

# Embryo culture and Transfer

## Introduction

We tend to think of embryo culture as a “modern” laboratory method associated with the advent of IVF in the late 1970s and early 1980s. It was in 1912 that the very first rabbit embryos (blastocysts) were described in culture. In 1949 mouse embryos could be grown from the 8-cell embryo stage to blastocysts in a complex embryo culture medium. Most embryo culture media used in early human IVF was based upon media that were successfully used in animal embryo culture. In 1985, an embryo culture medium called Human Tubal Fluid Medium (HTF) was first described as designed specifically for human IVF. Since the development of HTF, many modifications and advancements have been made in the recipes for human embryo culture medium. In addition to this one medium, we now have available, a number of multi-step media (sequential media) that have been developed and are used on different culture days in order to respond to the changing metabolic needs of the rapidly growing and changing embryo. Introduction of sequential media for human embryo culture have improved the success rates of many IVF programs worldwide.

## The Embryo Culture Media

The Laboratory at Victoria Fertility Centre (VFC) uses an improved sequential culture media system called Vitro Life. Vitro Life is manufactured in the USA and fresh batches of medium are acquired every few weeks.

## Oocyte Wash Buffer

On the day of egg retrieval (Day 0), this buffer is used for the retrieval of the eggs from the ovary. Oocyte wash buffer has an ingredient, which prevents a change in pH when the solution is exposed to air during the retrieval. The eggs are very susceptible to any minute changes in the pH of their environment. The eggs are washed in this buffer and then placed into the next medium for culture.

## Fertilization Medium

After the wash at retrieval, the eggs are put into the fertilization medium. This medium contains a variety of salts, sugars, amino acids, protein and other nutrients essential for the maintenance of the egg (and sperm in IVF) during the process of fertilization (IVF and ICSI). The fertilization medium and all of the other subsequent culture media, are buffered with the appropriate components in order to maintain the correct pH of the solution in the embryo incubator.

## **Cleavage Medium**

All of the eggs which undergo normal fertilization are next placed into cleavage medium, which is formulated specifically to support the growth requirements of the early cleavage stage embryo. The cleaving (dividing) embryo is cultured in this medium until Day 3. If the embryo transfer is scheduled for Day 3, the embryos are transferred to the uterus in a small amount of this medium.

## **Blastocyst Medium**

Embryos, that are to be cultured until Day 5 or 6, are placed, later on Day 3, into another medium referred to as blastocyst medium. The embryos are then maintained in this medium until embryo transfer on Day 5 or embryo cryopreservation on Day 5 or 6. This medium has additional components and/or different components required by the embryo in its transition from a cleavage stage embryo to a blastocyst. If the embryo transfer is scheduled on Day 5, the embryos are transferred to the uterus in a small amount of this medium.

## **Sperm Buffer**

The sperm buffer is formulated in order to maintain the correct pH when the solution is exposed to air. This buffer is used during the preparation of semen samples and solutions for semen samples, which will be washed and processed outside of the incubator.

## **Sperm Medium**

The sperm medium is similar to the Sperm Buffer except that the buffer is such that the correct pH of the solution is maintained whilst in the incubator. This medium is important for the final resuspension of sperm to be used in IVF because the process of fertilization occurs inside the incubator.

## **The Embryo Culture Equipment**

### **The Laminar Flow Hood**

The preparation of all media and solutions to be used in IVF, ICSI and IUI occurs inside this specialized hood, which blows air out towards the embryologist. The air is filtered and the outflow of air prevents any contaminants from blowing in and contaminating the solutions and embryo dishes being prepared. Preparation of semen samples to be used in IVF, ICSI and IUI also occurs in this sterile environment.

### **The Preparation Incubator**

All dishes and solutions to be used for an IVF, ICSI or IUI treatment are maintained in this incubator until use. The incubator is sterile inside, is at 37°C, has a carbon dioxide concentration of 6.0%, and the environment is fully humidified to prevent any

evaporation. All solutions and dishes to be used for treatment are equilibrated in this incubator for a minimum of 4 hours before use.

### **Embryo Culture Incubator**

All eggs and embryos are incubated here throughout their time in the VFC laboratory. The unit is infused with the proper levels of oxygen and carbon dioxide to ensure that the eggs/embryos are maintained under optimum conditions at all times. The environment in the incubator is also humidified and kept at 37°C. The temperature and gas levels are monitored continuously and the incubator is attached to a telephone based alarm system which will call out to the embryologist during off hours should an unsuitable or emergency condition arise.

### **IVF Chamber**

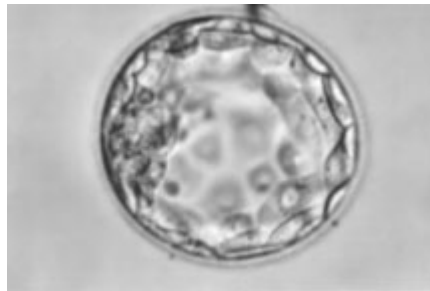
Whenever the eggs and embryos need to be outside of the incubator for any reason, they are handled in our IVF Chamber. The chamber looks like an isolate that you would see in a special care newborn nursery in the hospital. This chamber however is specially modified and adapted for the purpose of maintaining eggs and embryos under optimum conditions even when they are being handled outside of the incubator.

## **Blastocyst (5 Day) Culture**

Culture media are now available that are specifically designed to support the growth of the embryo from Day 3 to Day 5-7 of culture. From Day 3 to Day 5, the embryo undergoes profound changes while it transforms from a totipotent cleavage stage embryo into the more differentiated blastocyst.



**Human embryo on Day 3 of culture**



**Human embryo on Day 5 of culture**

The culture of blastocysts was introduced to reduce the high numbers of high order (triplets or more) multiple pregnancies, which have been an unfortunate result of IVF treatment. Traditional embryo culture methods have necessitated the transfer of more than two embryos to the uterus in order to obtain higher pregnancy rates. Growing the embryos to a more advanced stage (blastocyst), allows for better selection of the embryos that are able, in culture, to grow to blastocyst and are more likely to implant. In this way we can better select the embryos which have the potential to give us a baby and thereby reduce the number of embryos we transfer - and so maintain a decent chance of pregnancy, though, by transferring fewer embryos reduce the risk of high order multiple pregnancies.

There is however a significant attrition rate from day 3 ( when the embryos are usually between 6 and 8 cells ) until day 5 ( when the embryo often reaches a blastocyst stage of approximately 250 cells) In order to consider culturing the embryos to day 5 - we require that we have at least 4 - 5 or more good quality embryos. It is important to remember that most of the embryos that do not do well in extended culture ( i.e. do not develop in to good quality blastocysts) would not have given us a baby. HOWEVER - there is always a chance that some embryos might have survived in the perfect environment of a uterus rather than our laboratory. In other words we may lose some embryos during extended culture - that may have given us a baby if they had been transferred to the uterus earlier.

As a rule - we at VFC will always consider culturing the embryos to day 5 - unless we think it is in your best interests to do the transfer on day 3.  
This is a complicated subject and will always be discussed with you - the parents.