



# Karolinska Institutet

**Institutionen för klinisk vetenskap, intervention och teknik  
Enheten för obstetrik och gynekologi**

## CULTURE AND VITRIFICATION OF HUMAN PREEMBRYOS

**AKADEMISK AVHANDLING  
som för avläggande av medicine doktorsexamen vid Karolinska Institutet  
offentligen försvaras på engelska språket**

**Onsdagen den 4 Juni 2014, kl 13.00,  
Föreläsningssal B64.  
Karolinska Universitetssjukhuset, Huddinge.**

**Av  
Fredwell Hambiliki, embryolog**

*Huvudhandledare:*

**Docent Anneli Stavrénus-Evers**

Institutionen för Kvinnors och Barns Hälsa,  
Uppsala Universitet

*Bihandledare:*

**Professor Outi Hovatta**

Karolinska institutet  
Institutionen för klinisk vetenskap,  
intervention och teknik

*Opponent:*

**Professor Arne Sunde**

Norwegian University of Science and Technology  
Department of Laboratory Medicine, Children´s  
and Women´s Health

*Betygsnämnden:*

**Docent Kersti Lundin**

Göteborgs universitet  
Kliniska vetenskaper, Obstetrik och gynekologi

**Docent Håkan Wramby**

Lunds universitet  
Institutionen för obstetrik och gynekologi

**Professor emerita Pia Ek**

Uppsala university  
Institutionen för medicinsk biokemi och  
mikrobiologi

## **ABSTRACT**

Despite improvements in stimulation protocols, culture media formulations and laboratory protocols, the success rates in human IVF remain disappointingly low. The ability to successfully cryopreserve supernumerary embryos in a given IVF cycle without losing significant embryo viability is essential to maximize the cumulative benefit of a given treatment cycle. Therefore, studies on culture, cryopreservation and gene expression of human embryos fertilized *in vitro* were performed.

In these studies the impact of culture media on fertilization of human oocytes *in vitro* was investigated. Furthermore, the impact of growth factor supplementation to *in vitro* culture media and embryo survival and cryodamage after vitrification were studied. Using *in situ* hybridization and immunohistochemistry methods, the expression of genes in the human Fallopian tube, endometrium, and pre-implantation embryos and in human embryonic stem cells (hES) cells was studied.

The findings can be summarized as follows: *in vitro* culture media has impact on normal fertilization. Supplementation of growth factors to *in vitro* culture media implicates a physiological role in regulating pre-implantation development. Vitrification of embryos is an effective way of cryopreservation. *In situ* hybridization, immunohistochemical and matrix assisted laser desorption/ionization time of flight mass spectrometry methods are versatile tools in reproductive medicine research.

These findings will help to identify markers for embryo development and characterisation of hESC. Furthermore, knowledge obtained will give us tools to improve formulations of culture and cryopreservation media, which in turn might increase the overall results in IVF treatment and maximise the usage of hESC.