

em^{ART} 2.0

International Online Symposium
for the Best Practices in Clinical Embryology

An Initiative by **ihera**
INTERNATIONAL HUMAN EMBRYOLOGY
RESEARCH ACADEMY

In association with **Cipla**

THEME - *FROM BELL JARS TO BENCHTOPS*

ABSTRACT BOOKLET

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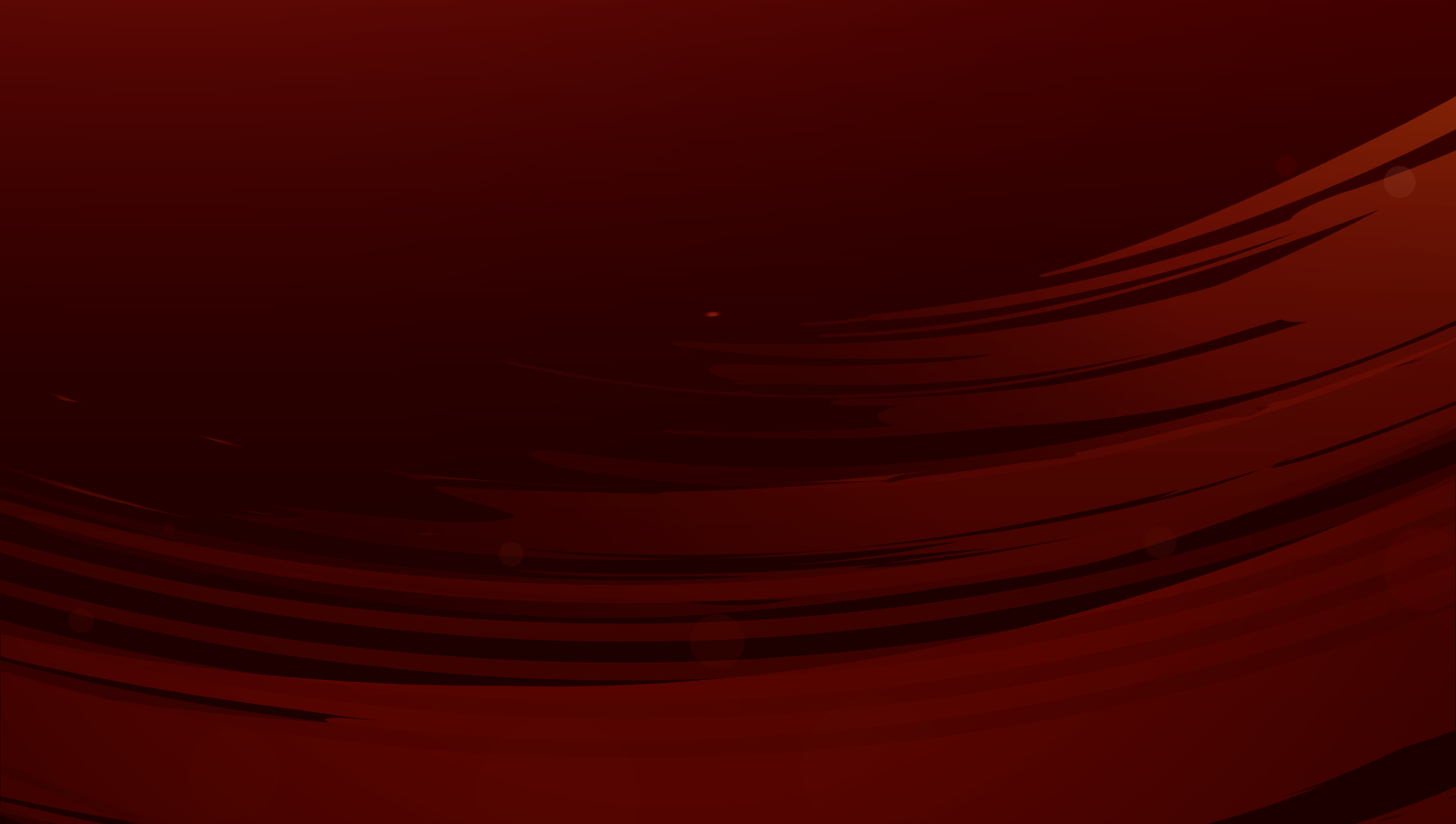
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01 Welcome Address



 embART 2.0

**International Online Symposium
for the Best Practices in Clinical Embryology**

Dear Colleagues,

The field of Assisted Reproductive Technology (ART) has grown by leaps and bounds. Still a very nascent and evolving field, the need for active research in the field is understated.

