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Analysis of the embryotoxic effects of blastocyst culture medium in vitro fertilization technology

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Abstract. The culture medium for blastocysts contains components that may exhibit embryotoxicity, potentially affecting the developmental process of blastocysts and leading to abnormalities in the growth and development of offspring. This paper reviews and discusses the impact of blastocyst culture medium on the development of blastocysts and later-stage embryos. The results presented in the literature indicate that blastocyst media with different compositions not only affect development during the incubation period but may also have long-term effects on post-implantation development and maturation of embryos. The paper primarily summarizes the effects of the composition and concentration of blastocyst culture medium on embryonic development, aiming to further improve the quality of in vitro culture systems and optimize the culture conditions.

Keywords: Embryo, Blastocyst, Embryotoxicity, Developmental Arrest.

1. Introduction

In 1978, the birth of the world's first test-tube baby in the UK marked a significant milestone, providing the most crucial technical means to address infertility and achieve optimal childbirth [1]. As the name suggests, In Vitro Fertilization and Embryo Transfer (IVF-ET) is an assisted reproductive technology that involves separately retrieving mature eggs and sperm from the human body, artificially fertilizing the eggs with sperm outside the body, and allowing for early embryonic development, followed by the transfer of the embryo into the uterus for further development and the birth of a baby. However, due to the international consensus and ethical requirements of the "14-day rule," the culture of human embryos outside the body must not exceed 14 days. We are unable to experimentally verify whether the in vitro culture phase before in vitro fertilization and embryo transfer affects the subsequent development of the fetus. Therefore, model organisms such as mice are commonly used for validation and exploration [2]. The process of IVF-ET mainly includes in vitro fertilization, early development, and implantation. During the early stages of in vitro culture, the blastocyst culture medium serves as the extracellular environment for the contact between oocytes and sperm. The blastocyst culture medium has nutritional, osmotic, and immunosuppressive effects, containing a variety of nutritional factors and immunosuppressive substances that can improve the maturation rate of oocytes and stimulate cell proliferation to promote embryonic growth and development [3]. However, some articles have pointed out that different components added to the blastocyst culture medium in the in vitro culture system can affect long-term embryonic development [4]. These results indicate that different blastocyst culture media have caused varying degrees of damage to the early development of embryos. Since mouse

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embryonic development is a complex process influenced by multiple factors, it is primarily regulated by cytokines, hormonal level changes, and immunosuppressive responses during the early stages of development, which directly affect the quality of the early developing embryos and the rate of organ formation [5]. Therefore, understanding the factors influencing the early development of mice under in vivo or in vitro culture conditions and the mechanisms by which they cause damage to embryos is very necessary and important. This paper reviews and compares the changes in hormone levels within mice and the cell proliferation capacity and apoptosis levels after stimulation by different concentrations of blastocyst culture medium, providing a theoretical basis for optimizing in vitro culture conditions.

2. In Vitro Fertilization

In vitro fertilization research made groundbreaking progress in the 1980s, and today the technology has become one of the core techniques in human reproductive medicine. In vitro fertilization is an artificially operated process where eggs and sperm are fertilized outside the body, also known as the fertilization process or oocyte injection. It is a special biomedical experimental process, and for mammals, its success depends not only on the combination and fertilization capabilities of sperm and eggs but also on their viability in the environment. Blastocyst culture involves obtaining early embryos with complete structure, morphology, and function outside the body, simulating the embryonic development process within the body under in vitro culture conditions. Some studies have pointed out that compared to the naturally developing group within the body, the implantation rate of the in vitro culture group is somewhat lower, but there is no statistical difference. However, while in vitro fertilization does not affect the implantation rate of embryos, the survival rate after implantation is significantly reduced. This suggests that compared to in vitro culture, the operation of in vitro fertilization is more likely to affect the quality of the embryos and is the main reason for the decline in embryonic quality and adverse pregnancy outcomes [4]. The blastocyst culture medium contains a variety of substances that can affect embryonic development and directly impact the health of mice. Mice (Organorus rabbit) are one of the earliest and most widely used animal models in research. By observing the effects of immune substances and cytokines in the embryonic culture medium on the development and health of mouse embryos, important information about embryonic development can be obtained and factors affecting embryonic development and health can be identified.

3. In Vitro Culture

The primary goal of in vitro embryo culture is to obtain embryos with high developmental potential and to acquire the biological characteristics necessary for normal development. To achieve normal growth and development, it is essential to obtain embryos from the in vitro fertilization process and cultivate them until they form a normally functioning embryo that has developed for a certain period, after which it can be transplanted into the body to continue its development. There are two main methods of in vitro culture: In Vitro Fertilization-Embryo Transfer (IVF-ET) and In Vitro Culture of Full-term Embryos (ICF) [1]. IVF-ET involves various methods of combining eggs and sperm with culture medium or fertilized egg cells outside the body 2 to 4 days after fertilization to form early embryos and to cultivate them in vitro until 7 to 8 days post-fertilization, ensuring they have normal structure, function, and developmental potential during the fertilization process. On the other hand, ICF involves fusing a functional or active sperm with an egg cell into one cell through microinjection technology, allowing the formation of a mouse embryo with embryonic layer structure, morphology, and functional architecture during the fertilization process. Currently, IVF-ET and ICF are widely used internationally to obtain tissues and organs needed for normal growth and development.

