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Embryo Culture Media And It's Effect

Alisha Lepcha, Department of Clinical Embryology and Reproductive Genetics, Rayat-Bahra University.
Dipneet Kaur (HOD), Department of Clinical Embryology and Reproductive Genetics, Rayat-Bahra University.
Dr. Sunil Kumar (Professor), Department of Clinical Embryology and Reproductive Genetics, Rayat-Bahra University.
Email: Lepchaalisha123@gmail.com

ABSTRACT

Embryo culture media play a crucial role in assisted reproductive technology (ART), particularly in in vitro fertilization (IVF) procedures. The primary function of these media is to mimic the natural environment of the female reproductive tract, providing the necessary nutrients and conditions for the development of embryos from fertilization to the blastocyst stage. Over the past few decades, the formulation and optimization of embryo culture media have been the subject of extensive research, as they directly impact the success rates of IVF and the overall health of the resulting offspring. Early embryo culture media were relatively simple, consisting of basic salt solutions supplemented with glucose, pyruvate, and amino acids. Modern culture media are now tailored to support different stages of embryo development, from the initial fertilization phase through to the blastocyst stage, and often include components such as growth factors, vitamins, antioxidants, and specific ions that have been shown to enhance developmental competence.

One of the key aspects of embryo culture media is the balance of energy substrates. Early-stage embryos rely heavily on pyruvate and lactate as energy sources, while later stages, particularly the blastocyst stage, require increased levels of glucose. This shift in metabolic requirements has led to the development of sequential media systems, where different formulations are used at various stages of embryo culture to better meet the changing needs of the developing embryo. The effects of embryo culture media on IVF outcomes are profound. High-quality media can significantly improve the rates of fertilization, cleavage, and blastocyst formation.

In conclusion, the evolution of embryo culture media has been pivotal in enhancing the success rates of ART procedures. By providing a supportive environment that closely mimics natural conditions, these media facilitate proper embryonic development and improve the chances of successful implantation and healthy pregnancy outcomes. Ongoing research and innovation in this field hold promise for further improvements in reproductive medicine, offering hope to many couples struggling with infertility.

KEY WORDS: *Embryo culture media, Assisted reproductive technology, In vitro fertilization, Fertilization stage, cleavage, Blastocyst stage, Female reproductive tract, Metabolic requirements, Sequential media, Implantation, Infertility*

GENERAL DESCRIPTION

Subfertility has been described as the inability to achieve a pregnancy in spite of regular intercourse aimed at conception for more than 12 months (Evers, 2002). Subfertility is a major clinical and social concern. The most frequently used treatments of subfertility are IVF (Invitro fertilization) and ICSI (Intracytoplasmic sperm injection).

Since the birth of Lewis Brown, the first child conceived by IVF (invitro fertilization) at Oldham Cambridge by Edward and Steptoe in 1978 (who received the 2010 Nobel prize), the use of ART has drastically increased worldwide. Even for India IVF has been a game changer. This year it marks 46 years since the first IVF baby arrived as a beacon of hope, changing the history of infertility medicine forever.

India is climbing fast in the global infertility chart. At present India performs an average of 2-2.5 lakhs IVF cycles per year. The future looks bright as the projections indicate the potential of conducting 5-6 lakhs IVF cycles by 2030. The rising

prevalence of late marriage and parenthood, increasing rate of infertility, clinical factors, racial, ethnicity and greater awareness for infertility treatment. The increasing success rates of IVF is one of the factors that causes the growth (Ekta Batra,2023). Over the past 5 years IVF field have contributed 20% growth in Indian fertility field with 60-70% success rate.

During an IVF treatment, women are routinely stimulated with hormones. Hormonal medications known as Follicle stimulating hormones (FSH) and Luteinizing hormones (LH) are used during ovarian stimulation to help with the increased production, maturity and release of eggs. Over a period of two weeks, these hormones are injected to start the process. The stage may last longer, on the basis of time of growth of the eggs. Oocytes are retrieved from the woman and are prepared for fertilization. Also, the semen containing the spermatozoa is prepared and fertilization takes place either by conventional IVF (i.e. co-incubation of the oocyte and spermatozoa in a culture dish) or by intracytoplasmic sperm injection (ICSI) (i.e. injection of a single spermatozoon directly into the oocyte). The resulting zygotes are cultured for two to six days after fertilization in embryo culture medium, before embryos of good quality are selected for transfer or cryopreservation. Thus, the optimum embryo culture system and culture media are required.