The history of in vitro fertilisation (IVF) goes back more than half a century. The first birth in a nonhuman mammal resulting from IVF occurred in 1959, and in 1978, the world's first baby conceived by IVF was born.

Louise Brown, born 43 years ago on 25th July 1978, made history by being the first test tube baby conceived through IVF. This was led by Patrick Steptoe and Robert Edwards, the latter of which received the 2010 Nobel Prize for Physiology or Medicine.

To commemorate this success in the field of ART, iHERA, in association with Cipla, announces a two-day scientific fest. We take immense pleasure in inviting you to the 2nd Virtual “**embART 2.0**” – International Online Symposium for the Best Practices in Clinical Embryology to be held on 24th & 25th July, 2021.

The theme of this Symposium is “**FROM BELL JARS TO BENCHTOPS**”. **embART 2.0** will feature world renowned faculties from across the globe on a single platform to spread the knowledge, with wide range of synchronized sessions focusing on infertility and reproductive biology guaranteed to stimulate and motivate the audience.

A 2-day Symposium consisting of Pre-Congress Masterclass, Keynote Lectures, Oral Presentations, Interactive Debates and Panel Discussions on Cutting-Edge Research in the field of Human Reproduction and Fertility. The Pre-Congress Masterclass is specially designed for in-depth knowledge and enables you to choose the deliberations specific to your area of interest and clinical practice.

In totality, **embART 2.0** is an innovative and informative event that talks about latest research and techniques used in the field of Clinical Embryology across the world, informs about the processes in clinical governance and Quality improvement and exposes audiences to Clinical Embryology innovators and experts.

We welcome you and look forward to your participation.

A platform to educate yourself..... listen, get inspired, learn, practise and flourish.

Regards,



02 Team iHERA

About iHERA

International Human Embryology Research Academy or iHERA is an online, not for profit platform, which envisions for enhancing the knowledge for anyone who wants to expand their information horizon right from basics to expert level. iHERA being in its initial phase; we aim high yet keep our feet grounded. We believe in growing together and welcome all interested in growing with iHERA.

iHERA aims to provide a platform for researchers to share their work from the field of embryology and increase their audience and reach. By providing this means iHERA believes to fulfil the basic need of every aspiring embryologist and researcher – the need to get recognized.

Founders



Dr. Ved Prakash
Founder



Dr. Sanjay Shukla
Co-Founder



Dr. Charudutt Joshi
Co-Founder

Advisory Board

Dr. Jayant Mehta

Dr. Jaffar Ali

Dr. Yousef Alhelou

Dr. Charulata Chatterjee

Dr. Krishna Chaitanya

Dr. Gaurav Majumdar

Dr. Sarabpreet Singh

Dr. Shubhangi Gangal

Dr. Pranay Ghosh

National Coordinators

Dr. Rahul Sen Dr. Sanketh Dhumal Satya Dr. Paresh Makwana Dr. Akash Agarwal

Team Members

Dr. Tarika Deora	Ms. Yoshita Tanwar	Mr. Deepu Gupta	Dr. Nidhi Singh
Ms. Tejaswini G Sanketh	Mr. Kripal Singh Rawat	Mr. Sumit Singh Rana	Ms. Jyoti Pandey
Mr. Rahul Sisodiya	Ms. Sazda Khatoon	Mr. Lakshmi Narasimharao Putla	

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03 Organising Committee & Scientific Committee

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International Online Symposium
for the Best Practices in Clinical Embryology

