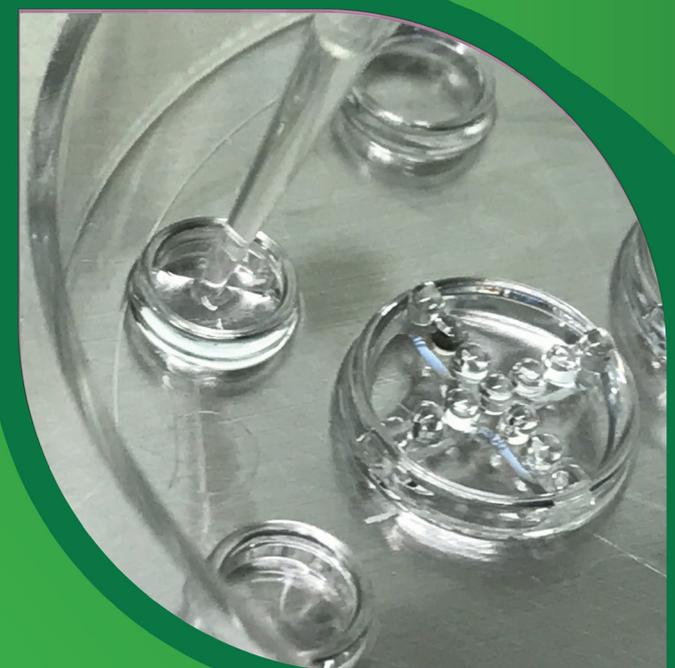


# NEXUS

Indian Fertility Society &  
ORIGIO India Initiative

"Ideal culture media:  
An Enigma?"





Culture media are an essential part of a culture system that aims to minimize insults and stress to gametes and embryos. They are designed to provide an optimal In vitro environment and offer a balance of ions, energy substrates and nutrients thus safeguarding the best possible clinical outcome. A well-functioning culture system is influenced by multiple factors including air quality, temperature, CO<sub>2</sub>, pH levels, and the level of expertise of laboratory Team.

The constantly changing in vivo environment symbolizes a specific chemical composition at every stage of embryonic development, together with factors such as culture embryonic density and an ideal CO<sub>2</sub> environment along with pH, to name just a couple of critical parameters

In this issue, we discuss the nuances of embryo culture and we are sure that the bulletin would benefit embryologist, andrologist and lab directors alike.

It is a great privilege and pleasure to write this message for the 5<sup>th</sup> E-bulletin of IFS-Nexus. I also sincerely thank “ORIGIO India Private Ltd” for supporting us in this academic endeavour.

**Dr. Sohani Verma**  
**President-IFS**



This is an honour for me to write best wishes message for this very special Nexus E-bulletin on “ Understanding Embryo culture”.

As the science of culture media for Assisted Reproductive Technology (ART) develops, paralleling further advancements in the reproductive medicine field, we find ourselves at a critical Juncture at which increasing research and strive for perfection is directing further exploration in this field to help improve clinical outcomes in human in vitro fertilization (IVF). We commonly ask our self, is there an ideal culture medium? Is any media better than another?

This bulletin would cover various steps involved in the procedure of embryo culture and try to find answers to these complex unanswered questions.

Indian fertility Society feels proud and congratulates the editors on the launch of the 5<sup>th</sup> edition of Nexus E-Bulletin. It would not only help to disseminate scientific & ethical content but also constantly update everyone with new researches and developments across the world.

I wish the editorial team best of luck in this endeavour.

**Dr. K.D. Nayar**  
**Secretary General-IFS**



At the very onset, the editorial team would like to thank all of you for positively appreciating our previous E-bulletins of Nexus. Team 'Nexus' sincerely hopes to bring out such teaching material for you regularly and the bulletin is appropriately named NEXUS - which means building bridges.

Such bulletins are the call of the day and enormously bridge the gap between the existing knowledge and recent advances. Our present edition is focused on simplifying the process of embryo culture and covers all essential details with nice algorithms. Part 1 deal with

commonly asked questions and part 2 with bench side work. I am sure it will immensely benefit you all.

The aim of this bringing out this bulletin is educating us about the finer aspects of Embryo culture and its nuances.

An interesting evolution has occurred in the development of IVF culture media, which provides perspective for the future of this subject. The development of culture media for human IVF remains on a forever-evolutionary path.

A medium designed for particular cells must come as close as possible to providing the nutritional and physical milieu that allows the unique genetic and epigenetic functional capacities of the cell to be expressed. Moreover the practical use of such media is also very essential and important to understand.

We would make an endeavor to simplify the process of media handling and usage for the beginners and experienced alike and hope the bulletin would help you to improve your results.

Our motto is "knowledge empowers" and we sincerely hope that you would enjoy reading this edition of Nexus.

Feel free to communicate with us at any point of time and contribute critically. Your comments would be published in the next bulletin, which is titled " Vitrification in ART".

We would also like to place on record our truthful thanks to Origio India limited who are helping us in the publication of this bulletin and off course I promise that there is no conflict of interest at any level.

Wish you happy reading and yes don't forget to file this issue.

I would formally like to thank my friend Dr Ashish Fauzdar of New Delhi who has worked un-relentlessly towards bringing out this issue from conception to end.

**Prof (Dr) Pankaj Talwar**  
**Joint Secretary-IFS**  
**Editor NEXUS**

# Index

<b>S.No.</b>	<b>Topic</b>	<b>Page No.</b>
1.	Origin of Culture Media	6
2.	Requirement of Embryos	6
3.	Composition of Media on Day 3, Day 5	7
4.	Advancement in culture media	7
5.	Regular culture media for cell culture	
6.	Sequential Culture media	9
7.	Monoculture systems-Single Step media	9
8.	Recombinant additives in culture media	9
9.	Advantage of one media over another	9
10.	Paraffin and mineral oil	10
	Different types of Oils	10
	Pitfalls of oil during cleaning and transportation	10
	Correct storage condition of Oil	10
	Thickness of Oil for embryo culture	11
	Right Oil for embryo culture	11
11.	ICSI and PVP media	11
12.	Expiry of media	11
13.	Cold chain maintenance during transportation	11
14.	Role of cytokines in culture media	11
15.	QA & QC of media	12
16.	Different type of culture media used in embryo culture	12
	Flushing/Rinsing IVF Media	12
	Fertilization Media	12
	Cleavage Stage Media	12
	Blastocyst Stage Media	13
	Embryo transfer media	13
17	Role of different constituents of IVF media	13
	Amino acids	13
	Carbohydrates	14

<b>S.No.</b>	<b>Topic</b>	<b>Page No.</b>
	Chelators	14
	Buffers	14
	Macromolecules	15
	Phenol red	15
	Inorganic salts	15
	Vitamins	15
	Antibiotics	15
18.	<b>EmbryoGen</b> <sup>®</sup> range of media	16
19.	EmbryoGlue <sup>®</sup> media	16
20.	Embryo density of culture media	16
21.	Available methods for embryo culture	17
	Micro-drops embryo culture	17
	Specific Culture dishes	17
	Universal GPS Dishware <sup>®</sup>	
	Embryo Corral <sup>®</sup> Dishes	18
	Volume of Mineral Oil Overlay	18
22.	Protocol for Embryo Culture: Hands On	19
	Day -1 Activity	19
	Dish preparation	19
	Oocyte Collection & Insemination Dish Day 0 Activity	19
	Ovum Pickup	19
	Insemination or ICSI	20
	Preparation of Single Well Dish for post denudation culture on Day 1	20
	Day 1 Activity	20
	Embryo culture post fertilization/denudation	
	Day 2 Activity	20
	Day 3 Activity	20
	Day 5 Activity	20
24.	References	20
23.	Tips and Tricks about Incubator & Gas System	21

## Frequently asked questions

### 1. How did the Culture media for IVF originate?

Culture media are an essential part of a culture system that aims to minimize insults and stress to gametes and embryos. Designed to provide an optimized in vitro environment, culture media provide a balance of ions, energy substrates and nutrients, safeguarding the best possible clinical outcomes.

A well-functioning culture system is influenced by many factors including air quality, temperature control, CO<sub>2</sub>, pH levels, and the level of expertise of laboratory staff.

Culture media for embryo growth was first described in 1912 for the growth of an embryo of a rabbit. Later on in 1949 mouse embryos were grown in culture media from the 8-cells stage to blastocysts. These culture media, like Earle, Ham's F10, Tyrode's T6 and Whitten's WM1 were based on different salts and were constructed to support the development of somatic cells and cell lines in culture. These culture media, known as physiological salt solutions were used by Robert Edwards for his first successful IVF attempt.

Historically human embryos were cultured in either Simple salt solution or Complex tissue culture media.

- **Simple Salt media** → Earle's, T6 and HTF medium with balanced salt solutions with added carbohydrates, glucose, pyruvate and lactate supplemented with patient serum.
- **Tissue Culture Media** → Ham's-F 10, MEM, TCM-199 designed to support immortal cell lines supplemented with carbohydrates, amino acids, vitamins, nucleotides and metal ions.

None of these earlier culture media were designed to support embryo development. They contain many components that were detrimental to embryo development in vitro such as high levels of glucose, divalent metal ions, nucleotides and hormones. Although these different culture media support the development through the first three-cleavage division, they are not able to support blastocyst development.

### 2. What are the requirements of embryos as they grow?

The mammalian embryo undergoes significant changes in physiology, molecular regulation and metabolism as it grows. The human embryo is a highly dynamic entity with its changing needs from one of the most quiescent tissues in the body (the oocyte) to being the most metabolically active (the blastocyst).

- **Mature Oocyte** → Low biosynthetic activity, Low level of Oxygen consumption (QO<sub>2</sub>), needs Pyruvate as primary energy source with Non-essential amino acids.
- **Day 5 Embryo (blastocyst stage)** → High Biosynthetic activity, High oxygen utilization (QO<sub>2</sub>), Glucose as preferred energy source and requires both Non-essential and essential amino acids.

**Table 1. Difference in the physiology of the mammalian embryos for development from the zygote to the blastocyst stage.**

Precompaction stage	Postcompaction stage
Low biosynthetic activity	High biosynthetic activity
Low QO <sub>2</sub>	High QO <sub>2</sub> (Metabolic Quotient)
Pyruvate based metabolism	Glucose based metabolism
Maternal genome	Embryonic genome
Low ability to maintain cellular homeostasis	Complex systems for maintenance of cellular homeostasis

The nutrient available within the human female reproductive tract fluid also mirrors the nutrient preference of embryo:

- **Fallopian Tube fluid** → Needs, High concentration of Pyruvate (0.32 mmol/l), high concentration of lactate (10.5 mmol/l), low concentration of glucose (0.5 mmol/l)
- **Uterine fluid** → Needs Low level of Pyruvate (0.1 mmol/l), Low level of lactate (5.87 mmol/l) and high concentration of glucose (3.15 mmol/l)

### 3. What are the Contents of media on Day 3 , Day 5.?

Embryo culture media includes with following main constituents in varying concentration at different stages of development.

1. Glucose, Organic acid, Lactate & Pyruvate
2. Essential and Non-essential amino acids
3. Electrolytes and Metals

**Table 2. Composition of human embryo culture media G1 (Cleavage Day 2/3) and G2 (blastocyst Day 5/6).**

Component (mM)	G1 (Cleavage Day 2/3)	G2 (blastocyst Day 5/6)
NaCl	85.16	85.16
KCl	5.5	5.5
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	0.5	0.5
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1.8	1.8
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0	1.0
NaHCO <sub>3</sub>	25.0	25.0
Na pyruvate	0.32	0.10
Na lactate (L-isoform)	10.5	5.87
Glucose	0.50	3.15
Glutamine	1.0	1.0
Taurine	0.1	0.0
Non-essential amino acids	All	All
Essential amino acids	None	All
EDTA	0.01	0.0
Human serum albumin	2 g/l	2 g/l

[\*Adapted from David K.Gardner and Michelle Lane1, Human Reproduction Update 1997, Vol. 3, No. 4 pp. 367–382]

### 4. Advances in the media.

In recent years there has been change in the way medias are formulated and several media have been designed to support development upto blastocyst stage such as DM1/2/3, G1/G2 media, KSOM and Quinns Advantage. Until recently, it was common practice for clinics to prepare their own culture media in house, either by making them from individual chemicals for preparing simple media or by reconstituting preformulated powder in case of tissue culture media.