EMBRYO CULTURE SYSTEM

An embryo culture system is a specialized environment designed to support the growth and development of embryos outside the organism, typically in a laboratory setting. These systems are essential in assisted reproductive technologies (ART) such as in vitro fertilization (IVF). Key components and considerations for an effective embryo culture system include:

1. Culture Media: A carefully formulated solution that provides the necessary nutrients, energy sources, and environmental conditions for embryonic development. Media are often designed to mimic the natural conditions within the reproductive tract.

2. Incubators: Controlled environments that maintain optimal temperature (37°C), humidity (90%), and gas concentrations (such as oxygen, nitrogen and carbon dioxide) to support embryonic growth.

3. Sterility and Cleanliness: To prevent contamination and ensure the health of the embryos, all equipment and materials must be sterile, and laboratory practices must adhere to strict aseptic techniques.

4. Monitoring and Assessment: Regular observation of embryo development is crucial. This often involves using microscopes and imaging systems to monitor cell division, morphology, and other developmental markers.

5. Osmolarity and pH Control: Maintaining proper osmotic conditions and pH levels (7.2) in the culture media is essential for preventing stress and damage to the embryos.

6. Additives and Supplements: Depending on the specific needs of the embryos, culture media may be supplemented with growth factors, hormones, or other agents to support development.

7. Gas Atmosphere: Embryo culture typically requires a controlled atmosphere with reduced oxygen levels (5-7%), nitrogen (89-90%) and elevated CO2 levels (5-6%) to simulate the conditions in the reproductive tract.

Effective embryo culture systems have led to significant advances in reproductive medicine, improving the success rates of IVF and other assisted reproductive technologies.

EMBRYO CULTURE MEDIA

Embryo culture media is a complex solution that is used to support the growth of embryos. Embryo culture media is used to mimic the composition of oviduct and uterine fluid to closely approximate the natural environment of developing human embryo. They are composed of a basic salt solution with the addition of other components, such as carbohydrates (pyruvate, lactate and glucose) and amino acids, which together are the main source of energy for developing embryos.

Human embryo is metabolically dynamic and their nutrition requirements change from day to day and from stage to stage. Cleaving embryos normally develop in the fallopian tube, whereas the natural environment for morulae and blastocysts is the uterine cavity thus, imitating the internal environment requires embryo culture media provided with nutrients required.

Embryo culture refers to the process of nurturing and developing embryos outside the natural environment of the mother's womb, typically within a laboratory setting. This technique is commonly used in assisted reproductive technologies (ART), such as in vitro fertilization (IVF), where fertilization occurs outside the body. Embryo culture is a crucial aspect of ART, as it provides a controlled environment for the early development of embryos, allowing healthcare professionals to optimize conditions for successful implantation. The advancements in laboratory techniques and culture media have

contributed to improvements in success rates for IVF procedures. However, it's important to note that not all embryos will develop normally, and the success of IVF can vary among individuals and couples.

With the advancement of the recent embryo culture media, we can efficiently maintain the viability of the developing embryo. The main perspectives of embryo culture in an assisted reproductive technique (ART) programme are to improve the quality of embryos that develop in the laboratory and to develop the chances of successful delivery of a healthy baby.

Embryo culture media are often designed for specific stages of embryo development. For example, there are separate media formulations for the early stages of development (cleavage stage) and for the blastocyst stage. The choice of culture media can impact the success rates of IVF procedures, and laboratories may choose media based on their specific protocols and preferences.

It is important for these media to be of high quality and free from contaminants to ensure the best possible conditions for embryo development. Research and development in this field continue to refine and improve embryo culture media to enhance the outcomes of ART procedures.

Previously, it was conventional to use media permitting culture of human in-vitro fertilized embryos for 2 to 3 days to reach the four-to-eight cell stage, with additional embryo transfer to the patient. Premature replacement of the human embryo to the uterus may in part account for the low implantation rates associated with human IVF, with only approximately 10% of embryos transferred leading to a live birth. Further basic research on the metabolism of invitro fertilized embryos revealed that there are specific needs, depending on the developmental stage of the preimplantation embryo. In addition, improvements in culture media resulted from an increased understanding of the environment of the oviduct and uterus. Since 1997, the extended culture in sequential serum-free culture media has attracted more attention. The ability to culture zygotes to the blastocyst stage should help to synchronize the embryo with the female reproductive tract, and to help to identify those embryos with little development potential.

