Abstract

Invitro fertilization of mice oocytes cryopreserved using a newly developed manual freeze-thaw protocol

Introduction

Cryopreservation of oocytes could have numerous advantages. Excess oocytes obtained after hyperstimulation could be used for subsequent cycles in invitro fertilization programs, or used as donar oocytes. A women undergoing treatment which would damage or require the removal of her ovaries could cryopreserve her oocytes for future fertility treatment. The aim of this study was to develop a cost effective manual oocyte freeze-thaw protocol which would not require a expensive programmable freezer.

Method

Female albino mice aged 8-10 weeks were super ovulated with an intraperitoneal injections of 5 IU of Pregnant Mare Serum Gonadotrophin followed by 5 IU of human Chorionic Gonadotrophin (hCG) at 46-48 hrs. Oocyte cumulus masses were retrieved 18-20 hours after the hCG injection.

In 41 experiments the media used was either M_2 culture medium, or PBS or EBSS, and the oocyte cumulus masses were cryopreserved with varying concentrations of Dimethyl sulphoxide (DMSO), Ethylene Glycol (EG),Glycerol, 1-2 Propanediol(1-2 PROH) and Sucrose. In the two other experiments, the commercially available Embryo Freeze and Sperm Freeze media were used. The oocyte cumulus masses were placed in 0.5 ml paillets which were cooled

at 0^0 celsius for 20 minutes and transferred into liquid nitrogen (LN_2) vapour for five minutes and then immersed into LN_2 tanks slowly for storage. One week later the oocyte cumulus masses were thawed and inseminated with fresh sperms. Fresh oocyte cumulus masses were also inseminated as controls. The inseminated cumulus masses were incubated at 37^0celsius in 5 % Carbon Dioxide for six hours in a locally designed 5% Carbon Dioxide modular incubator. After six hours the oocytes, (visible after digestion of the cumulus by the sperms) were examined. The presence of two Polar Bodies with two Pronuclei were considered as confirmation of fertilization.

Results

The combination of 3.5M DMSO with 0.25M sucrose gave the best fertilization rate (FR) of 71.4% which was comparable with the 72.7% FR in the control group. The next best was 1.5M DMSO and 0.3M sucrose with a FR of 66.6%. Glycerol gave better fertilization rates than EG. The best FR with glycerol (45.5%) was obtained with a combination of 2% glycerol with 0.2M sucrose. The best FR with EG was only 33.3% which was obtained with a combination of 1.5M EG and sucrose with 20% human serum in PBS. Sperm Freeze and Embryo Freeze had FR of 9% and 17.6% respectively. The experiments using 1-2 PROH did not result in any fertilization.

Conclusions and recommendations

The newly developed manual oocyte freeze- thaw protocol using 3.5M DMSO with 0.25M sucrose and a relatively cheap locally designed 5% Carbon Dioxide modular incubator has produced acceptable invitro fertilization rates. This protocol does not need an expensive programmable freezer, and can be recommended for use in centres with limited resources.