In 1984-1985 special media were developed for human IVF. Menezo and his colleagues published a paper, in 1984, describing a new concept in Human Embryo culture. They suggested adding serum albumin as a source for amino acids. The serum protein ensures that oocytes and embryos do not adhere to the glass surface of the pipette used to manipulate them.

In 1985 Quinn et al. published in the journal Fertility and Sterility a formula entitled Human Tubal Fluid (HTF), which mimics the in vivo environment to which the embryo is exposed. The formulation of HTF was based on the known chemical composition of the fluids in human fallopian tubes as known at that time. This medium is based on a simple balanced salt solution without amino acids; however, the concentration of potassium was adjusted to that measured in the human female reproductive tract. This medium was found to be better compared with earlier media developed. In last decade there has been major shift away from in house media preparation to commercially produced media manufacturing procedure with strict regulatory and quality control. Currently there are essentially two types of media that are widely used for clinical IVF: sequential culture systems or monoculture systems.

## 5. Requirement of embryos as they grow.

The regular culture media for human embryos include simple salt solution **Earle's, T6 and HTF medium** with balanced salt solutions with added carbohydrates, glucose, pyruvate and lactate supplemented with patient serum. Alternatively complex tissue culture media like **Ham's -F 10, MEM, TCM-199** designed to support immortal cell lines supplemented with carbohydrates, amino acids, vitamins, nucleotides and metal ions were used for human embryo culture.

**Table 3. Composition of human tubal fluid (HTF) and Ham's F-10 media**

HTF		Ham's F-10			
Component	Concentration (mM)	Component	Concentration (mM)	Component	Concentration (mM)
NaCl	101.60	NaCl	126.60	Lysine	0.20
KCl	4.69	KCl	3.82	Methionine	0.03
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.20	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.62	Phenylalanine	0.03
		Na <sub>2</sub> HPO <sub>4</sub>	1.31	Proline	0.10
KH <sub>2</sub> PO <sub>4</sub>	0.37	KH <sub>2</sub> PO <sub>4</sub>	0.61	Serine	0.10
CaCl <sub>2</sub> ·2H <sub>2</sub> O	2.02	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.30	Threonine	0.03
		CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.00001	Tryptophan	0.003
		FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.0030	Tyrosine	0.12
		ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.0001	Valine	0.03
NaHCO <sub>3</sub>	25.00	NaHCO <sub>3</sub>	14.28	Biotin	0.0001
Sodium pyruvate	0.33	Sodium pyruvate	1.00	Ca pantothenate	0.0015
Sodium Lactate	21.40	Calcium Lactate	2.23	Choline chloride	0.005
Glucose	2.78	Glucose	6.11	Cyanocobalamin	0.001
		Alanine	0.10	Folic acid	0.003
		Arginine	1.21	Inositol	0.003
		Asparagine	0.11	Nicotinamide	0.005
		Aspartic acid	0.10	Pyridoxine	0.001
		Cysteine	0.26	Riboflavin	0.001
		Glutamate	0.1	Thiamine	0.003
		Glutamine	1.0	Hypoxanthine	0.03
		Glycine	0.1	Lipoic acid	0.001
		Histidine	0.14	Thymidine	3.00
		Isoleucine	0.20	Leucine	0.10

## 6. What are Sequential media?

The sequential media were developed to accommodate the dynamics of embryos nutrition and to mirror the environment of the female reproductive tract. The embryos are exposed to a gradient of nutrients as it passes along the fallopian tube in to the uterus.

Sequential media systems are designed initially to provide optimal support for the development of the cleavage stage embryos. The culture media is subsequently changed to allow for optimal conditions for the extended culture to the blastocysts stage. These media systems are formulated to address the changing requirement of the developing embryos, thereby reducing the cellular stress and limiting the need for the embryo to undergo adaptation to grow to the blastocyst stage.

Cleaving embryos use pyruvate and lactate as energy sources and non-essential amino acids (NEAA) for protein metabolism. 8-cell stage onwards the major energy source is glucose and the embryos essential amino acids (EAA) are required for protein metabolism.

## 7. Monoculture systems-Single step media

Monoculture systems are a single medium formulation that is used to support zygote development till blastocyst stage. Monoculture is based on the principle of allowing the embryo to choose what it wants during preimplantation development. A monoculture system provides continuous support to the embryo throughout extended culture; hence there is no need to refresh the medium.

**Single step media** have optimized energy substrates like D-glucose and L-lactate supporting uninterrupted continuous culture. These are designed to reduce ammonium build-up through the use of a stable glutamine source. In addition they have High  $Mg^{2+}$  to  $Ca^{2+}$  ratio to limit detrimental stress-induced calcium influx into the embryo. Use of bioactive L-lactate helps in better control of intracellular pH. The medium can also be used for embryo transfer.

## 8. Recombinant additives in the culture media

For an oocyte that is removed from its natural environment, a culture medium that meets the essential requirements for its survival and growth in vitro is required.

Albumin is the most abundant macromolecule in the human oviduct, and for that reason alone, it is certain to remain a recommended supplementation of media as a protein source.

- **Recombinant Human Serum Albumin**

Recombinant albumin has been proven to be structurally identical to plasma-derived human serum albumin (HSA). It possesses several advantages including higher LOT-to-LOT consistency, greater homogeneity, and less endotoxin levels than HSA. Moreover, recombinant albumin is free from viral/prion contamination risk and plasma-derived impurities.

- **Hyaluronan**

is a high molecular mass polysaccharide that can be obtained endotoxin and prion free through recombinant DNA technology. It has been demonstrated that hyaluronan improve mouse and bovine embryo culture systems but also its use for embryo transfer results to increase the embryo implantation. In one of the largest prospective trail to date, which enrolled 1282 cycles of IVE, it was determined that use of hyaluronan enriched medium was associated with significant increases in clinical pregnancy rate and implantation rates, both for day 3 and day 5 embryo transfer.

## 9. Advantages of one media over others. Is any media better?

Early embryos are capable of adjusting to a range of nutrients in the oviduct and uterus as a way of surviving the variable conditions encountered by the mother during gestation.

Every medium provides nutrients within a sufficiently narrow range of concentrations that do not disrupt embryo homeostasis or metabolism to such a degree that embryonic viability or offspring phenotype is affected.

It is still unclear how the composition of the media affects embryo quality and IVF/ICSI success rates and which if any culture medium leads to the highest IVF/ICSI success rates. Mantikou et al. (2013) performed a systematic review that included 22 studies of 20 different culture media from 11 commercial companies, but was unable to find a superior culture medium from the pooled data. It is unlikely that a perfect medium composition exists or that only one formulation is suitable for human embryos.

## 10. Paraffin and mineral oil.

Most clinics today use oil to cover embryo culture dishes in order to maintain stable temperature, osmolality and pH. The quality of oil plays a very important role for IVF success.

The oil types commonly used are **mineral oil, paraffin oil or a mixture of both** these oil types. Paraffin oil and mineral oil are chemically slightly different. The polycarbon lipid tail of mineral oil contains more unsaturated bonds than the more saturated paraffin oil. This makes mineral oil more unstable and prone to attack by free oxygen radicals and photo-oxidation than paraffin oil.

The paraffin oil is pre-washed with HSA-containing medium and have very low endotoxin and peroxide levels. **Many studies have revealed that Paraffin oil gives better development of embryo's.**

### • What are different types of oils exist and how do they differ?

Oil is a term that only specifies that the substance is a non-polar and viscous liquid. It may have been produced from animal or plant materials, it can come from crude oil (often named mineral oil) or it can be synthetic. The two main types of synthetic oil are silicon oil and poly alpha olefin (PAO) oil.

	Plant/Animal derived Oil	Mineral oil	Paraffin oil	Synthetic oil
<b>Origin</b>	Plant/animal derived	Petroleum derived	Type of mineral oil	Artificially produced
<b>Structure</b>	triglycerides Hydrophobic carbohydrate chains linked as esters to a hydrophilic molecule of glycerol	Alkanes that can be straight, branched or ring shaped.	Alkanes with straight and branched shape	Synthesised by polymerising siloxane with side-chains
<b>Purity</b>	Sensitive to oxidation	Highly purified	Refined, increased purity- less reactive to chemical attacks such as e.g. oxidation	Contain low molecular weight chain residues that are probably toxic
<b>Usage</b>	Not recommended for IVF	Can be used for IVF	Can be used for IVF	Tested in IVF

### • Potential pitfalls during cleaning and transportation

Most oil are clean immediately after the refining. The oil may also be filtered in order to remove waxes that might otherwise make the oil look hazy.

One common method of cleaning is a quick rinse using lower molecular weight hydrocarbons but tensides may be used as well. The result is oil that is contaminated with an increased level of low molecular weight hydrocarbons or that contains tensides. Both have a negative influence on the gametes or the developing embryo.

### • Is the correct storage condition of oil secures embryo viability?

The paraffin oil for IVF is fully saturated and is still at risk of been oxidized during storage. This process is accelerated when the oil is stored at an elevated temperature and exposed to light. The first step involves the formation of peroxide. Peroxides are very reactive and will do a lot of damage to any cells that they come in contact with.

Food grade oils are considered good until the peroxides level as high as 10 – 12 meq/kg but the peroxide concentrations needed to have a negative influence on embryos is just above 0.02 meq/kg.

Such low levels are hard even to measure unless special equipment is used. When the oxidation continues, the peroxides turn into aldehydes or ketones, molecules that also have a large potential for biological activity.

The last two steps, oxidation to carboxylic acid and carbon dioxide are actually making the oil better again for the embryos.

#### • How thick layer of mineral oil is needed to cover the culture?

Even the thinnest 1mm layer of oil, reduces the evaporation but it takes a 2mm layer to fully protect the media from evaporating. Once the layer is 3 mm thick, an additional thickness will not further reduce the rate of evaporation.

#### • Which is the right oil for embryo culture?

The oil is produced by distillation of petroleum followed by purification steps to remove contaminants such as sulphur, and finally hydrogenation where hydrogen molecules are added to all the double bonds, creating fully saturated oil. Mineral oil is the type of oil currently used in IVF. There is however a lot of variance between these oils and they vary in quality. A good mineral oil for IVF consists of similar sized aliphatic molecules

Fully saturated oils are fairly inert and will not react quickly at normal temperatures with e.g. oxygen. They are however still sensitive to oxygenation, especially if they are kept warm or in sunlight. Usually the oil that is used in IVF is already saturated with water so that it won't absorb water from the culture media.

## 11. ICSI and PVP media

**ICSI medium** is utilized for sperm immobilization and the selection of mature sperm for ICSI through hyaluronic acid binding. It is natural and biodegradable alternative to PVP. One step immobilization and selection of mature sperm for ICSI and have Improved embryo quality and development. This can also used for approaching “physiological” ICSI.

**PVP Medium** is used for slowing down the movement of the spermatozoa for ICSI.

## 12. Expiry period of media

In-vitro fertilization (IVF) laboratories all over the world rely heavily on the utilization of commercially available media for assisted reproductive procedures.

A common characteristic of all these commercial media is that they possess a **short life span, usually expiring in 6-8 weeks**. From a regulatory point of view it is illegal to use expired media in clinical procedures involving patients. Two vital issues, **depletion of nutrients** and **weakening of the buffering capacity as time progresses**, guide the manufacturers to set the shelf life for the safe use of the media.