HISTORY OF EMBRYO CULTURE MEDIA

The early days of ART, the main energy sources in the culture media were glucose, lactate and pyruvate. The buffer solutions were supplemented with these compounds. Later on, the culture media were supplemented with serum albumin as the source of energy. Albumin is an important protein constituent for embryo development. Fatty acid plays a major role in the early embryo metabolism required for membrane synthesis. They are mainly bound to albumin but some albumins are highly contaminated with liquid peroxidase which are highly embryonic toxic. In the late 1800, the idea introduced by Bernard, that the environment surrounding living tissues is an active one gave the notion that organs and tissues could be studied outside their setting in a suitable fluid formulated to facilitate these studies. In less than 10 years later, Ringer devised a solution of salts. It is an interesting aspect that the culture media evolved and used in the clinical setting were construed to support the development of somatic cell culture applications. The first success of fertilization of the human oocyte in vitro by Robert Edwards was accomplished in a simple, chemically defined media. These commercially available media were a modified Earle's balanced salt solution, and a modified Ham's F10 or T6. They were supplemented with maternal serum thus converting them into biological media. Menezo et al broke with the tradition of using balanced salt solution and produced a medium containing amino acids without the need of a serum supplement. In the year 1985 Quinn et al. developed a medium specifically designed for human IVF was human tubal fluid (HTF). The HTF resembled the composition of the fluids in the human fallopian tube. Human tubal fluid, supplemented with either whole serum or with serum albumin, gained great popularity for the use of day 2 or day 3 human embryo cultures, and has remained in use ever since. A culture medium is a foreign environment for the human embryo. Hence, it is very important to understand the physiology and metabolic needs of any type of the cell in order to culture it well in the laboratory. During preimplantation period several physiological changes occur in the human embryo as it undergoes successive stages of development. Till 8-cell stage, the embryo development is still under maternal control and the developing embryos have pyruvate lactate preference for its energy requirements. After the 8-cell stage, the embryo moves towards the glucose-based metabolism once the embryonic genome has been activated which then support the growth up to the blastocyst stage. Two investigators responded to these findings by modifying the HTF media. Quinn removed glucose and inorganic phosphate, QB 11, for obtaining the first glucose-free medium. Pool also generated a HTF variant, called Preimplantation Stage 1 or P-1 medium, a glucose- and phosphate-free medium, but additionally containing the amino acid taurine. The improved understanding of both the physiological changes in oviduct and uterus and the different metabolic needs of the cleavage-stage and blastocyst-stage embryo led to the development of stage-specific or "sequential" complex media G1/G2.

ENERGEY REQUIREMENTS OF EMBRYO CULTURE MEDIA

The level of glucose, lactate and pyruvate vary between the oviduct, uterus as well as within the cycle. The composition of these media is continually evolving as researchers strive to optimize conditions for successful embryo development in vitro. Additionally, the specific requirements may vary based on the stage of embryo development (e.g., cleavage-stage vs. blastocyst-stage).

Carbohydrates:

The preimplantation embryo is subjected to a diverse gradient of carbohydrate during the course of its development. In short, the early embryo shows a rather simplistic physiology and maintains only low levels of oxidative metabolism, whereas it exhibits a somatic-cell like physiology after compaction utilizing a wider spectrum of nutrients, biosynthetic rates are increasing, along with an increased respiratory capacity and an ability to utilize glucose. This involves a shift in the energy requirements at the time at which the embryonic genome is activated or at the post-compaction stage. Zygotes and subsequent cleavage stages prefer pyruvate as the primary source of energy, while the eight-cell-stage embryo uses glucose. Glucose is a key anabolic precursor and is required for the synthesis of triacylglycerols and phospholipids, and as a precursor for complex sugars and glycoproteins. Glucose also metabolized by the pentose phosphate pathway (PPP) generates ribose moieties required for nucleic acid synthesis.

