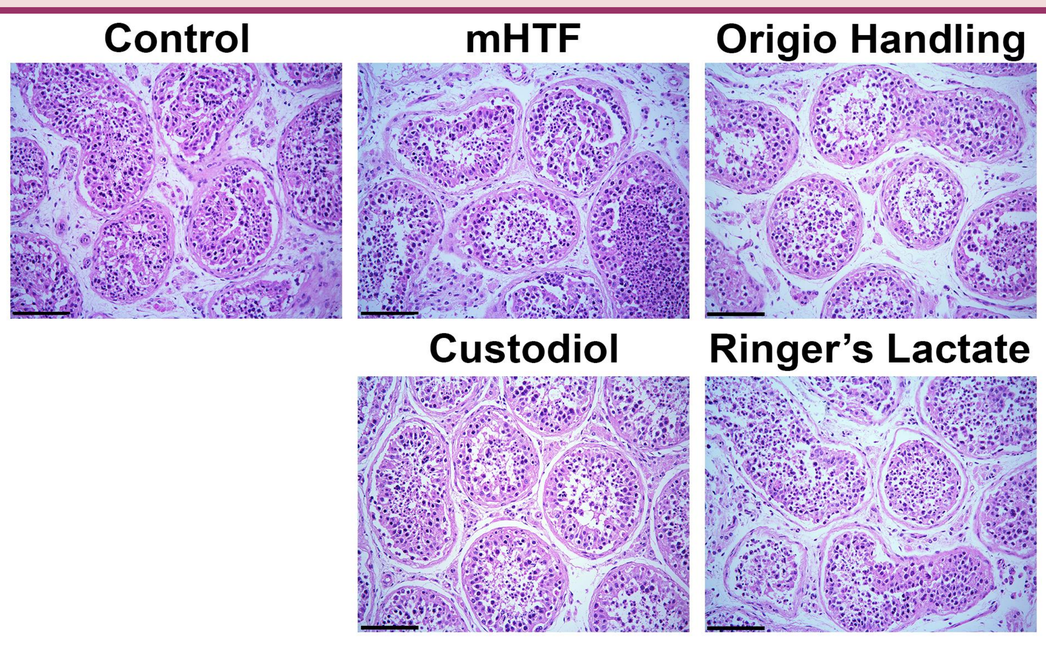
Medium	General information	Availability
mHTF	IVF medium	Commercially available $
Origio Handling	IVF medium	Commercially available $
Custodiol	Used for gonadal transport in Europe	Commercially available $$$ (in US)
Ringer's Lactate	Cheap	Already in every hospital

MATERIAL & METHODS



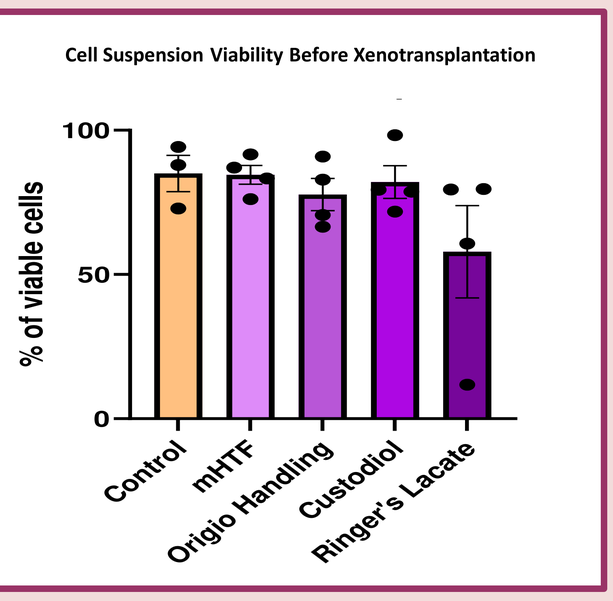
RESULTS

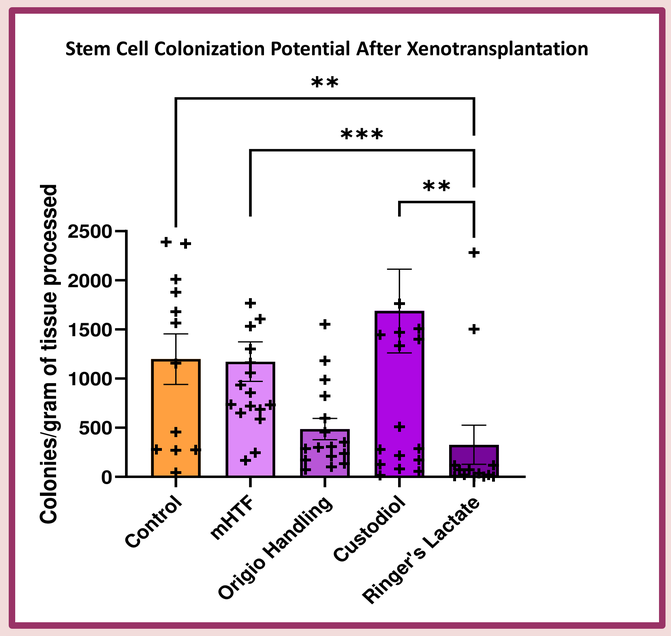
From testicular tissues processed on arrival- Time 0 (Control) and tissues incubated in transport medias (mHTF, Origio Handling, Custodiol, Ringer's lactate) for 24 hours. All analyses were done after cryopreservation and thawing of these conditions.