## 13. Cold storage and cold chain from shipment to the receiving unit.

IVF personnel's working in the laboratory must check for the temperature of embryo culture media to ensure the maintenance of cold chain from the manufacturing unit to laboratory during transportation. Always ask the vendor for certificate for maintenance of cold chain management for ensuring appropriate delivery condition on receipt with every new lot.

## 14. Role of cytokines in the IVF culture media

Cytokines drive the dialogue between the embryo and endometrium and are increasingly expressed throughout embryo development. The cytokine Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is a natural signalling molecule that allows for both autocrine and paracrine communication between the embryo and endometrium.

Communication between the embryo and endometrium is crucial in creating the right environment for a successful pregnancy.

Compromised embryo competence, impacting the maternal-embryo dialogue, may lead to an increase in implantation failure, preclinical pregnancy loss and miscarriage.

Such media closely mimics the environment found in the female reproductive tract at conception. Creating the best possible in vitro conditions for the embryo, with the use of a cytokine, will promote successful implantation through improved endometrial receptivity.

## 15. QA and QC of the media

The Ideal media should be adequately tested and should meet the following features.

pH (in air): 7.3-7.5

Osmolality: 285-295 mOsm/kg

MEA:  $\geq 80\%$

Endotoxins:  $< 0.4$  EU/mL

Shelf Life: 8 weeks from date of shipping

Sterile Filtered: (SAL  $10^{-3}$ )

## 16. What are different types of culture Media's we routinely use in the laboratories?

### • Flushing / Rinsing IVF Media

HEPES or MOPS buffered media are designed specifically for rinsing and flushing needles, tubes, washing of the cervix before embryo transfer and for flushing of OCC complex after oocyte collection. Such media does not need incubation but only warming for one hr before use. G-MOPS PLUS is also supplemented with human serum albumin.

These maintain the pH of the cells at  $37^{\circ}\text{C}$  and are designed to be used specifically in ambient atmospheric air conditions and not an enriched  $\text{CO}_2$  environment.

The media contains nonessential amino acids to assist the maintenance of homeostasis within the oocyte-cumulus complex during the collection procedure. Amino acids have a protective role that helps the embryo to maintain homeostasis. This is of particular importance during early stages of development, before the formation of the transporting epithelium. Media with amino acids also aids in minimising intracellular stress. By having amino acid in the media the gametes and embryos are better protected against variations in the environment.

Flushing media containing HEPES may be used for various semen washing procedures also.

### • Fertilization Media

Fertilization medium contains all components necessary to support oocyte and sperm function during fertilization. It contains glucose and fructose to support both cumulus cells and sperm functionality.

These are Bicarbonate buffered medium, which provides a glucose-rich environment for efficient oocyte-cumulus complex and sperm cell metabolism. Helps provide a suitable environment for gamete fusion that includes antioxidants and nonessential amino acids.

After the oocyte-cumulus complex has been washed with flushing HEPES/MOPS based buffers, they are placed in Fertilization Medium, where insemination is carried out.

These media contains glucose to assist sperm function and provides a metabolite for the cumulus and coronal cells. The inseminated oocyte can remain in this medium for up to 20 hours. After checking for the presence of Pronuclei, the PN are then transferred into Cleavage Medium.

### • Cleavage Stage Media

Bicarbonate-buffered media cultures human embryos from the PN stage to day 2 or day 3. This medium contains amino acids to support embryo viability and is buffered by bicarbonate.

Being Low in glucose and high in pyruvate helps in optimizing early cleavage stage development. These are recommended for post ICSI Culture of oocytes to reduce stress when cumulus cell metabolism and sperm cell movement are no longer critical. ICSI can be performed in this medium, as glucose is required only for sperm function and the cumulus complex. The media can be used for embryo transfer on day 2/3 or we can shift the embryos to blastocyst media for further culture

for Day 5 culturing of the embryos.

#### • Blastocyst Stage Media

The media is used for culture of human embryos from the 8-cell stage / D3 until the blastocyst stage and embryo transfer. Once an embryo has reached the Day 3 (eight-cell) stage, it is then transferred into Blastocyst Medium. This media has been metabolically well adjusted to maximize blastocyst development rate and are suitable for blastocyst transfer also. Bicarbonate- buffered medium has a higher glucose concentration to maximize blastocyst differentiation and expansion.

Media contains amino acids to support embryo viability and hyaluronan may be added to improve development and cryo-survivability and to facilitate implantation. It supports metabolic needs after embryo genome activation.

These Includes essential and nonessential amino acids for improved blastocyst development and are Ideal for use in a low-oxygen environment that replicates the human reproductive tract.

#### • Embryo Transfer Medium

Embryo transfer is one of the most sensitive and critical procedures in IVF treatment. Media have been developed exclusively for embryo transfer for increasing the implantation-enhancing effect.

These medias have the basic composition of a rich blastocyst culture medium and contains high concentration of hyaluronan and recombinant human albumin and GM-CSF cytokine.

**EmbryoGlue** is a medium developed exclusively for embryo transfer. It has the basic composition of a rich blastocyst culture medium and contains a high concentration of hyaluronan and recombinant human albumin. It is uniquely developed to mimic the conditions in the female uterus in order to help embryos implant after transfer. When EmbryoGlue was used for embryo transfer the clinical pregnancy rate was significantly increased from 41% to 50% compared to when a conventional culture medium with low or no hyaluronan was used as per a study.

**EmbryoGen**<sup>®</sup> and **BlastGen**<sup>™</sup> have a positive effect on embryo transfer success rates . Transferring using **BlastGen**<sup>™</sup> ensures that GM-CSF is present in the reproductive tract at the time of implantation. This cytokine is known to play an important role in the regulation of the mother's immune response and can facilitate implantation.

Similarly **SAGE 1-Step media** can be used for embryo transfer with good results.

## 17. Role of different components of IVF media?

### • a. Amino acids

Amino acids are the building blocks of proteins, and thus are obligatory ingredients of all known cell culture media. Essential amino acids must be included in the culture media, as cells cannot synthesize these by themselves.

Supplementation of the culture medium with amino acids is necessary for embryo development.

Media that support the development of zygotes up to 8-cells are supplemented with non **essential amino acids** - Proline, serine, alanine, asparagine, aspartate, glycine, glutamate.

Media that support the development of 8-cell embryos up to the blastocyst stage are supplemented with **essential amino acids**: Cystine, histadine, isolucine, leucine, lysine, methionine, valine, argentine, glutamine, phenylalanine, therionine, tryptophane.

AA has a protective role that helps the embryo to conserve homeostasis. This is of particular importance during early stages of development, before the formation of the transporting epithelium. These also offer gametes and embryos protection against variations in the environment and help in minimising intracellular stress.

AA are required for the proliferation of cells and their concentration determines the maximum achievable cell density. L-glutamine, an essential amino acid, is particularly important.

L-glutamine provides nitrogen for NAD, NADPH and nucleotides and serves as a secondary energy source for metabolism. L-glutamine is an unstable amino acid that, with time, converts to a form that cannot be used by cells, and should thus be added to media just before use Remember that its degradation results in the build-up of ammonia, which can have a deleterious effect on embryos. Alanyl-glutamine – a stable form of glutamine that does not break down into ammonium –

also provides alanine, which is necessary for embryo development. Taurine acts as an antioxidant, eliminating the extreme effects of cell metabolism

### b. Carbohydrates

Carbohydrates are existent in the female reproductive tract but their concentrations fluctuate throughout the length of the fallopian tube and uterus. Together with the amino acids they are the chief energy source for the oocytes, embryo and blastocyst.

Energy source requirements evolve from a pyruvate-lactate preference in the embryos, up to the 8-cell stage, which are under maternal genetic control, to a glucose-based metabolism after activation of the embryonic genome that supports their development from 8-cells to blastocysts.

### c. Chelators

Defence of embryos against oxidative insult throughout the culture is crucial to maintain viability. Generation of unwarranted levels of reactive oxygen species (ROS) is triggered by various components of the In-Vitro environment, to most of which embryos do not normally encounter In-vivo.

To counterweigh these deficiencies in the culture environment, antioxidant and chelators are frequently used to control or suppress ROS levels as embryos develop. However, there is no consensus regarding doses, time of exposure and proper combinations of anti-oxidants and chelators in embryo culture.

EDTA is used as a chelator in medium that supports the embryo from the zygote stage to 8-cells and prevents abnormal glycolysis. EDTA probably acts in several ways, including -

1. Chelation of heavy metal divalent cations that may be present as trace contaminants in media components and plastic culture ware.
2. Impede the enzyme 3-phosphoglycerate kinase, thus reducing glycolytic activity in an early embryo. Increased glycolysis in an early embryo seems to be associated with deprived development

### d. Buffers

The buffering capacity of the IVF culture media, in assisted reproductive procedures, is critically vital. Ideal buffer reassures and sustains the required pH in the culture system for growth of the embryos. The bicarbonate buffer emerged as the ideal buffering agent for the human embryo culture

Technically speaking a pH buffer is a substance that acts as a weak acid and/or a weak base so that the pH of the solution to which it is added will be resistant to a change in pH in response to culture conditions and stresses. Such occurs throughout during IVF culture, which buffers respond by accepting or donating hydrogen ions, which are ultimately responsible for establishing pH.

In IVF, the most common buffer used is sodium bicarbonate. The pH of sodium bicarbonate-containing media is sensitive to the amount of carbonic acid formed from the relative amounts of CO<sub>2</sub> in the atmospheric conditions surrounding the culture dish. The pH of the media can be maintained as long as levels of CO<sub>2</sub> remain constant in the incubators.

This can be challenging with repeated incubator door openings and closings for embryo grading as well as for manipulations performed under IVF workstation. For procedures performed in room atmosphere, like gamete collection, ICSI, cryopreservation, and embryo transfer, many labs choose to utilize handling media with reduced bicarbonate levels and inclusion of another pH buffer to maintain the pH outside the incubator. Common buffers are HEPES and MOPS.

**HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)** is a zwitterionic organic chemical buffering agent. HEPES can be utilized as a free acid, or may be conjugated to various salts. HEPES provides no nutritional benefit to the dividing cells. It is added to the media exclusively for extra buffering capacity when the IVF cell culture requires prolonged periods of manipulation outside of a CO<sub>2</sub> incubator

HEPES is having following characteristics:

- pKa values between 6.0 and 8.0
- High solubility,
- Membrane impermeability
- Limited effect on biochemical reactions

**MOPS (3-(N-morpholino)propanesulfonic acid)** is a buffer which is a structural analog to MES. Its chemical structure contains a morpholine ring. With a pKa of 7.15 at 20°C, it is very useful for buffering of IVF biologic systems where stable pH is required for a longer period.

#### e. Macromolecules

Human IVF culture systems have conventionally been supplemented with proteins, which offer a source of energy required for embryo development. They also act as reservoirs for vitamins and minerals.

The most commonly used protein source for human in vitro embryo culture systems is albumin. Albumin, which is abundant in the female reproductive tract, serves as a source of the free amino acids and fixed nitrogen that support preimplantation embryo development in in vitro culture media. It can be extracted from various sources such as the patient's own serum, commercially available as pooled human serum.

Human serum albumin (HSA) is an unstable substance because its biological composition may vary between sources and batches and also on the degree to which diverse molecules bind to albumin during its preparation.

Defined non-protein or recombinant protein supplements are becoming a viable alternative during gamete and embryo manipulation procedures. Biological protein supplements are still preferred for any extended period of embryo culture. Understanding the goals and purpose of supplemented macromolecules in embryo culture media during each step of the laboratory IVF process should assist us in choosing the safest and most consistent macromolecule for each step, but also selecting a product that has the capability of delivering the best clinical outcome.