Amino acids:

It is the most vital constituent of culture media. The "amino acids-(AA)", a term which includes all 20 common and naturally occurring amino acids, are important regulators of mammalian preimplantation development. Studies revealed that the human fallopian tube fluid contains high amino acids like taurine and glutamine. Prior to embryonic genome expression, the embryo utilizes carboxylic acids and AA as energy sources. In addition, certain AA are known to function as biosynthetic precursor molecules, osmolytes, buffers of internal pH, antioxidants and chelators, especially for heavy metals. It is important to note that there are also specific changes in the nitrogen requirements of the embryo.

The nonessential amino acid and glutamine are highly present in oviduct fluid. The seven nonessential AA (NEAA) and glutamine stimulate the growth of the early cleavage embryo. NEAA are used for protein metabolism while pyruvate and lactate are used as energy sources by the cleavage stage embryos. In contrast, an inhibitory effect was seen on blastocyst development and viability if the thirteen essential AAs are presented at an early stage. At the post-compaction stage, both groups of AAs act stimulatory to the inner cell mass of blastocysts, while the nonessential AAs and glutamine lead to stimulation of the trophectoderm and hatching from the zona pellucida.

However, AAs in culture media also spontaneously undergo breakdown to release ammonium into the culture medium with concentration being time dependent. Ammonium is toxic to the embryo and reduces viability. Especially L-glutamine (Gln) is highly unstable in solution, where it breaks down rapidly into equimolecular amounts of ammonium and pyrrolidine-5-carboxylic acid. Therefore, Lane and Gardner (introduced a two-step (sequential) culture media protocol to remove the accumulated ammonium. Another possibility is replacing Glutamine with a stable dipeptide of Glutamine. It must be noted that culture media should include sufficient levels of sulphur containing amino acids to minimize apoptosis leading to monozygotic twinning

Chelators-EDTA (Ethylenediaminetetraacetic acid):

The beneficial effect of the divalent cation EDTA were first reported by Ambruzak et al. over 20 years ago. The positive effect of EDTA is limited to the cleavage stage embryos. EDTA is shown to reduce the inner cell mass of the human embryos past the cleavage stage and also negatively affect the foetal development. A reason for this biphasic effect of EDTA is the inhibitory role that plays in the glycolysis pathway by inhibiting cytosolic kinases during the cleavage stage of the embryos. However, in contrast to the cleavage stage embryos, the inner cell mass uses glycolysis as the main source of energy producing pathway and the presence of EDTA has a great effect on the ICM growth and the subsequent foetal development.

Its usefulness is based on its role as a ligand and chelating agent, i.e. its ability to "sequester" metal ions. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. The addition of EDTA to culture media alleviates the 2-cell block in mice embryos and inhibits premature utilization of glycolysis by cleavage stage embryos, thereby preventing any Crabtree-like effect that is associated with arrest in culture. However, EDTA at a concentration of 0.1mmol/L reduces blastocyst development and cell number. Other investigators indicated that an EDTA concentration of 0.005–0.01 mmol/L did not have a deleterious effect on murine preimplantation or post implantation development.

Macromolecule:

The most Common sources for macromolecules are proteins for culture media such as human serum albumin or synthetic serum. Both are added at concentrations of 5 to 20%. Today, most commercial media include synthetic serum in which the composition is well known. Protein in the form of albumin is thought to maintain the stability of cell membranes

and chelate trace amounts of toxic components presented in culture water, media components and culture dishes. Other functions include capillary membrane permeability and osmoregulation. The presence of macromolecules in embryo culture media serves to facilitate manipulation of gametes and embryos. However, the uses of any blood products involve the risk of potential contamination and infection of preimplantation embryos.

Some investigators have used synthetic polymers such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) in ART but neither can be considered a physiological alternative to protein.

When the human embryo culture media were supplemented with serum some harmful effects have been attributed in the embryo development. These harmful effects are morphological changes, early blastulation and disturbance in the energy metabolism. Due to these disadvantages the media was supplemented with human serum albumin instead of whole serum. Albumin is mostly found in the reproductive tract thus it has a lot of advantages over whole serum. It also helps in invitro handling of the gametes. It helps the embryo not sticking into the bottom the culture dishes due to the effect on the surface tension.

Hyaluronan is another molecule that is found in the tract that is used for the preparation of the culture media. Hyaluronan is said to increase the implantation as well as the aid in the survival of the embryos after the cryopreservation.

