

ENVIRONMENTALLY CONTROLLED IN VITRO INCUBATOR

FIELD OF THE INVENTION

This invention relates generally to incubators for providing a controlled environment for maintaining cells, tissues and organisms in vitro during examination and manipulation, and more particularly to incubators in which the atmosphere and ambient light are controlled to eliminate conditions which may be toxic to such cells, tissues and organisms.

BACKGROUND

Advances in vitro fertilization ("IVF") techniques have raised hopes for its widespread use in permitting infertile human patients to bear children, and hold promise for significant commercial benefits in animal breeding. A problem that has prevented the more widespread use of IVF, however, is the disappointingly low pregnancy rates that have been achievable through the technique, even with the transfer of several preembryos. With humans such rates seldom reach 28% and the ongoing pregnancy rate is usually well below 22%.

Y. Hirao and R. Yanagimachi have reported in their article entitled *Detrimental Effect of Visible Light on Meiosis of Mammalian Eggs In Vitro*, J. Exp. Zool. v. 206, p.365 (1978) that short wavelength visible light (below about 480 nm) emitted from fluorescent lights of the type widely used in laboratories can inhibit the maturational changes (meiosis) which an egg must undergo before fertilization can occur.

Other articles, such as *Effect of Room Florescent Light on the Deterioration of Tissue Culture Medium* by R. L. Wang at page 19 of *In Vitro*, v. 12 n. 1 (1976), reports that tissue culture medium of the type used in cloning cell lines deteriorates more rapidly when subjected to fluorescent light.

The article *Apparatus For the In Vitro Fertilization and Culture of Human Oocytes* by J. Testart et al at pg. 372 of *Fertility and Sterility*, v. 38, n.3 (September 1982), acknowledges, as have others, that IVF of mammalian eggs should be carried out under conditions as close as possible to those occurring in vivo. The article describes an incubator in which oocytes and zygotes are maintained in culture tubes stored in metal buckets so as to remain in darkness except when being manipulated or examined. A filtered light source for use with a compound microscope in the incubator is described in which the filter restricts the illumination to the longer wavelength, on the order of 500 to 750 nm, which the article states do not harm mammalian eggs. No filter is used however with the light source extending into the incubator for a stereozoom microscope. In addition, the biological materials are exposed to ambient light each time they are removed from the buckets.

The incubator described in the Testart et al article also provides a controlled atmosphere for the culture media in the culture tubes by bubbling a controlled gas mixture (5% CO₂, 5% O₂, 90% N₂) from a commercially available premixed gas cylinder through a bottle of distilled water and distributing the humidified gas through a series of culture tubes. The tubes are stoppered and the gas is conducted from tube to tube by tubing extending through holes in the stoppers. However, any examination of the biological materials per-

formed outside of the culture tubes results in their exposure to air.

An article entitled *Development of One-Cell Ovine Embryos In Two Culture Media Under Two Gas Atmospheres* by Betterbed et al, at page 547 of *Theriogenology*, v. 23, n.3(March 1985), reported experiments in which one-cell and two-cell sheep "embryos" were cultured in either 5% or 20% O₂. The article stated that the reduction of oxygen from 20% to 5% had no effect on embryo development.

Earlier work reported by W. K. Whitten in a paper entitled *Nutrient Requirements for the Culture of Preimplantation Embryos In Vitro* given at a symposium in April 1970 in Venice and included in a book entitled *Advances in Biosciences* published by Pergamon Press found that a higher percentage of mouse embryos reached the blastocyst stage when cultured in an atmosphere containing 5 to 10% oxygen concentration than when cultured in higher or lower ambient oxygen concentrations.

Applicants have also observed the toxic effects of oxygen concentration of greater than 10%. Applicants have extended such findings to show that exposures to 20% oxygen, the oxygen concentration of air, for as little as two hours or less can also result in toxicity.

In addition, applicants have shown that short wavelength light as well as atmospheric oxygen concentrations is also toxic to mammalian zygotes and pre-embryos. It is believed that the short wavelength light, such as produced by fluorescent lights, and atmospheric concentrations of oxygen adversely effect both the culture medium and the zygotes and pre-embryos themselves. This phenomenon has not previously been observed in human zygotes and pre-embryos since viable pre-embryos are normally replaced in the patient after two or three divisions, while the deterioration does not become apparent until after about 3 or more divisions.

The above referenced Testart et al article in *Fertility and Sterility* provides a partial but unsatisfactory solution to the above noted problems. Storing the culture tubes in the dark buckets reduces the exposure to short wavelength florescent light and the controlled atmosphere within the culture tubes prevents the exposure to toxic levels of oxygen while a culture is maintained in the tube.

Using the system, however, a culture is exposed to ambient florescent light whenever the culture tube is removed from the bucket. Additionally, the use of culture tubs strung together by gas supply tubing is a particularly inconvenient and disfavored way to maintain cultures which has not found wide acceptance. It is preferred by most researchers, particularly in the United States, to maintain the zygotes and pre-embryos in culture dishes which are easier to manipulate.

SUMMARY OF THE INVENTION

In accordance with the invention there is provided an incubator for use in maintaining and examining cells, tissues and organisms including mammalian eggs, zygotes and pre-embryos in culture media which includes an environmentally closed chamber having an enclosure, a portion of which is transparent to light having a wavelength above about 500 nm and which incorporates filter means for strongly attenuating transmission of shorter wavelength light. Control means are provided for maintaining the oxygen concentration within the chamber at a level substantially lower than in air.