The recombinant form of albumin is a recently developed macromolecule that has been shown to be very effective in mammalian embryo culture. It has several advantages, including batch-to-batch consistency and biological stability; it is free from viral and prion contaminants, and has lower endotoxin levels. The beneficial effect of more complex protein supplements is evident after the activation of the embryonic genome and probably due to the presence of growth factors.

Glycosaminoglycans (GAGs) such as hyaluronan (HA, hyaluronic acid) are found in abundance in the female reproductive tract of several species, including humans and have a role in fertilization, adhesion, proliferation of several cell types. It has been described that HA with recombinant human albumin, can appreciably improve embryo development and implantation rates.

#### f. Phenol red

Large number of the commercially available culture media includes phenol red as a pH indicator, which allows constant monitoring of pH. Phenol red is bright red at pH of 7.4, the optimum pH value for cell culture during the cell growth. It changes color as pH is changed due to the metabolites released by the cells or media contamination.

At low pH levels, phenol red turns the medium yellow, while at higher pH levels it turns the medium purple.

#### g. Inorganic salt

Inorganic salt in the media help to retain the osmotic balance and help in regulating membrane potential by providing sodium, potassium, and calcium ions.

#### h. Vitamins

Vitamins are essential for growth and proliferation of cells. Vitamins cannot be synthesized in sufficient quantities by cells and are therefore important supplements required in tissue culture. Media are also enriched with different vitamins making them suitable for a particular cell line.

#### i. Antibiotics

Though not required for cell growth, antibiotics are frequently used to control the growth of bacterial and fungal contaminants. Regular use of antibiotics for cell culture is not recommended since antibiotics can mask contamination by bacteria.

Most of ART laboratories use culture media containing antibiotics to minimize the risks of microbial growth.

Streptomycin and gentamycin belong to the aminoglycoside group of antibiotics which utilize their antibacterial outcome by inhibiting bacterial protein synthesis.

## 18. What is EmbryoGen® and BlastGen™ range of

Communication between the embryo and endometrium is crucial in creating the right environment for a successful pregnancy. Compromised embryo competence, impacting the maternal-embryo dialogue, may lead to an increase in implantation failure, preclinical pregnancy loss and miscarriage.

**EmbryoGen®** and **BlastGen™** is a novel culture media suite containing the Cytokine GM-CSF and closely mimics the environment found in the female reproductive tract at conception. The inclusion of cytokines aims to reduce stress caused to the embryo by creating a physiological in vitro environment, increasing the chances of a successful implantation. It is a natural signalling molecule that allows for both autocrine and paracrine communication between the embryo and endometrium.

**GM-CSF appears to assist embryo development in several ways:**

- Regulates cell survival, proliferation and differentiation.
- Regulates immune response.
- Enhances cell signalling.
- Benefits cell-to-cell communication.
- Promotes blastocyst formation.
- Pre-implantation embryos express receptors for GM-CSF.
- Helps in the regulation of implantation.

**Such medias are Beneficial to all patients, but are recommended for women with:**

- Recurrent clinical & biochemical pregnancy loss
- Recurrent implantation failure

## 19. What is embryo Glue?

Culture media are not specifically designed for embryo transfer. The use of a defined embryo transfer medium can impact the success of embryo implantation. Keeping this in mind Vitrolife has developed an embryo transfer media called as embryo glue.

Embryo Glue has the essential composition of a blastocyst culture medium and contains a high concentration of hyaluronan and recombinant human albumin. The synthesis of hyaluronan increases noticeably on the day of implantation and decreases to near basal levels by the next day, indicating that it is imperative for implantation.

It has been exclusively developed to simulate the conditions in the female uterus in order to help embryos implant better by transferring them in high viscosity environments of implantation promoting hyaluronan, which is the major glycosaminoglycan present in the follicular, oviductal and uterine fluids.

## 20. What do you understand by Embryo density?

**Definition:** *Embryo density is defined as the embryo to volume ratio achieved during in vitro culture.* It is the number of the embryo in the defined volume of culture medium.

Embryo density is achieved by manipulating either the number of embryos in a given volume of medium, or manipulating the volume of the medium for a given number of embryos:

**Example:** A microdrop with five embryos in a 50µl volume under oil has embryo to volume ratio (1:10 µl) and a microdrop with one embryo in a 10-µl volume under oil (1:10 µl) has the similar embryo density.

## 21. What are the available methods for embryo culture?

### a. Micro drops embryo culture for OCC's Insemination

Flat sterile plastic petri dishes are available from a number of manufacturers. In recent year, few manufacturers have offered dishes that are labelled specifically for use in human IVF and as such must meet minimum quality assurance such as CE-marked, FDA cleared and MEA tested, Produced under ISO 13485 with full lot traceability, Sterile (SAL  $10^{-6}$ ), Non-pyrogenic (LAL test), USP Class VI test, Release test results (MEA) Standard 60 mm flat sterile plastic petri dishes with micro-drops of culture media under a mineral oil overlay are the common platform for embryo culture.

**Theory:** Micro-drops are prepared to control embryo density with consistency in dish preparation.

#### Embryological Utility:

The standard 60 mm dishes, using 50  $\mu$ l and 15  $\mu$ l drops are utilized for embryo culture or for oocyte culture after ICSI.

For conventional insemination of the OCC's, the format, with four peripheral insemination and two central washing drop of 100  $\mu$ l, can be used.

The volume of medium for each peripheral microdrop can be increased to 150  $\mu$ l for the purpose of insemination.

#### Preparation of micro-drop plate for Embryo culture

##### Micro-droplet plate:

Multiple micro-drops per dish are recommended, as embryos should be rinsed through at least one spare micro-drop before being placed into the micro-drop intended for culture. Microdrops should have Embryo density of 1:16  $\mu$ l.

Make six peripheral micro-drops: 50  $\mu$ l each (for culture of 3-4 embryos per drop).

Also make one central micro-drops: 50  $\mu$ l (for rinsing or holding embryos selected for transfer).

Cover the micro drops with 12 ml mineral oil. Pipette the oil carefully so as not to disrupt the structure of the drops.

Place the lids onto each labelled culture dish and equilibrate in the incubator overnight.

##### Points to Remember for micro-drop preparation:

Rapidly pipette the desired volume of culture medium onto each position on the surface of the labelled dish.

Dehydration of microdrops is more of a problem with smaller volumes, e.g., for individual embryo culture.

Smaller microdrops may be beneficial with regard to embryo density.

Dishes should be prepared on a cool surface, and placement of mineral oil should be done as soon as the microdrops are ready.

### b. Specific culture dishes

**Universal GPS Dishware®:** New type of dishes that are precisely designed for embryo culture. These can also be used for insemination.

**Theory:** These newer dishes rely on fabrication of conical and smaller volume wells into the dish design, so that embryos rest at the lowest point in the wells, where putative embryotrophic factors may concentrate. Embryo density should be no more than four embryos in a 50- $\mu$ l volume.

#### Embryological Utility

GPS dishware can culture and hold 12 embryos, although placing fewer embryos per well, and using more dishes is advisable.

The larger volumes of wells of this plate make the dish suitable for conventional insemination of oocytes and/ or culture of embryos.

#### Preparation of GPS dishware

Pipette up to 150  $\mu$ l of culture medium into the eight peripheral wells.

Then pipette up to 200  $\mu$ l of culture medium into the two central wells.

Pipette 13 ml of mineral oil into the dish, carefully so as not to disrupt the medium within the raised wells.

Placed the lids onto each labelled culture dish and equilibrate in the incubator overnight.

## Embryo corral® Dishes

The central wells of this dish are designed so that each quadrant will have an embryo density of 1:30  $\mu\text{l}$ , where four individual embryos, separated by posts, share a total volume of 120- $\mu\text{l}$  culture medium.

### Embryological Utility

This dish is less suited towards conventional insemination of oocytes but better suited for embryo culture.

### Volume of Medium Embryo Corral® dish

The manufacturer recommends using 30  $\mu\text{l}$  of culture medium in each of the four quadrants.

Higher volumes of culture medium risk creation of a dome over the entire well, where an embryo would be able to migrate over the posts into an adjacent well,

### Preparation of Embryo corral plate:

Pipette 6  $\mu\text{l}$  of culture medium into the eight peripheral wells.

Pipette 30  $\mu\text{l}$  of culture medium into each quadrant of the two central wells, one well at a time (there are four wells divided with posts, in each central well). Do not pipette the total volume of 120  $\mu\text{l}$ , as this will result in an uneven distribution of medium in each well.

Pipette 13 ml of mineral oil into the dish, carefully so as not to disrupt the medium within the raised wells.

Placed the lids onto each labelled culture dish and equilibrate in the incubator overnight.

## Mineral Oil overlay

The volume of mineral oil recommended for overlay is 12 ml for 60 mm dish or 13 ml for the GPS® dishware.

The larger volumes can spill over the rim with movement of the dish.

Smaller volumes of mineral oil are acceptable, as long as the microdrops are completely covered, but the overlay acts as gas and heat sink, and maximizing volume of mineral oil will take advantage of this property.

For dishes smaller than 60mm, e.g., 35 mm, the concept is the same although fewer microdrops can be used in a dish, and the volume of mineral oil dispensed for the overlay is 2 ml per dish.

## 22. Protocol for Embryo culture: Hands on

### LABORATORY AND MEDIA PREPARATION

To ensure proper equilibration of medium and oil, all IVF dishes prepared on (Day - 1) and (Day 0) are pre-warmed to 37°C and equilibrated overnight at 6% CO<sub>2</sub> atmosphere in an incubator the day prior to oocyte collection (Day - 1) and on the day of oocyte collection (Day 0). Minimum incubation period of the media should be no less than 8 hours in a CO<sub>2</sub> Incubator.

Tri-gas Incubator with 6% CO<sub>2</sub>, 6% O<sub>2</sub> and 88% N<sub>2</sub> concentrations having reduced oxygen may also be used for optimal fertilization and embryo growth with good results. The acceptable pH is in range of 7.25-7.4.

#### DAY -1 (DISH PREPARATION)

##### Oocyte Collection and Insemination Dishes (60 x 15 mm)

On the day before oocyte retrieval (Day -1), prepare the dishes with four to five 0.1 mL (100 µL) volume Insemination drops of Fertilization media along the perimeter of the dish with, one or two central Wash drops of 50 µL volume.

Ideally 2 and maximum three harvested oocytes (OCC's) may be inseminated and cultured in a 100-µL drop. A 12 mL volume oil overlay is recommended for such dishes.

Also prepare a 35 mm dish with 2 ml Fertilization media for rinsing the harvested oocytes before inseminating them in the 60 mm plate, which has been prepared earlier.

#### DAY 0 (OVUM PICKUP)

Immediately after the OCC's harvesting, the oocytes are washed in appropriate HEPES/MOPS based media.

##### Fertilization Media culture plates

1-3 OCC's are then placed into the pre-equilibrated oocyte collection dish with insemination droplets (Prepared on Day -1). The dishes are placed in the incubator for latter insemination and further culture.

##### Insemination or ICSI

Oocytes should be incubated for a minimum of 1 hour prior to insemination or denuding if ICSI is to be performed.

It is recommended that we add 75,000 – 175,000/mL motile sperm per 150 µL micro droplet containing 1-3 oocytes.

We normally add 6 µL with approx 10 million /ML counts to the Insemination droplets.

If ICSI is performed the injected oocytes are placed in 50 µL cleavage media droplets and further cultured.

##### Preparing single well Embryo Culture Dishes for post denudation culture on Day 1

Prepare micro drop dishes by dispensing at least three (50 µL) drops of cleavage medium. Three micro drops should be placed at the 3, 6 and 9 o'clock positions in a centre well of a single well dish. One of these drops in the SW dish is to be latter used as washing drop. We culture approx 04 embryo's per such 50-µL droplets.