Antibiotics:

Embryo culture medium is prone to contamination due to the presence of numerous components. Embryo culture media are routinely supplemented with antibiotics to prevent bacterial contamination. Nowadays, commonly used antibiotics are penicillin (β-lactam; 100U/ml), streptomycin (aminoglycoside; 100 µg/ml) and gentamycin (aminoglycoside; 50 µg/ml). The anti-bacterial effect of penicillin is attributed to its disturbance of cell wall integrity through the inhibition of the synthesis of peptidoglycan. Penicillin has no direct toxic effects on the preimplantation embryo. Streptomycin and gentamycin disturb bacterial protein synthesis. However, the aminoglycosides show more toxic effects.

Protein supplement:

Working without albumin has been attempted several times but protein or similar macromolecule (polyvinyl alcohol or bicarbonate) is required for IVF procedures for several reason:

1)Sperm capacitation: albumin acts as the sterol acceptor molecule in the media.

2)Handling: prevents the embryos and sperms from sticking to pipettes and tubes.

3)Protein as a peptide and lipid carrier: Recombinant human serum albumin (HSA) is used in the medium as a source from 2002.HSA contains approximately 4.5% albumin (45mg/ml).

Vitamins:

The addition of vitamins as antioxidants to the culture media containing glucose and phosphate helped to prevent a loss in respiration and metabolic control. The following possible vitamins are components of different ART culture media: ascorbic acid, cyanocobalamin, folic acid and tocopherol. Their optimum concentrations were determined using mouse zygote assays. Moderate dosages of vitamins C and E were seen to reduce oxidative damage in mouse embryo culture and improve their blastocyst development rate.

Growth factors:

Mammalian embryos are naturally exposed to a complex mixture of growth factors that play a key role in growth and differentiation from the time of morula to blastocyst transition. However, defining their role and potential for improving in-vitro pre implantation development is complicated by factors such as gene expression of both the factors and their receptors. The blastocyst expresses ligands and receptors for several growth factors, many of which can cross-react thus making it difficult to interpret the effect of single.

KEY COMPONENTS OF MODERN CULTURE MEDIA

Components	One media system Gynemed GM501®	Sequential media G-1™PLUS	Sequential media G-2™PLUS
Salts	Sodium chloride	Sodium chloride	Sodium chloride
	Potassium chloride	Potassium chloride	Potassium chloride
	Calcium chloride	Calcium chloride	Calcium chloride
	Monopotassium phosphate	Sodium citrate	Sodium citrate
	Magnesium sulphate	Magnesium sulphate	Magnesium sulphate

Components	One media system Gynemed GM501®	Sequential media G-1™PLUS	Sequential media G-2™PLUS
		Sodium dihydrogen phosphate	Sodium dihydrogen phosphate
Buffer	Sodium bicarbonate	Sodium bicarbonate	Sodium bicarbonate
Energy Substrates	Glucose	Glucose	Glucose
	Sodium lactate	Sodium lactate	Sodium lactate
	Sodium pyruvate	Sodium pyruvate	Sodium pyruvate
Non-Essential AA's	NEAA's 8	NEAA's 9	NEAA's
Glutamine Dipeptide	Alanyl-Glutamine		
Essential AA's	EAA's 2	EAA's 11	EAA's
Chelator	EDTA	EDTA	none
Macromolecules	none	Hyaluronan, HSA	Hyaluronan, HSA
Fatty acid	none	Lipoic acid	none
Vitamins	none	none	4 Vitamins
Indicator	Phenol Red optional	none	none
Antibiotic	Gentamicin	Gentamicin	Genamicin
Water	yes	yes	yes

SYSTEMS OF MEDIA

Selection of the proper culture media in embryo culture is the key factor. It can influence the development of the embryos in vitro culture. Other factors influencing are Carbon dioxide, oxygen level and the number of incubator chamber.

Sequential culture media "back-to-nature"

Back to nature is the sequential media principle. It has two phases of the first phase medium mimics the environment in the fallopian tubes to support the human oocyte fertilization and the early embryonic development and the second phase medium mimics the environment in the uterus where the embryo develops to blastocyst stage.

The "back-to-nature" attempts to mimic the changing needs of the developing zygote and embryo in a media should approximate the concentration to which the embryo is naturally exposed. The embryo is capable of actively controlling ionic gradients etc, and is able to regulate its internal environment. Therefore, with regard to embryo physiology, it is appropriate to consider the preimplantation period in at least two phases: pre-compaction and post-compaction. Such a breakdown of the preimplantation period is of importance when one considers changes to medium formulations. Other considerations include the time at which the embryonic genome is activated.