4. The Impact of Blastocyst Culture Medium Components

4.1. The Impact of Blastocyst Culture Medium Components on Early Development of Mouse Embryos

In the in vitro development system, the blastocyst culture medium (BEM) is one of the most important media, and many animal models require the application of BEM for in vitro embryo culture to obtain

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normal offspring. The main components of the blastocyst culture medium generally include: abundant nutrients (sugars, proteins, and lipids), immune cells, growth factors, and other cytokines, the composition of which determines the development of the embryo in vitro. Some literature has pointed out that certain components added to some culture media to support embryonic development have actually been proven to be detrimental to embryonic development in vitro, such as high levels of glucose, divalent metal ions, nucleotides, and certain hormones. High levels of glucose during the culture process are the cause of slow development or developmental arrest of cleavage-stage embryos [6]. This is because, in the early stages of fertilized egg development, the initial metabolic and biosynthetic activities of the embryo are relatively low, and the embryo almost entirely relies on pyruvate, carboxylic acids, and lactic acid as its preferred energy substrates [7], as well as specific amino acids, such as aspartic acid [8]. ATP is produced based on mitochondrial metabolism. However, the embryo still absorbs low levels of glucose [9], which is considered to maintain the production of glutathione to prevent oxidative stress and nucleic acid and lipid biosynthesis through the pentose phosphate pathway. It is not until the activation of the embryonic genome begins and the accompanying increase in biosynthesis that the nutritional preference shifts to glucose-based metabolism. By the blastocyst stage, glucose becomes the preferred nutrient [10]. This suggests that in the use of components in the blastocyst culture medium, attention should be paid to conforming to the changes in nutritional needs under normal development conditions in the body. In addition, attention should also be paid to the pH range of the culture medium. In the first few hours after the combination of oocytes and fertilization, the intracellular pH regulation and calcium transport system have not yet started to operate, and the ability of the embryo to regulate ionic homeostasis gradually emerges as development progresses [11]. The pH regulation transport system is not detected until 6-8 hours after fertilization and does not fully function until about 10 hours after fertilization [12]. The amino acid composition in the culture medium is also particularly important. Amino acids in the culture medium can promote the development of the embryo to the blastocyst stage and increase the subsequent survival and development capabilities [13]. Even a brief exposure (less than 5 minutes) to a culture medium lacking amino acids can also damage the subsequent developmental potential [3].

4.2. The Long-term Impact of Blastocyst Culture Medium on Embryo and Cell Development

Currently, numerous scholars have confirmed a variety of factors that involve damage to embryos and cells caused by blastocyst culture medium. Many factors may cause or exacerbate developmental damage to early embryos, such as environmental factors, cellular nutrients, and culture conditions (e.g., components of the culture medium, etc.). Although many studies have focused on the toxic effects of the blastocyst culture medium on embryos or cells during the culture period, several components in the culture medium may not affect the development of the blastocyst but can significantly impact the viability of the fetus and its development post-transplantation [14]. A study found that the level of ammonium ions produced in the culture by a medium containing glutamine inhibited the development of in vitro human blastocysts [15]. In an amino acid-containing culture medium, especially one with glutamine, ammonium is spontaneously generated through decomposition at 37°C [16]. A medium containing 1 mM glutamine can produce approximately 150 mM of ammonium within 24 hours at 37°C, significantly higher than the level proven to inhibit embryonic development [17]. Although this level of ammonium does not always change the development of the blastocyst, it significantly affects the health of the blastocyst's cells [14-16]. More importantly, it significantly reduces the ability of the embryo to implant, leading to a large number of fetal absorptions and underdevelopment. There are also many studies that have proven the beneficial effects of EDTA on embryonic development starting from the fertilized egg stage [18]. However, the beneficial effects of EDTA are limited to embryos at the cleavage stage [3]. When cultured with 100 mM EDTA, the development of the ICM of compacted embryos and the development of the fetus post-transplantation are reduced [19]. This is because the ICM of the embryo primarily uses glycolysis as the energy production pathway [20], and the presence of EDTA inhibits its development, thereby inhibiting fetal development. Therefore, culture media specifically designed for the development of embryos at the post-compaction stage omit EDTA from its formulation.

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5. Conclusion

Due to the various substances contained in the blastocyst culture medium, there are still significant differences in the degree and mechanisms of impact on embryos by different culture media. Even with the use of high-quality blastocyst culture media, maternal factors after transplantation play an important role in the early development of blastocysts. The presence of immune substances and pro-apoptotic cytokines within the embryo can lead to defects in germ layer differentiation and cell death, resulting in defects in blastocyst formation. Maternal factors can also affect embryonic differentiation by influencing the expression of pro-apoptotic and anti-apoptotic molecules within the mother. Additionally, compared to other cytokines, cytokines with anti-proliferative, angiogenesis-inhibiting, and angiogenesis-promoting effects vary across different culture media. Although current research has shown that the blastocyst culture medium has a significant impact on mouse embryo development with multiple mechanisms and specificity, further refinement is still needed. Future research directions mainly include: 1) conducting a more comprehensive analysis of maternal factors; 2) studying the impact of immune substances and pro-apoptotic molecules on blastocysts of different embryo types; 3) investigating whether multifunctional culture media can promote multiple pregnancies in mice and reduce birth defects and improve health status through the combined action of these immune substances; 4) developing corresponding in vitro culture conditions in conjunction with the developmental characteristics of blastocysts and maternal factors. In summary, in vitro fertilization technology is an important technical means that can avoid some of the drawbacks of fertility methods and assist people with fertility defects. However, due to the variety of components and their concentrations in the culture medium, further research is needed; at the same time, there has been no related research on the addition of substances such as anti-proliferative, angiogenesis-inhibiting, and pro-apoptotic in the embryo culture medium. With the development of human assisted reproductive technology, the pregnancy rate has been greatly improved. To improve embryo quality and reduce the developmental toxicity of blastocysts, antioxidants, growth regulators, and other substances can be added to the culture medium, which can enhance egg quality and embryonic development potential. With the advancement of gene editing technology, genetically modified mice can also be used to better understand the impact of different components in the culture medium on embryonic development.

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