Application Note

MCQ Gas Blender 100 Series Application

Effects of controlled atmosphere for *in vitro* embryo cultures

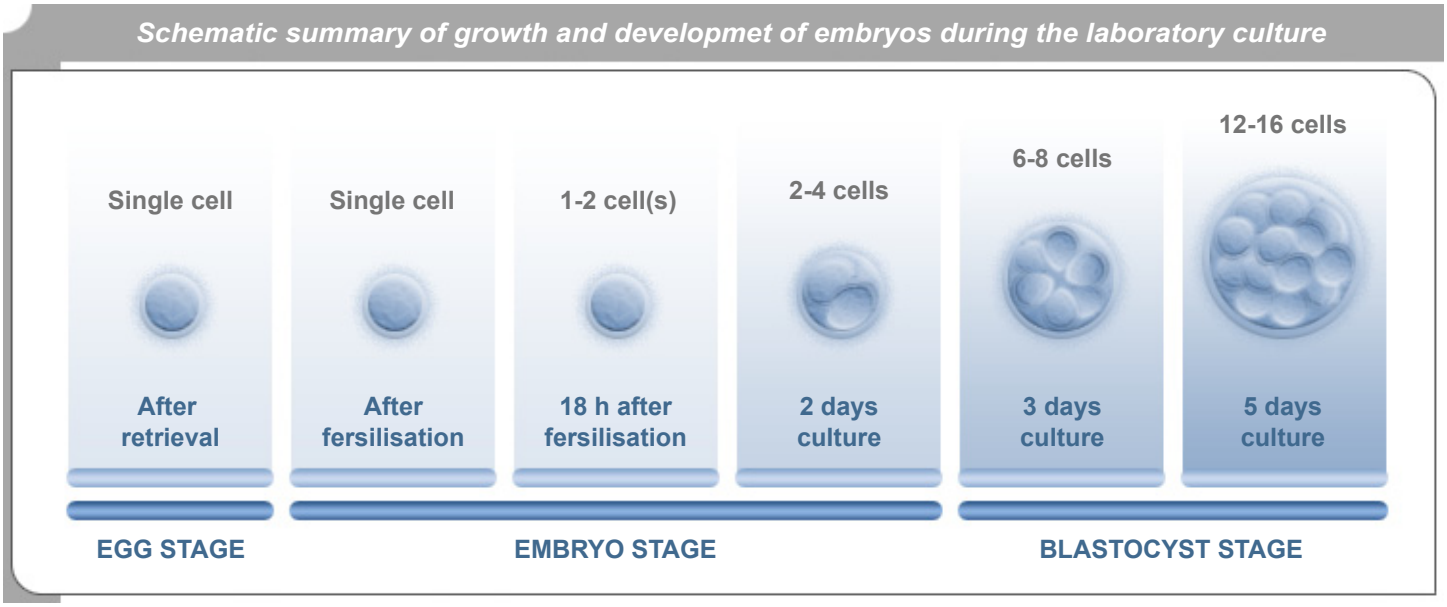
Introduction

Cell culture study currently represents one of the most important and promising application field for modern science and one of the most developed. Many of the latest remarkable achievements earned in medical and biological fields arisen from researches conducted on lab-cultured cells. In order to obtain the best results, culture parameters must be accurately set and controlled and the importance of the gas mixture for in vitro cell development has been particularly discussed in one of our previous application note (link). This work aims to highlight the crucial role of gas mixtures when undertaking In Vitro Fertilization (IVF) processes, which includes the critical step of embryo culture. For these applications MCQ suggests the use of its MCQ Gas Blender 100 Series, a professional instrument designed for three-components gas mixtures management.

In Vitro Fertilisation

In vitro fertilization (IVF) and other several assisted reproductive techniques, have been developed by modern medicine to face the problems related with couple infertility. Female infertility in women is mainly connected with problems with the fallopian tube, while male infertility mostly derives from men defect sperm quality. IVF is a sophisticated process designed to overcome these problems and to help couples establishing a successful pregnancy. Basically the IVF involves retrieving eggs from woman's ovaries, letting sperm fertilise them in a controlled environment and transferring the fertilised eggs back in the patient's uterus. However, without additional techniques, this simple process would provide small chances of pregnancy. In order to enhance the successful rate of process, other additional steps has been developed and are now routinely used in IVF:

- **Ovarian hyperstimulation**
  Through a 10-16 days-long cycle of Human Menopausal Gonadotropin (HMG) injections, the patient ovarian status in stimulated in order to achieve the simultaneous maturation of several eggs.
- **Egg retrieval**
  Once the egg follicles have reached a particular size (about 16 mm of diameter), the patient is ready to undergo egg retrieval. Time is another important factor since the retrieval must be performed just before ovulation occurs.
- **Egg preparation**
  Eggs are separated from the follicles fluid and then stored in incubator under physiologic conditions (both atmosphere conditions and nutrient mixture are crucial for the correct storage and development of cells). The eggs will remain in the incubator until fertilization is ready to take place.
- **Fertilisation**
  Sperm and eggs are placed in incubators which enables fertilization to occur. The eggs are monitored to confirm



that fertilization and cell division are taking place. Once this occurs, the fertilized eggs are considered embryos.

- **Embryo culture**
  Embryos are left to develop for several hour, waiting for the cell division to occur. The importance of gas mixture during the embryo culture will be discussed later.
- **Embryo selection**
  Embryos are analysed and the ones not adequately grown are rejected. At this stage, a pre-implantation genetic diagnosis (PGD) can optionally be conducted, to check any possible predisposition to genetic diseases.
- **Embryo transfer**
  In order to establish the best condition for embryo implantation, the patient is administered with estrogens and progesterone. Once the uterine lining is appropriately prepared, the selected embryos are transfer to the patient's uterus.