There has been a remarkable rise in the usage of sequential media system where in the first 3 days one medium is required while the other 2 days requires the other medium to support morula compaction and blastocyst development. Since the composition of the oviduct fluid varies from uterine, two different medias are used so as to imitate in vivo conditions of embryos. Also, the harmful byproducts are eliminated such as ammonia and to ensure adequate supply of the substrates replenishing of the media is involved in the method.

Single-step media "let-the-embryo-choose"

The principle of single-step media is to let the embryo decide the needs during development and differentiation. This culture media has all the mixture of all required components. In this media system the entire components required for the embryo is provided. Thus, there is no need of replenishing the media in a daily basis. Thus, during the development of the embryo till the blastocyst a single formulation is used.

The design of a culture medium involves the simultaneous use of all the concentrations in a mixture because the effects of each component in the medium may depend on the concentrations of the other components. As long as concentrations

are within 'tolerable ranges', the embryo itself will adapt and utilize whatever it requires. This philosophy led to a family of media in which all of the substances necessary to early embryological development is provided, and there is no need for a media change. One-step formulation is applied throughout the entire in-vitro development from fertilization to the blastocyst stage of the embryo.

COMMERCIALLY AVALABLE HUMAN IVF CULTURE MEDIA

One media system			
Company	**Medium**	**Culture period**	**Website**
Life Global	global®	day-1 to day-5/6	www.lifeglobal.com
Gynemed	GM501	day-0 to day-5/6	www.gynemed.de
IrvineScientific	SSM™	day-0 to day-5/6	www.irvinesci.com
Sequential media system			
Company	**Medium**	**Culture period**	**Website**
Cook Medical	Cleavage K-SICM	day-1 to day-3	www.cookmedical.com
	Blastocyst K-SIBM	day-3 to day-5/6	
CooperSurgical	Quinns Advantage®Cleavage	day-1 to day-3	www.coopersurgical.com
	Quinns Advantage®Blastocyst	day-3 to day-5/6	
FertiPro	FERTICULT™IVF Medium	day-1 to day-2	www.fertipro.com
	FERTICULT™ G3 Medium	day-3 to day-4	
InVitroCare	IVC-TWO™	day-0 to day-3	www.invitrocare.com
	IVC-THREE™	day-3 to day-5	
Irvine Scientific	ECM®	day-0 to day-3	www.irvinesci.com
	MultiBlast®	day-3 to day-5	
Origio	EmbryoAssist™	day-0 to day-3	www.origio.com
	BlastAssist™	day-3 to day-5	
	ISM1	day-0 to day-3	
	ISM2	day-3 to day-5	
Vitrolife	G-1™PLUS	day-1 to day-3	www.vitrolife.com
	G-2™PLUS	day-3 to day-5	
	IVF™	day-0 to day-3	
	CCM™	day-3 to day-5	

TYPES OF EMBRYO CULTURING SYSTEM

There are two types of embryo culturing systems, they are

*Coculture system

*Open and close culture system

Coculture system

In humans, the first attempts to obtain blastocysts with classical culture media were disappointing, and the use of a coculture strategy was naturally tempting: the first significant results of successful blastocyst development were obtained in the early 1980s, using trophoblastic tissue as a feeder layer in order to mimic an autocrine embryotropic system.

Embryo coculture in the cleavage and blastulation, reduced the fragmentation, with good implantation rates. The media supplemented are maternal serum or protein substrate. If the implantation is failed even if the embryo quality is good then, the use of coculture is recommended because they can produce embryotropic factors. Apart from oviduct cells, endometrial cells, granulosa cells and Vero cells are also used.

Coculture of the human embryos cell with the autologous and heterologous somatic cell lines offered a means of successfully culturing human blastocyst in vitro. In the early 1990s Menezos group in France and Bongso in Singapore coculture human embryo with viro cells and tubal ampullary cells respectively.

Open and close system

In the close system the microdroplets are covered with paraffin oil to maintain the osmolarity, PH and temperature of the media while in the open system the media is not overlayed with the oil. The main difference between the two systems are the amount of media required. In the close system we require small volume of the media (25 -100 µl/drop) while in the open system we require larger volume of the media (0.5-2ml/dish).