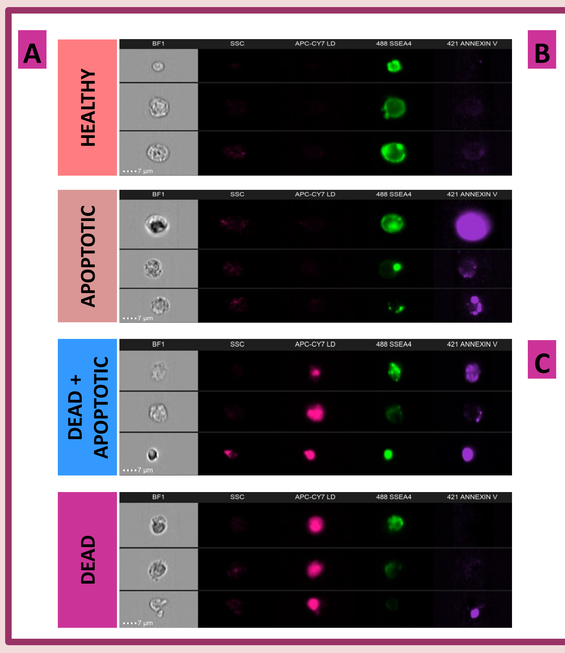
Histological Evaluation of Testicular Tissue: Samples were fixed in 4%PFA overnight and embedded in paraffin. Slides were cut and stained with haematoxylin and eosin (H&E). *Note: The testicular tissue incubated in Ringer's Lactate shows morphological impairments in the tubules. Scale bar, 100 µm.

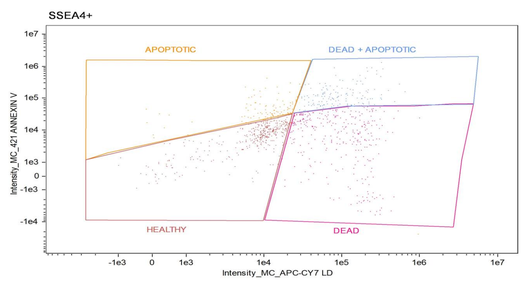
Viability of Testicular Cell Suspensions: Thawed tissue was digested with Collagenase IV, 0.25% Trypsin-EDTA and DNAse in preparation for xenotransplantation. Samples were stained 1:1 with trypan blue and live/dead cells were counted.

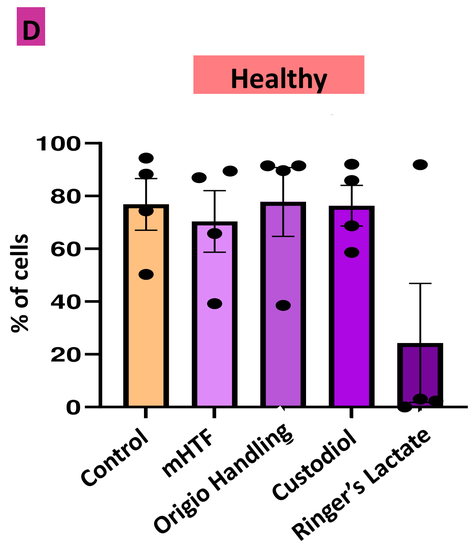


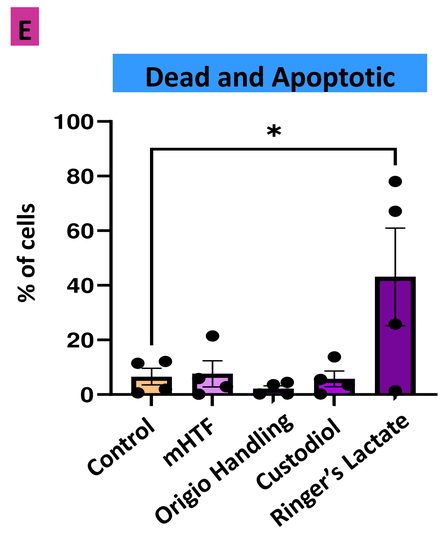


Stem cell colonization potential of testicular cell suspensions after xenotransplantation in mice: cell suspensions were transplanted into mice testes, removed after 2.5 months and analysed with human-specific antibodies









ImageStream and Flow Cytometry Analysis of Testicular Cell Suspensions: A- ImageStream cell differentiation using different markers: SSEA-4 (stem cells), Annexin V (apoptosis), and Ghost dye (cell death). Panels **B** and **C-** provide examples of quantifications via ImageStream and flow cytometry, respectively. Panels **D** and **E-** summarize the results of the flow cytometry analysis for four biological replicates. **D-** Percent of Healthy cells (negative for Annexin V and Ghost dye). **E-** Percent of Apoptotic and Dead cells (positive for Annexin V and Ghost dye).

CONCLUSION

- ✓ Enhanced viability of SSEA4+ spermatogonia and a higher yield of colonies were observed following a 24-hour incubation in mHTF and Custodiol media.
- ✓ mHTF and Custodiol are recommended as the preferred media for transporting testicular tissue prior to cryopreservation