Embryo Culture

The embryo culture is vital for the success of any IFV procedure. This delicate step involves the development or fertilized eggs under highly accurate and controlled conditions. After the retrieval, embryos are fertilized and left developing for 18 hours. Embryos are then monitored in order to select the ones in which the fertilization process has been successful. After the selection, embryos are usually cultured until having reached the 6-8 cell stage, three days after the fertilisation. Alternatively embryos can be placed into an extended culture system, allowing them to develop until blastocyst stage (12-16 cells, overall a five-day-long culture) before the transferring. The culture, as well as the preparation of all media and solutions to be used in IVF, occurs inside specialized hoods, in which the process parameters are controlled and monitored. The main factors affecting embryos are: dishes and solutions sterility (absolutely needed to avoid environmental contaminations), adequate growth medium (substances required for a correct development may change depending upon the embryo stage), constant temperature (maintained at 37°C to reproduce the optimal uterine conditions) and proper levels of carbon dioxide and oxygen in the culture atmosphere.

Gas Mixture effects

Gas mixture composition, is particularly important for the correct development of cultured cells. Even small changes in the atmosphere composition can drastically affect the

Fertilisation methods: intracytoplasmic sperm injection (A); sperm and eggs incubation (B).

Each embryo stage requires differente medium

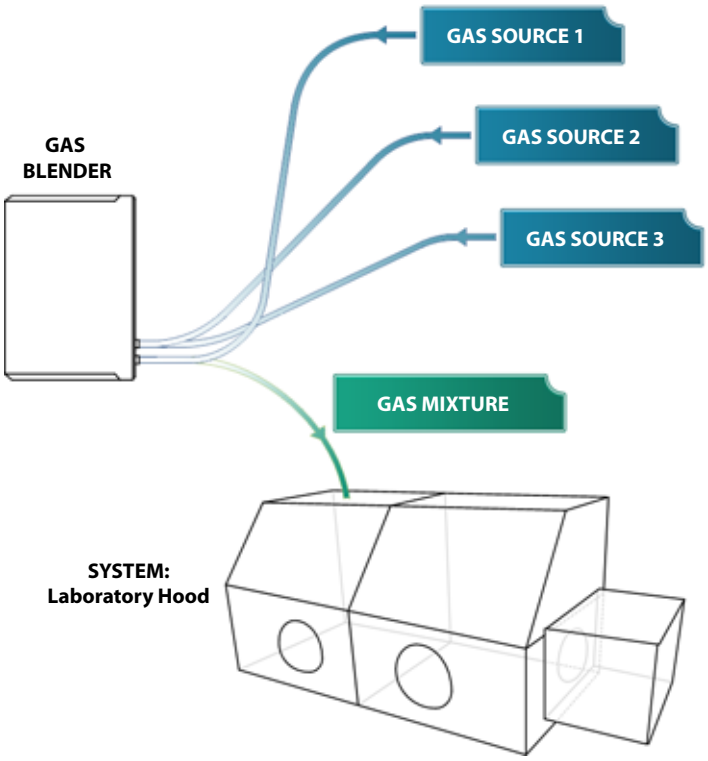
embryos and thus the entire IVF process. The standard culture procedure involves the growth of embryos under physiological conditions. Compared with the atmospheric value, carbon dioxide concentration is increased up to 5-6%, while the oxygen amount is variable. In facts, the effect of oxygen concentration on in vitro cultures is a crucial topic, still debated by the scientific community [1,2]. A low-oxygen gas mixture configuration (5% O_2 and 5% CO_2 in N_2) and a high-oxygen configuration (20% O_2 and 5% CO_2 in N_2) are the two commonly used options. Use of low-oxygen configuration has been proved especially suitable for 2-3 days cultures, since the oxygen concentration reduction from atmospheric levels has generally improved embryonic development [3,4]. A more complex issue arises performing the 5 days cultures. For these applications some studies have highlighted good performances of the low-oxygen configuration [5-7] while other works have reported no real benefits [8].

MCQ solution for gas blending

Literature works show how crucial the gas mixture composition can be when culturing embryos, and how the atmosphere's optimization is still far from being accomplished. Undertaking embryo culture experimentation requires a professional instrument capable of blending highly accurate gas mixtures and suitable for dynamic management. For these applications MCQ suggest the use of its Gas Blender 100 Series, an instrument designed to work with high precision 3 components gas mixtures. The MCQ ensures a precision of 1% of the setpoint, a repeatability of 0,16% of the reading value and a 50 ms response time for setpoint changes (currently the fastest on the market). Unlike the standard gas blending configuration, which requires 3 single channels mass flow controllers connected each other with an external control unit, the Gas Blender 100 Series offers the advantages of 3 single-channel mass flow controllers all in a compact box, easily to handle and to install wherever it's needed. The Gas Blender 100 Series requires no external control unit, for all the mixture parameters and other gas settings can be managed by the user with the MCQ Gas Mixer Manager, the software specifically created to access all the Gas Blender features. The software only requires a desktop or laptop computer compatible with any Windows operative systems starting from Windows XP. This hardware configuration needs little lab-space, proven especially suitable for culture applications.

• Hardware Configuration

An example of MCQ Gas Blender 100 Series hardware configuration is represented in the scheme below. The gases in use must be dry and non-aggressive. The instrument works with pure or mixtures gas media (the example shows pure gases for simplicity). The gas cylinders are connected to the instrument through 6 mm diameter tubes and a check valve is installed along each line as backflow prevention device. Each gas is connected and controlled by a dedicated channel of the Gas Blender 100. Another 6 mm tube finally connects the instrument to the working system (a generic lab hood for cell culture) in which the experiment takes place.



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