

In vitro fertilization of mouse oocytes

CURRENT STATUS: POSTED

ADITYA SANKAR
University of Copenhagen

✉ adisankara2000@gmail.com *Corresponding Author*
ORCID: <https://orcid.org/0000-0002-1840-3356>

Javier Martin Gonzalez
University of Copenhagen
ORCID: <https://orcid.org/0000-0002-7075-6028>

DOI:

10.21203/rs.2.21562/v1

SUBJECT AREAS

Developmental biology *Genetics*

KEYWORDS

Embryology, IVF

Abstract

this protocol describes the procedure of *in vitro* fertilisation of mouse oocytes. The procedure is performed at the transgenic core facility by a specialist according to the protocols found here:

<https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols>

Introduction

This protocol refers to the MRC Harwell guideline procedure for performing *in vitro* fertilisation of mouse oocytes using fresh or frozen sperm. See attached supplementary documentation.

Reagents

See attached pdf file for detailed information on media

Equipment

See attached pdf file for detailed information on equipments needed

Procedure

1. Sperm is either thawed from a cryopreserved stock or extracted fresh from the cauda epididymis of the male mouse and incubated for 30 min. at 37 °C in TYH medium.
2. Cumuli containing oocytes are transferred to a 90 µL HTF medium drop on a petri dish, covered by mineral oil (NidOil).
3. 10 µl sperm suspension are added to the HTF drop and the IVF dish is incubated at 37 °C for 4-5 hours. Afterwards, fertilization is assessed by second polar body formation.
4. Sperm thawing and IVF procedures as well as TYH and HTF media preparation are performed according to the protocols described at <https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols>
5. Following successful IVF, the zygotes are supplemented with fresh KSOM Embryomax media everyday until E5.5

Troubleshooting

cautionary notes are placed wherever necessary in the protocol.

Time Taken

2 hours from the time of oocyte isolation

Anticipated Results

Successful fertilization of mouse oocytes resulting in healthy embryos

References

Takeo T, Nakagata N.Reduced glutathione enhances fertility of frozen/thawed C57BL/6 mouse sperm after exposure to methyl-beta-cyclodextrin. Biol Reprod. 2011 Nov;85(5):1066-72. doi: 10.1095/biolreprod.111.092536.

Acknowledgements

Javier Martin Gonzalez, Transgenic Core Facility, University of Copenhagen

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

IVF protocol MBCD+GSH Fresh and Frozen - September 2018.pdf