One mL of Embryo Culture Oil should be used to completely cover the micro drops in central well of such Single well dish.

To avoid evaporation and changes in osmolality do not prepare multiple dishes at the same time. Immediately place the dish in a CO<sub>2</sub> incubator.

### DAY 1

#### Embryo culture post fertilization/denudation

Following fertilization assessments with the identification of the presence of normal fertilization (two PN), transfer 4-5, 2PN into each of the two pre equilibrated 50  $\mu$ L microdrops in the single well culture dish previously prepared on Day 0.

It is recommended that embryos to be group cultured (maximum 5 embryos per micro drop) in an uninterrupted culture system (without dish change or medium renewal), until the desire stage of embryo development is achieved.

### DAY 2

Embryo assessment is optional but not recommended unless embryo transfer is planned so that Embryos are not unduly stressed during assessment.

Embryo Transfer and cryopreservation are optional depending on the individual lab protocol.

### DAY 3

Embryo assessment is optional but not recommended unless embryo transfer is planned so that Embryos are not unduly stressed during assessment.

This step is not required if we are carrying out day 5 culture using single step media.

Other wise embryo transfer and cryopreservation are optional depending on the individual lab protocol.

### DAY 5

Assess embryo morphology and do Embryo Transfer and / or Cryopreserve Blastocysts.

## REFERENCES

- David K.Gardner and Michelle Lane<sup>1</sup>, Human Reproduction Update 1997, Vol. 3, No. 4 pp. 367–382
- Menezo Y, Testart J, Perrone D. Serum is not necessary in human in vitro fertilization, early embryo culture, and transfer. Fertil Steril. 1984, Nov;42(5):750-5
- Quinn P, Kerin JF, Warnes GM. Improved pregnancy rate in human in vitro fertilization with the use of a medium based on the composition of human tubal fluid. Fertil Steril. 1985 Oct;44(4):493-8.
- Mantikou E, van der Veen F, Mastenbroek S et al, Embryo culture media and IVF/ICSI success rates: a systematic review. Hum Reprod Update. 2013 May-Jun;19(3):210-20.

## 23. Tips and tricks about Incubator & Gas System

01 Routine Box incubators work at  $\text{CO}_2$  gas concentration of 6 %, which reaches the incubator from manifold along copper or stainless steel tubing's from the cylinders. Balance of the air in the incubator is the laboratory ambient air, which is composed of Nitrogen - 78.084%, Oxygen -20.9476 %, Carbon Dioxide- 0.0314% and many other minor gases. Most culture media utilize bicarbonate/ $\text{CO}_2$  buffer system to keep pH in the range of 7.2-7.4. The osmolarity of the culture medium should be in the range of 275-290 mosmol/kg.

02 Incubators for maintaining the atmosphere with reduced oxygen are often named triple-gas incubators and are supplied by premixed gases  $\text{O}_2$  (5%) and  $\text{N}_2$  (79%) and 6% ( $\text{CO}_2$ ). Triple gas incubators have the advantage of rapid recovery of gas pressures and temperature as it has small compartments and dishes maintain close contact with the heating surface of the incubators.

03 Always maintain the humidity in the incubators by regularly topping of water levels. Keep the humidity levels around 95 % in IVF culture incubators.

04 Calibrate the  $\text{CO}_2$  levels in the incubators by checking the pH. Once we are sure of the pH the further monitoring may be done by weekly  $\text{CO}_2$  measurements and minor calibration, if required may be carried out. Commercial gas analysers may be used to analyse the gas pressures. Use of fyrite kit is not recommended for the same.

05 Gas incubator may have two types of sensors to monitor the gas concentrations i.e IR sensor or Thermal Conductivity sensor. To safeguard stable conditions, an infra red (IR) sensor is recommended as the IR sensor works independently of humidity and temperature in the incubator.

06 When setting the gas value in the incubator menu, the altitude has to be taken into consideration. There are many differences in laboratory Incubator settings established at Srinagar and Chennai. Not all IVF laboratories have the luxury to be located at an at sea level. It is easy to set the  $\text{CO}_2$  value in the incubators in IVF laboratories near sea level with proper pH of their culture media as compared to incubators set at high altitude. This is important, since there is a relationship between altitude and atmospheric pressure. At sea level, atmospheric pressure is 760 mmHg. At higher altitudes the atmospheric pressure decreases, but the partial pressure of gases must be maintained for proper equilibrium. It is the amount of  $\text{CO}_2$  that drives the pH in culture media. The more the carbon dioxide present in our incubators, the more carbonic acid,  $\text{H}_2\text{CO}_3$ , is formed thus decreasing the pH. The Henderson-Hasselbalch equation best describes the relation between the pH-value and the reaction between acidic and basic components in culture media with variation in altitude.

07 Incubators should be equipped with a display that permits for the continuous monitoring of fluctuations in gas concentrations, temperature and humidity.

08 The repeated opening of the door in triple gas incubators with a low ability to regulate and synchronize gases can lead to worse culture conditions compared to that in a classic  $\text{CO}_2$  incubator. A long recovery time for  $\text{CO}_2$  can change the pH of the medium. It is therefore recommended that additional measurements of pH in the medium be performed once fortnightly.

09 Embryos should be cultured under paraffin oil, which prevents evaporation of the medium preserving a constant osmolarity. The oil also minimizes fluctuations of pH and temperature when embryos are taken out of the incubator for microscopic assessment. Paraffin oil can be toxic to gametes and embryos; therefore, batches of oil must be screened and tested on mouse embryos before use in culture of human embryos.

Table A. Commercially available Sequential Human Embryo IVF Culture Media from different companies

Culture Media/ Companies	Sperm preparation	Rinsing/ Flushing media	Fertilization Media	Cleavage Stage Media (D2)	Blastocyst Stage Media (D5)	Embryo Transfer
<b>Vitrolife</b>	Sperm rinse, G-IVF Plus	G-RINSE G-MOPS	G-IVF Plus	G-1 Plus	G-2 Plus	G1/ G2 Plus Embryo Glue
<b>COOK Sydney</b>	Sydney IVF Sperm preparation media	COOK Sydney Gamete Buffer	COOK Sydney Fertilization	COOK Sydney Cleavage	COOK Sydney Blastocyst	COOK Sydney Cleavage Blastocyst
<b>Origio EmbryoGen® Origio BlastGen™</b>	Sperm Preparation Media Origio	Flushing	ORIGIO Sequential Fertilization If ICSI is done shift oocytes to <b>EmbryoGen®</b>	<b>EmbryoGen®</b>	<b>BlastGen™</b>	<b>BlastGen™ EmbryoGen®</b>
<b>Origio Sequential</b>	Origio Sperm preparation Medium	Flushing	ORIGIO Sequential Fertilization	ORIGIO Sequential Cleavage	ORIGIO Sequential Blastocyst	ORIGIO Sequential Cleavage/ Blastocyst
<b>Sage-Quinn's Advantage™</b>	Quinn's Sperm Washing media	Quinn's Advantage medium with HEPES	Quinn's Advantage Fertilization Medium	Quinn's Advantage Cleavage Medium	Quinn's Advantage Blastocyst	Quinn's Advantage Cleavage/ Blastocyst
<b>Irvine Scientific</b>	Sperm preparation Medium Modified sperm washing Media	Modified HTF media with gentamicin HTF media Multipurpose Handling Medium® (MHM)	Continuous Single Culture® Complete (CSCM-C)	Complete Early Cleavage Medium® (sss)	Complete MultiBlast Medium® (sss)	Complete Early Cleavage Medium® (sss) Complete MultiBlast Medium® (sss)

Table B. Sequential IVF Culture Media dispensing

Activities on (Day -1)	Activities on Day of OPU (Day 0)	Activities on (Day 1)	Transfer day (D2)	ET (Day 3)	ET (Day 4)	ET (Day 5)
Activities	Activities	Activities	Activities	Activities	Activities	Activities
<p><b>1. Preparation for Rinsing and oocyte handling</b></p> <p>Flushing Media -Origio Gamete Buffer- Cook GMOPS – VITROLIFE Quinn's Advantage medium with HEPES Irvine Multipurpose Handling Medium® (MHM) GLOBAL® W/ HEPES VITROMED HEPES Plus</p> <p><b>2. Preparation for Sperm preparation</b></p> <p>Quinn's Sperm Washing media Irvine Modified sperm washing Media Sydney IVF Sperm preparation media Origio Sperm preparation Medium VITROLIFE Sperm rinse</p> <p><b>3. Preparation for IVF</b></p> <p>Quinn's Advantage Protein Plus Fertilization Medium Irvine Continuous Single Culture® Complete (CSCM-C) ORIGIO Sequential Fertilization G-IVF Plus COOK Sydney Fertilization GLOBAL® Fertilization VITROMED ONESTEP</p> <p><b>4. Preparation for ICSI is planned</b></p> <p>EmbryoGen® G-1 Plus COOK Sydney Cleavage ORIGIO cleav Sequential Quinn's Advantage Protein Plus Cleavage Medium Irvine Complete Early Cleavage Medium® (sss) GLOBAL® Total® VITROMED ONESTEP</p>	<p><b>1. Rinsing and oocyte handling</b></p> <p><b>2. Insemination or ICSI</b></p> <p><b>3. Preparation of Denudation plates for day 1</b></p> <p><b>Media required for making denudation plates</b></p> <p>G-1 Plus COOK Sydney Cleavage ORIGIO Sequential cleavage Quinn's Advantage Protein Plus Cleavage Medium Irvine Complete Early Cleavage Medium® (sss) EmbryoGen® GLOBAL® Total® VITROMED ONESTEP</p>	<p><b>1. Denudation / PN Assessment day</b></p> <p><b>2. Shift PN to post denudation culture plates</b></p> <p><b>3. Plan for ET on day 2 at 8 cell stage</b></p> <p><b>4. Dispense media for ET on day 2.</b></p>	<p><b>1. Embryo Transfer Day</b> Do transfer in following media.</p> <p>G-1 Plus COOK Sydney Cleavage ORIGIO Sequential cleavage Quinn's Advantage Protein Plus Cleavage Medium Irvine Complete Early Cleavage Medium® (sss) VITROMED ONESTEP</p> <p><b>2. Plan for day - 3 ET</b></p> <p><b>3. Plan for further culture to Day 5</b></p> <p><b>4. Freeze Day</b></p> <p><b>Dispense media for Day 3 ET</b></p> <p>G-1 Plus COOK Sydney Cleavage ORIGIO Sequential cleavage Quinn's Advantage Protein Plus Cleavage Medium Irvine Complete Early Cleavage Medium® (sss) EmbryoGen® GLOBAL® Total® VITROMED ONESTEP</p> <p><b>Dispense media for Blast culture</b></p> <p>G-2 Plus COOK Sydney Blast ORIGIO Sequential blast Quinn's Advantage Protein plus blast Medium Irvine Complete Early blast Medium® (sss) BlastGen™ GLOBAL® Total® VITROMED ONESTEP</p>	<p><b>1. ET Day</b></p> <p><b>2. Change media for further culture</b></p> <p><b>3. Freeze Day</b></p>	<p>Dispense media for Blast culture</p> <p>G-2 Plus GLOBAL® Total® COOK Sydney Blast ORIGIO Sequential blast Quinn's Advantage Protein Plus blast Medium Irvine Complete Early blast Medium® (sss) BlastGen™ VITROMED ONESTEP</p>	<p><b>1. ET Day</b></p> <p><b>2. Freeze Day</b></p> <p><b>Do transfer in following media</b> GLOBAL® Total® G-2 Plus Embryogluce COOK Sydney Blast ORIGIO Sequential blast Quinn's Advantage Protein Plus Blast Medium Irvine Complete Early blast Medium® (sss) BlastGen™ GLOBAL® Total® VITROMED ONESTEP</p>