In the open system there is a rapid fluctuation of the of temperature, Ph, osmolality. Due to homogenous there is distribution a better rate of fertilization in the close system culture media.

CULTURE CONDITION

Each off the culture conditions affect directly to the fertilization process of the embryos. They include as follows:

Osmolality

The osmolality of human fallopian tube fluid is more than 360 mOsmol. The osmolality of the culture media is usually kept around 300 mOsmol. If osmolarity is higher than this then there is the chance that it can harm the embryo development. If amino acid is added to the culture media, then the culture media becomes the natural osmolyte.

Studies demonstrates that various amino acids provide protection against high osmolality and thus it is recommended in all the culture media to aid this regulation. In addition, choice of culture volume can impact media osmolality due to evaporation. Use of larger volume of media helps in combat resulting osmolality rise, oil overlay and use of humidified incubators helps to prevent dramatic change in osmolality.

Impact of Ph

The pH only refers to hydrogen ion concentration and is only meaningful when applied to aqueous (water-based) solutions. When water dissociates it yields a hydrogen ion and a hydroxide ion. It must be noted that pH is dynamic. The balance of pH depends on the association or dissociation of compounds. The most important ions are sodium, potassium, magnesium, chloride and lactate and also the AA glycine which acts as an intracellular zwitterionic buffer.

An acceptable pH range for embryo culture media may be set between pH 7.2 and 7.4. Culture media pH is regulated by the balance of CO2 concentration, supplied by the media and by the concentration of bicarbonate in the media. However, the intracellular pH in human cleavage embryonic cells is pH=7.2 and pH is an important cellular function which is necessary to maintain intracellular homeostasis. Moreover, after the compaction stage the preimplantation embryos appear to have more control over their intracellular pH, because of the formation of tight junctions between cells. Hence, there is a trend to culture cleavage stage embryos in a slightly lower pH and morulae and blastocysts in a slightly higher pH.

Temperature and light

Temperature plays a vital role in the embryo culture. When the embryos are exposed to low temperature there is a chance of aneuploidy. High temperature more than 39 °C, it can also disrupt the microtubule organization. The exposure to suboptimal temperature also affects transmembrane transport and metabolism and slows down the development of the embryos.so, it is essential to maintain the temperature at 37°C. Low light setting is preferred in the human IVF lab. The exposure of the embryos to the light should be kept at minimum.

REVIEW OF THE LITERATURE

Numerous recent studies have been conducted to compare the effectiveness of commercially available ART culture media types. Interestingly, most studies prefer 3-day human IVF embryo culture and embryo transfer for comparison of different media types. Differences in embryo quality were observed in studies that used modern formulated media versus standard media, but no differences in pregnancy rates were reported. Moreover, no differences between a single or one-step defined medium versus a cleavage-stage media with regard to fertilization, pregnancy implantation rates, and ongoing pregnancy were found by following studies. Ebert et. Only the rate of pregnancy losses was significantly lower in patients with the one-step medium GM501 as compared to the Universal IVF medium.

Two other studies compared a single-step versus a sequential media system for the development of the human embryos to the blastocyst stage. IVF-embryos cultured in the single-step medium showed cytoplasmic pitting. IVF-blastocyst formation rates were not significantly different between the two media systems.

CONCLUSION REMARK

In order to maintain the optimal conditions many parameters should be followed in IVF lab for the development of the embryos. Thus, routine control must be kept regularly at different stages so that the environment for embryo growth becomes favourable. Exposure to different stress situation to the developing embryos must be avoided.

Human embryos can develop in vitro in rather different types of media from basic systems to sequential complex culture media. ART culture media contain only a subset of parts which are found under in vivo conditions. Hence, embryos cultured in-vitro was exposed to constant stress. Suboptimal culture conditions force the embryo to undergo adaptations, and thus lead to lower pregnancy and higher abortion rates.

It is evident that all necessary steps in ART as part of the treatment of infertility can influence the epigenetic programming during early development. Therefore, it is essential that a high level of quality control exists in the laboratory, and it is suggested that further investigations are necessary to optimize environmental conditions.

The culture system and the culture media must be very particular. A detailed attention must be paid to them. However, it is our duty as reproductive scientist to take good care of the embryos to **create embryos with good quality having higher implantation rate.**

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