Table C. Culture plates required at different stage of Single step Embryo Culture for IVF/ICSI

Culture Media/ Companies	Sperm Preparation	Oocyte Retrieval	Fertilization Media	Cleavage Stage Media (D2)	Blastocyst Stage Media (D5)	Embryo Transfer
<b>VITROLIFE</b>	Sperm Rinse GIVF <sup>Plus</sup>	G MODS <sup>plus</sup>	Insemination to be done in GIVF Plus If ICSI is done shift injected oocytes in G- TL directly	G- TL	G- TL	G- TL
<b>SAGE/ORIGIO</b>	Sperm Preparation Media Origio	Quinns advantage medium with HEPES Flushing media	Insemination to be done in origio fertilization media. If ICSI is done shift injected oocytes in SAGE 1-Step <sup>™</sup>	SAGE 1-Step <sup>™</sup> allows uninterrupted culture following IVF or ICSI insemination through to the blastocyst stage, and is ideal for use with time lapse technology	SAGE 1-Step <sup>™</sup>	SAGE 1-Step <sup>™</sup>
<b>IRVINE SCIENTIFIC</b>	Sperm washing media	Modified HTF media with gentamicin HTF media Multipurpose Handling Medium <sup>®</sup> (MHM)	Continuous Single Culture <sup>®</sup> Complete (CSCM-C): This medium is optimized to be used in an uninterrupted culture system without dish change or medium renewal	Continuous Single Culture <sup>®</sup> Complete (CSCM-C)	Continuous Single Culture <sup>®</sup> Complete (CSCM-C)	Continuous Single Culture <sup>®</sup> Complete (CSCM-C)
<b>GLOBAL</b>	GLOBAL <sup>®</sup> W/ HEPES	GLOBAL <sup>®</sup> W/ HEPES	GLOBAL <sup>®</sup> FERTILIZATION If ICSI is done shift injected oocytes to GLOBAL <sup>®</sup> Total <sup>®</sup>	GLOBAL <sup>®</sup> Total <sup>®</sup>	GLOBAL <sup>®</sup> Total <sup>®</sup>	GLOBAL <sup>®</sup> Total <sup>®</sup>
<b>VITROMED</b>	Sperm wash	HEPES plus	ONESTEP	ONESTEP	ONESTEP	ONESTEP

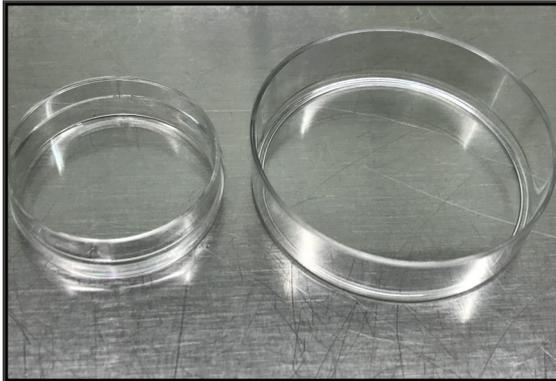


## – PART 2 –

### Index

<b>S.No</b>	<b>Topic</b>	<b>Page. No</b>
1.	Disposables and Culture plates for Embryo culture	27
2.	Commercially available Sequential Media for Human Culture	28
3.	Commercially available Single Step Media for Human Culture	30
4.	Daily activities from Day -1 to Day 2-3	31
5.	Company Related Information	38
6.	Acknowledgements	38

## Disposable and culture plates for embryo culture



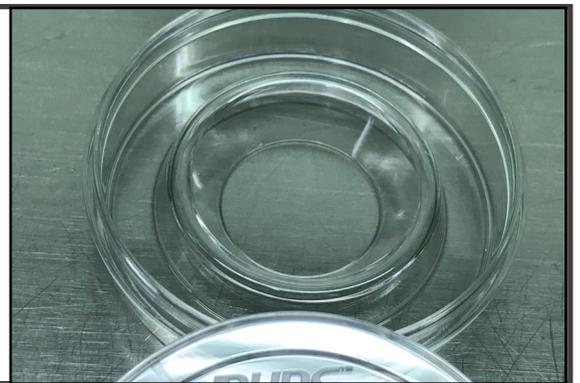
### Figure 1A

35 mm Cell Culture Dish with easy-grip design with flat surface and optically clear polystyrene surface (Cat. No: FAL-353001; Brand- Falcon®; dimensions 35x10 mm; 20/pack, 500/case). (Left)

Standard 60 mm flat sterile plastic petri dishes for Vitro Fertilization (IVF) us (Cat. No FAL-353652; Brand- Falcon®; dimensions 60x15 mm; 20/pack, 500/case). (Right)

### Figure 1B

Nunc™ Center Well Dish, CE- marked Class II for In Vitro Fertilization use with easy grip. The dishes have a perfect angle for reservoir well walls for locating and easy washing of samples and with true flat bottom for maintaining optimal temperature. (Cat. No-150260; Brand: Nunc™; dimensions 55 mm x 16mm, 1/120 pack/case).



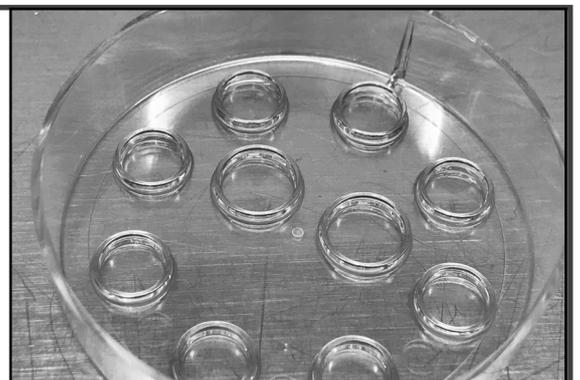
### Figure 1C

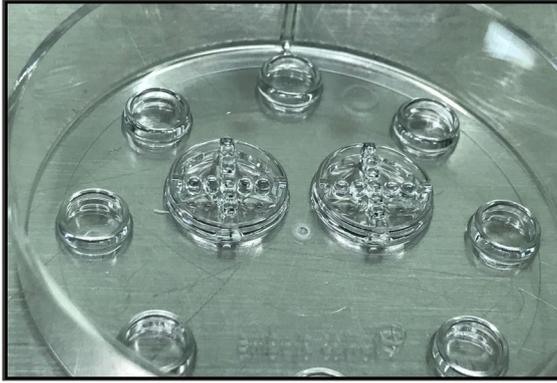
Nunc™ 4 Well Dish for IVF ensure proper orientation and prevent cross contamination with notched lid and dish. It also prevents scratches on well bottoms and maintains optimal temperature with 0.8mm raised edge. (Cat. No-144444; dimensions 66mm x 66mm, 4/120 pack/case).



### Figure 1D

Universal GPS® dish is designed for efficient oocyte and embryo handling and culture. The two (2) central wells are intended for washing oocytes or embryos. The eight (8) outer wells are intended for oocyte, and embryo culture at all stages. The gently sloped concave well bottoms allow oocytes and embryos to settle at a central location away walls, hence the name GPS. (Cat. No-UGPS-010; Brand: The Lifeglobal® group; Pack size: 10 dishes per sleeve).





## Figure 1E

Embryo Corral® dish has eight (8) outer wells designed for efficient oocyte, embryo handling and culture. The embryo corral® has two (2) central wells designed to take advantage of the potential benefits of group embryo culture. Each embryo corral® dish central well is divided into four quadrants. The quadrants are separated by posts to permit media exchange between quadrants without allowing movement of embryos. (Cat. No-EMBC-010; Brand: The Lifeglobal® group; Pack size: 10 dishes per sleeve).

### Plasticware information sheet for in vitro fertilization (IVF)

STYLE	ROUND DISH	ROUND DISH	SINGLE WELL DISH	FOUR WELL PLATE
Company	BD Falcon™ / Nunc	BD Falcon™ / Nunc	Nunc™/BD Falcon™	Nunc™/BD Falcon™
DIMENSIONS (Diameter x height)	35mm x10mm	54.81mm OD x 13.26mm	55mm OD x 16mm	13.4mm x 10.7mm per well
Product Package	-20 dishes/sleeve -Individually sealed plates	-20 dishes/ -Individually sealed plates	1/120 pack/case -Individually sealed plates	pack of 4/case of 120
Material	Non-cytotoxic: IVF Dishes and plates are manufactured from virgin polystyrene material tested. Tested for In-Vitro cytotoxicity			
Sterility	Sterile: Product are gamma irradiated, undamaged package guaranteed Sterile (SAL 10 <sup>-6</sup> )			
Endotoxin Testing	Nonpyrogenic: Product are nonpyrogenic validated as per USP Bacterial Endotoxins Limulus Amebocyte Lysate (LAL) test. Acceptance level is less than 0.5 endotoxin units/ml or 5 EU/device			
Embryotoxicity Testing	Product are tested for embryo toxicity using the one cell mouse embryo assay (MEA) for non-embryotoxicity assay. At least 75% of both test and control embryos must reach the hatched and/or expanded blastocyst stage.			

### Commercially available sequential media for human embryo culture



## Figure 2A

Range of embryo culture media available from SAGE as Quinn's Advantage Protein Plus™. This range of sequential embryo culture media bottles are packed in glass bottle in sterile condition with rubber stopper and cap of different color codes for differentiation between each media for different stages of embryo culture. The media range includes Fertilization media (Pink cap), Cleavage (Green cap), Blastocyst media (Blue cap) along with Oil for Tissue. The media bottles are packed in glass bottle in sterile condition with rubber cap in with different color codes to avoid mixing during media dispensing. (Manufacture: SAGE, In-Vitro Fertilization, Inc, a Cooper Surgical Company, Trumbull CT, USA)

## Figure 2B

Range of embryo culture media available from Origio® including media for Sperm preparation, Fert™, Cleav™ and Blast™ medium (Manufacture: Origio, a a Cooper Surgical Company, Knardrupvej 2-2760 Malov Denmark)



## Figure 2C

EmbryoGen® range of media developed exclusively for embryo culture and embryo transfer for increasing the implantation-enhancing effect. These medias have the basic composition of a rich blastocyst culture medium and contains high concentration of hyaluronan, recombinant human albumin and GM-CSF cytokine. EmbryoGen® have shown positive effect on embryo transfer success rates. The GM-CSF is present in the reproductive tract at the time of implantation and play an important role in the regulation of the mother's immune response. (Manufacture: Origio, a a Cooper Surgical Company, Knardrupvej 2-2760 Malov Denmark).

## Figure 2D

Range of embryo culture media available from Vitrolife® including media for Sperm Rinse™, G-MOPS™, G-IVF™ Plus, G-1™ Plus, G-2™ Plus media. (Manufacture: Vitrolife AB (publ) Box 9080,40092, Goteborg, Sweden)



## Figure 2E

Range of embryo culture media available from Life Global® total LP range including HEPES media for sperm preparation, Cleavage and blastocyst media and Lite Oil® (Manufacture: Life Global Group, 393, Soundview Road, Guilford, CT 06437, TEL: 800 720 6375/ 519 826 5800, FAX 519 826 6947)

## Figure 2F

The picture depicts range of embryo culture media available from COOK®. This range of sequential embryo culture media bottles are packed in glass bottle in sterile condition with rubber stopper and cap of different color codes for differentiation between each media for different stages of embryo culture. The range include Gamete Buffer™ (Blue), Sydney Fertilization™ (Red), Sydney Cleavage™ (Green), Sydney Blast™ (Orange) medium. (Manufacture: COOK Medical Inc, P.O. Box 4195, Bloomington, U.S.A)



## Commercially available single step medias for human embryo culture

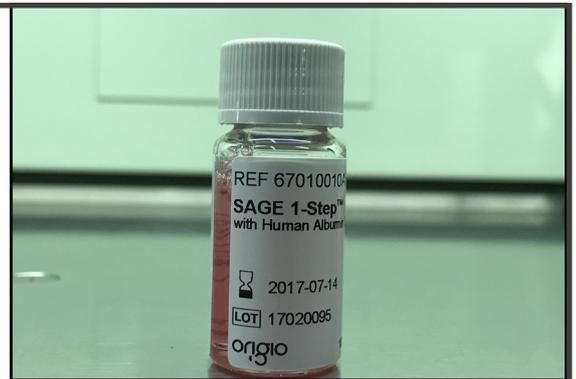


## Figure 3A

Range of single step culture media available from Origio® including Flushing Medium, Fert™, SAGE 1-Step™ medium (Manufacture: Origio, a Cooper Surgical Company, Knardrupvej 2-2760 Malov Denmark).

## Figure 3B

SAGE 1-Step™ Single step culture media available for continuous embryo culture from Fertilization to Blastocyst and embryo transfer. (Manufacture: Origio, a Cooper Surgical Company, Knardrupvej 2-2760 Malov Denmark).



## Figure 3C

Range of single step culture media available from Vitromed® including HEPES HTF Plus, Sperm Wash, One Step medium (Manufacture: VITROMED GmbH. Wildenbruchstrasse 15, 07745 Jena Germany)

## Figure 3D

One step media (Manufacture: VITROMED GmbH  
Wildenbruchstrasse 15, 07745 Jena Germany)

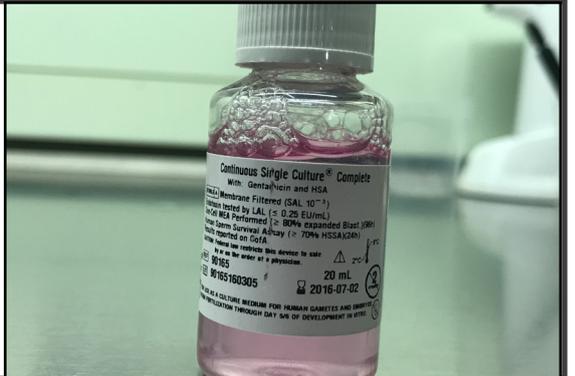


## Figure 3E

Complete Range of culture media available from Irvine Scientific® including HTF medium™, Continuous Single Culture® Complete (CSCM-C), Sperm Washing, Multipurpose handling Medium (MHM)® (Manufacture: Irvine Scientific-Corporate, 1830 E Warner Avenue, Santa Ana, CA 92705 USA)

## Figure 3F

Single step culture media available from Irvine Scientific® Continuous Single Culture® Complete (CSCM-C). CSCM-C is a complete, ready to use medium containing Human Serum Albumin (HSA) as the protein component. (CSCM-C) is optimized to be used in a uninterrupted culture system without medium renewal or dish change on day 3. (Manufacture: Irvine Scientific-Corporate, 1830 E Warner Avenue, Santa Ana, CA 92705 USA)



## Plates for Rinsing and Oocyte Handling (Day -1)

## Figure 4A(i)



Dispensing of media for Rinsing and Oocyte handling (DAY-1) in 35 mm petri dish. These plates maintain the pH of the cells at 37°C and are designed to be used specifically in ambient atmospheric air conditions and not an enriched CO<sub>2</sub> environment. To ensure proper equilibration of medium and oil, all IVF dishes prepared on Day - 1 are pre-warmed to 37°C

## Preparation of IVF Insemination Dishes (60 x 15mm) (Day -1)

### Figure 4B(i)



After the oocyte-cumulus complex has been washed with flushing HEPES/MOPS based buffers, they are placed in Fertilization Medium, where insemination is carried out. Fertilization medium contains all components necessary to support oocyte and sperm function during fertilization. Standard 60 mm flat sterile plastic petri dishes with micro-drops of culture media under a mineral oil overlay are the common platform for embryo culture. Microdrops are prepared to control embryo density with consistency in dish preparation. The standard 60 mm dishes are utilized for conventional insemination of the OCC's, the format, with four peripheral in-semination and two central washing drop of 100  $\mu$ l, each. Ideally 2 and maximum three harvested oocytes (OCC's) may be inseminated and cultured in a 100- $\mu$ L drop.

### Figure 4B(ii)

Pipette the oil carefully so as not to disrupt the structure of the drops and cover the entire micro-drops with mineral oil. A 12 ml volume of oil overlay is recommended for insemination of dishes. Dishes are prepared on a cool surface, and placement of mineral oil should be done as soon as the micro-drops are ready.



### Figure 4B(iii)



A well prepared insemination plates (60mm) for micro drop culture with oil overlay for oocyte insemination. These plates are prepared by rapidly pipetting the desired volume of culture medium onto each position on the surface of the labeled dish. Dishes are prepared on a cool surface, and placement of mineral oil should be done as soon as the microdrops are ready. As the dehydration of microdrops is more of a problem with smaller volumes for insemination. But smaller microdrops may be beneficial with regard to embryo density with six peripheral micro-drops of 100  $\mu$ l each (for culture of 3-4 embryos per drop)

### Figure 4B(iv)

The dishes smaller than 60mm, e.g., 35mm, are also utilized for group insemination of OCCs without preparing microdrops. These dishes uses minimum 02ml of fertilization media with 02ml of mineral oil dispensed for the overlay per dish. A complete 35mm petri dish prepared for group insemination





### Figure 4B(v)

A standard 60 mm flat sterile insemination dish with 100 $\mu$ L size of micro-drops of fertilization media under a mineral oil overlay. The fertilization media are bicarbonate-buffered, which provides a glucose-rich environment for efficient oocyte-cumulus complex and sperm cell metabolism as shown in the picture.

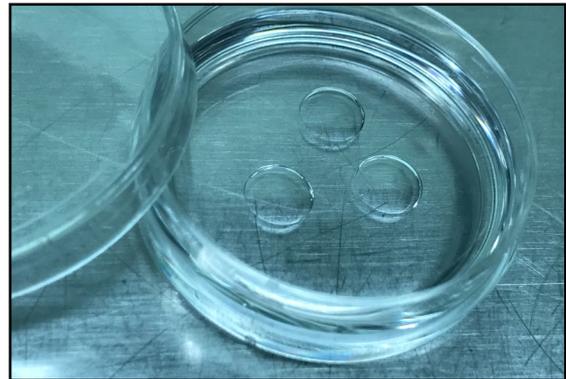
## Activities on day of pick-up (Day 0)

### Figure 4C(i)

Preparation of single well embryo culture dishes for post denudation culture. This dish is prepared by making micro drop dishes by dispensing at least three (50 $\mu$ L) drops of cleavage medium. Three micro drops should be placed at the 3, 6 and 9 o'clock positions in a centre well of a single well dish. One of these drops in the single well dish is to be latter used as washing drop. We culture approx 4 - 5 embryo's per such 50- $\mu$ L droplets. One mL of embryo culture grade oil is used to completely cover the micro drops in central well of such single well dish.



### Figure 4C(ii)



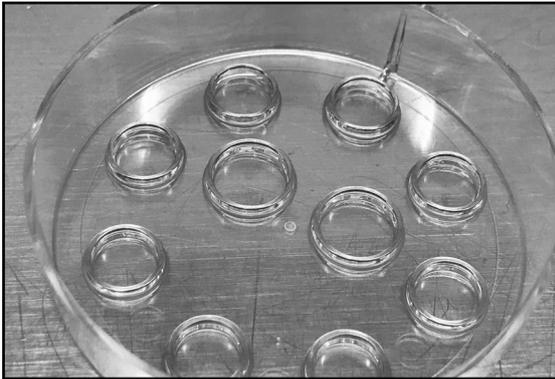
A 35mm dish for embryo culture. This dish can be alternatively used for embryo culture in absence of single well dish. This is also prepared by making micro drop by dispensing at least three (50 $\mu$ L) drops of cleavage medium placed at the 3, 6 and 9 o'clock positions in a centre of petri dish. Two mL of embryo grade oil is used to completely overlay the micro drops.

Figure 4C(iii)



A four well Nunc™ dish for embryo culture can also be used in absence of single well or 35mm petridish. This is prepared by making micro drop by dispensing four (50 $\mu$ L) drops of cleavage medium placed in the each well. This is further overlay with approximately 100 $\mu$ l of oil in each well of dish to completely cover the micro drops.

Figure 4C(iv)



Universal GPS Dishware® a new type of dishes that are precisely designed for embryo culture and insemination. These newer dishes rely on fabrication of conical and smaller volume wells into the dish design, so that embryos rest at the lowest point in the wells. GPS dishware can culture and hold 12 embryos, although placing fewer embryos per well, and using more dishes is advisable. The GPS dishware is prepared by pipetting up to 150  $\mu$ l of culture medium into the eight peripheral wells and up to 200 $\mu$ l of culture medium into the two central wells. A total of 13 ml of mineral oil is carefully overlay so as not to disrupt the medium within the raised wells.

Figure 4C(v)



Embryo Corral® dish that has eight outer wells designed for efficient , embryo handling and culture. The embryo corral® has two central wells designed to take advantage of the potential benefits of group embryo culture. Each embryo corral® dish central well is divided into four quadrants.

**Preparation of Embryo corral plate:**

Pipette 6  $\mu$ l of culture medium into the eight peripheral wells.

Pipette 30  $\mu$ l of culture medium into each quadrant of the two central wells, one well at a time (there are four wells divided with posts, in each central well). Do not pipette the total volume of 120 $\mu$ l, as this will result in an uneven distribution of medium in each well.

Pipette 13 ml of mineral oil into the dish, carefully so as not to disrupt the medium within the raised wells.

Placed the lids onto each labelled culture dish and equilibrate in the incubator overnight.

Figure 4C(vi)



The figure shows a calibrated micropipette for taking out the 30  $\mu$ l of fertilization medium to dispense each quadrant. The manufacturer recommends using 30 $\mu$ l of fertilization medium in each of the four quadrants. The central wells of this dish are designed so that each quadrant will have an embryo density of 1:30 $\mu$ l, where four individual embryos, separated by posts, share a total volume of 120 $\mu$ l culture medium

### Activities on day of Denudation : Day 1

Figure 4D

Following fertilization assessments with the identification of normal fertilization with presence of two PN stage. We prepare micro drop dishes for transfer of 4-5 fertilized embryo at 2PN stage into each of the pre equilibrated 50  $\mu$ l microdrops in the single well culture dish previously prepared on Day 0. It is recommended that embryos to be group cultured (maximum 5 embryos per micro drop) in an uninterrupted culture system (without dish change or medium renewal), until the desire stage of embryo development is achieved for embryo transfer.



## Activities on day of Embryo Transfer : Day 2-3



### Figure 4E(i)

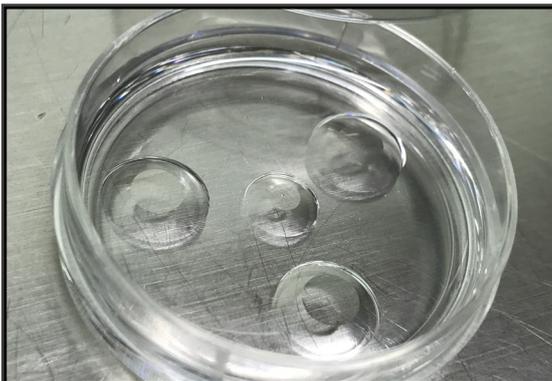
The figure shows a single well dish for embryo transfer prepared on the day of embryo transfer with 2 micro drops of 50 $\mu$ l each of cleavage medium for rinsing of embryos in the outer periphery of dish and centre well containing 01 ml of embryo transfer medium without oil. Embryo assessment is optional but not recommended unless embryo transfer is planned so that embryos are not unduly stressed during assessment.

### Figure 4E(ii)

The figure shows a four well dish for embryo transfer prepared on the day of embryo transfer with micro drops of 50 $\mu$ l each of cleavage medium in the left lower well for rinsing of embryos and left upper well containing 500 $\mu$ l off embryo transfer medium without oil. The figure shows loading of embryos in catheter just before embryo transfer under stereo zoom microscope.



## What mistakes to avoid while making IVF culture plates



### Figure 5A(i)

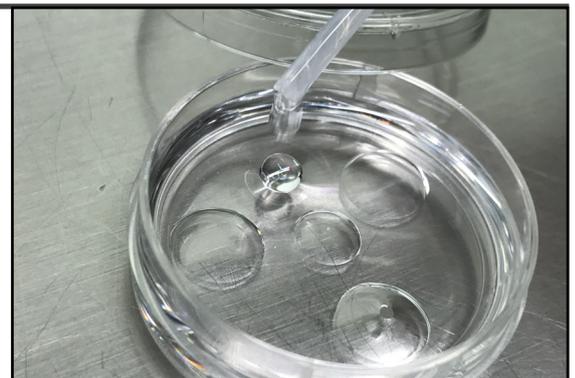
Very commonly the culture droplets are not covered appropriately with the oil. This may lead to the evaporation of the media and contamination of the small droplets of culture media. Such mistakes would also lead to PH changes in the embryo culture droplets.

On the other hand over layering the oil on the culture plates may lead to spillage of the oil or clogging of the rim of the culture plates. This may further lead to change in pH of the media droplets.

### Figure 5A(ii)

Do not leave air bubbles in the culture plates. Small air bubbles in the culture Media droplets leads to difficulty in identifying the embryos and oocytes.

The gametes tend to attach with the air bubbles and thus are many times lost during handling.



## Steps

This figure shows step wise opening of glass media bottle from COOK. Each media bottle is given unique color for identification with colored cap and a aluminum seal with rubber stopper to prevent leaking and contamination during transportation see Step 1- Step 6.

### Figure 5(a & b)



Appropriate use of cook handle to open the coloured polypropylene flip caps of the media vials .  
Use the specific edges on the handle to grip the cap edge and use the handle as fulcrum to remove the caps.

### Figure 5(c)

Removal of temper proof metal seal using cook handle. Using the handle ensures that the seal is removed by no touch technique thus avoiding iatrogenic contamination.



### Figure 5(d & e)



Fix the appropriate areas on the cook handle below the rubber cap on the borosilicate vial.  
Rotate the handle along the rim of the borosilicate vial to loosen the rubber cap slightly.  
Now again using the handle as fulcrum gently remove the cap aseptically. Do this step gently to remove the grey rubber plug .



### Figure 5(f)

Aseptically extracted rubber cap. This can be put back appropriately if required but the process of reselling is not recommended to avoid contamination of the media. Never touch the rubber cap during extraction.

### Companies Related Contact Information

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Vitromed, BD falcon™	Sar Healthline Pvt. Ltd	Mr. Atul Walia	+91-9958029696	info@sarhealth.com
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Irvine Scientific	Cryobiosystem India	Mr. Jitendra Kumar	+91-9650602424	jitender@cryobiosystem india.com

### Acknowledgment:

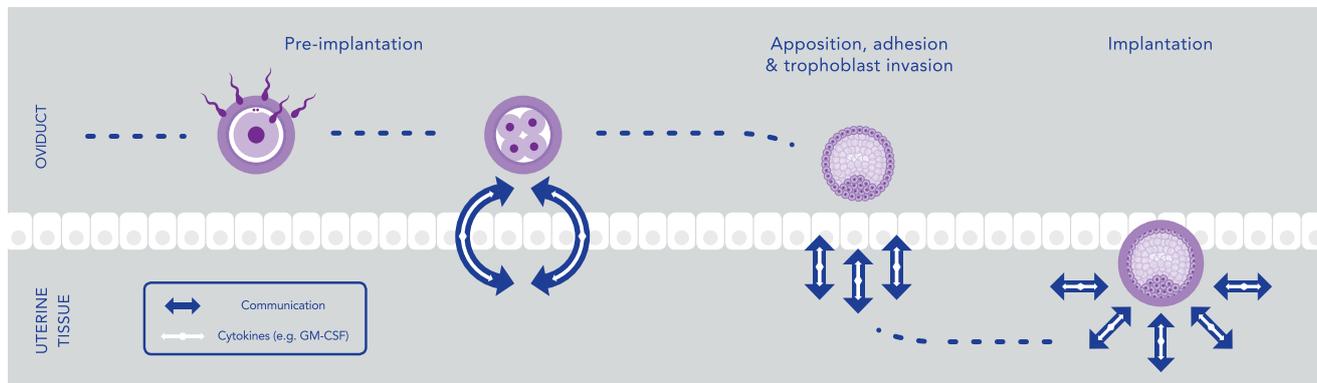
We would sincerely like to thank our industry partners for supporting us in bringing out this educational bulletin of human embryo culture.



# Breakthrough IVF technology : EmbryoGen and BlastGen

## Providing a more physiological in vitro environment

The EmbryoGen and BlastGen media suite closely mimics the environment found in the female reproductive tract at conception. Creating the best possible in vitro conditions for the embryo, with the use of a cytokine, will promote successful implantation through improved endometrial receptivity.



### Day 0-1 Fertilization

ORIGIO Sequential Fert™ is optimized to support sperm function and promote fertilization. It is recommended for gamete co-incubation before culture in EmbryoGen.



### Day 1-31 Initial dialogue

Cytokines are critical in the communication between the embryo and the endometrium prior to implantation.

EmbryoGen contains GM-CSF, a cytokine found naturally in the female reproductive tract. Exposure of embryos to GM-CSF has been shown to promote blastocyst formation and alleviate the negative effects of in vitro culture.



### Day 3-6 Ongoing support

Maternal-embryo communication is essential for recognition and implantation of the embryo.

BlastGen facilitates embryo culture through to the blastocyst stage with the added boost of GM-CSF. Also used for embryo transfer, BlastGen increases the presence of GM-CSF in the reproductive tract just prior to implantation.



### From embryo transfer onwards Sustained dialogue

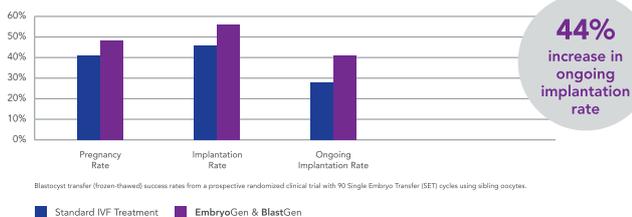
An environment that supports the dialogue between the embryo and endometrium is crucial for successful implantation, especially for women with repeated IVF failures<sup>1</sup>.

Transferring using BlastGen ensures that GM-CSF is present in the reproductive tract at the time of implantation. This cytokine is known to play an important role in the regulation of the mother's immune response and can facilitate implantation.

## EmbryoGen and BlastGen in the clinical setting

The introduction of a GM-CSF containing culture medium to the IVF world was based on the positive results of EmbryoGen in a prospective randomized clinical trial<sup>1</sup>, which showed its positive effect on ongoing implantation and live birth rates.

### 3 days of embryo culture in GM-CSF containing medium improved live birth rate<sup>1</sup>



## Make EmbryoGen and BlastGen part of your toolbox

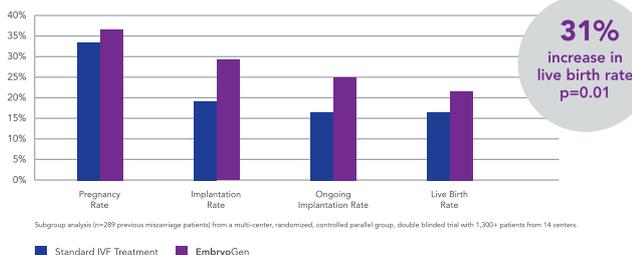
- Embryo-endometrial communication is key to successful pregnancy
- Cytokines drive communication
- EmbryoGen and BlastGen make up the first media suite containing the recombinant human GM-CSF cytokine
- EmbryoGen and BlastGen have a positive effect on embryo transfer success rates

**44%** increase in ongoing implantation rate with EmbryoGen and BlastGen<sup>1</sup>



Today early data on the clinical use of the full GM-CSF media suite, EmbryoGen and BlastGen, demonstrates that culturing in GM-CSF containing media until the blastocyst stage increases pregnancy and implantation rates<sup>2</sup>.

### EmbryoGen and BlastGen have a positive effect on pregnancy rate and increase the chances of obtaining a live birth<sup>2</sup>



**Definitions :** Pregnancy rate: Women with positive hCG at week 2 per women with transfer. Implantation rate: Number of sacs at week 7 per transferred embryo. Ongoing implantation rate: Number of sacs with heart beat at week 7 per transferred embryo. Live birth rate: Live births per transferred embryo.

# origio

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# The integral role of Culture Media in IVF

Culture media are an essential part of a culture system that aims to minimize insults and stresses to gametes and embryos.

Designed to provide an optimized in vitro environment, culture media deliver a balance of ions, energy substrates and nutrients, ensuring the best possible clinical outcomes are supported.

A well-functioning culture system is influenced by many factors including air quality, temperature control, CO<sub>2</sub> and pH levels, and the level of expertise of laboratory staff and these can all affect results.

From continuous media that offer uninterrupted culture and ease of use, to a sequential series optimized for each step in the process, our culture media range has been specifically designed to support optimal development of healthy embryos. This facilitates clinical management tailored to the individual needs of patients, whether this relates to day of transfer, postponed transfer, or additional procedures such as biopsy and PGS.

Clinical research has proven that cytokines play a critical role in communication between the embryo and endometrium, which is key for a successful pregnancy. Based on this knowledge we have developed EmbryoGen and BlastGen, a novel media suite for day 3 and day 5 culture that is unique within the industry.

## Developing high quality culture media for 30 years

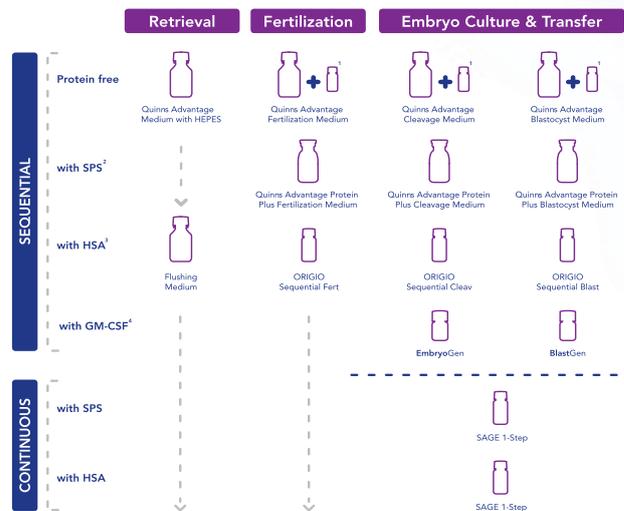
A market leader with strong heritage, and growing portfolio of innovative products, ORIGIO has developed and produced high-quality media since 1987.

We employ industry experts and partner with leading scientists to produce the best possible solutions. Our expert teams approach each challenge with one ultimate aim – enabling you to maximize your success rates.

ORIGIO media are manufactured in a purpose built, state-of-the-art production facility. Our world class ISO 13485 and ISO 9001 certified manufacturing site consistently maintains the highest standards for product quality and reliability.

## ORIGIO high quality culture media for every protocol

ORIGIO create media that support every step of the IVF process including oocyte retrieval, fertilization, culture and transfer. Our diverse product range has been carefully designed to meet your individual needs.



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