ORGANIZING COMMITTEE

Organizing Chairpersons



Organizing Chairperson

Dr. Ved Prakash



Co-Chairperson

Dr. Charudutt Joshi



Co-Chairperson

Dr. Sanjay Shukla

Scientific committee embART 2021



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Dr. Rahul Sen



Dr. Sanketh Dhumal Satya



Dr. Akash Agarwal

Coordinators



Dr. Paresh Makwana



Dr. Tarika Deora



Ms. Yoshita Tanwar



Mr. Deepu Gupta



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Ms. Tejaswini G Sanketh



Mr. Kripal Singh Rawat



Mr. Sumit Singh Rana



Ms. Jyoti Pandey



Mr. Rahul Sisodiya



Ms. Sazda Khatoon

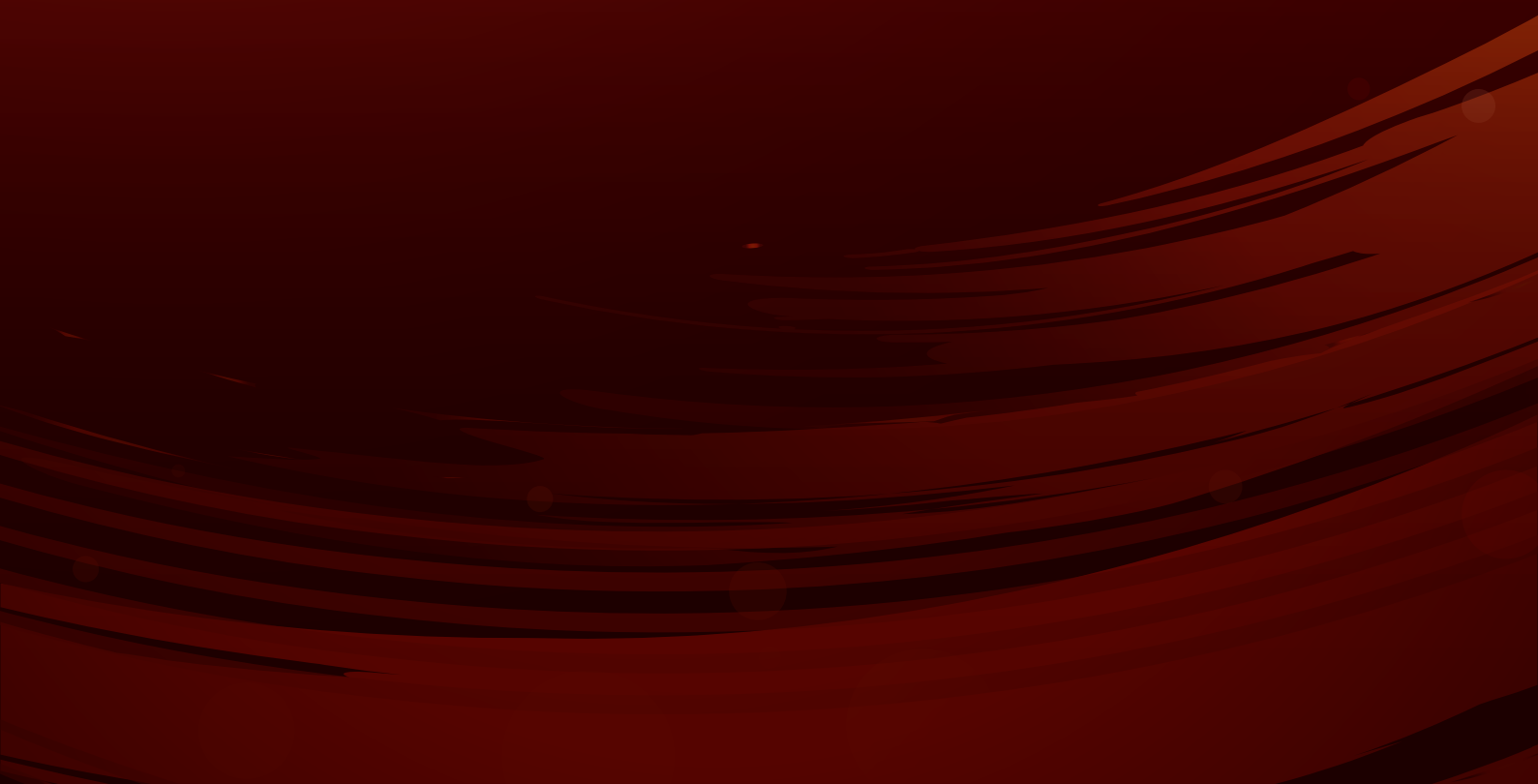


Mr. Lakshmi
Narasimharao Putla



04 Introduction To Faculty

International Faculty



International Faculty



Dr. Alpesh Doshi

Consultant Clinical Embryologist
and a Co-Founder of IVF London.



Dr. Antonio Alcaide Raya

Senior Embryologist & European
Technical Applications Scientist
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Dr. Carol Lynn Curchoe

Founder of ART Compass &
Repro AI, San Diego



Dr. George Liperis

Deputy Scientific Director
Westmead Fertility Centre,
Australia



Dr. Danilo Cimadomo

Science and Research
Manager of the Genera Life
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Canada



Dr. Yousef Alhelou

Director of ART Lab
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Freelancer, Consultant in Embryology,
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China



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Scientific Director at Pearl
Singapore Fertility Centre and
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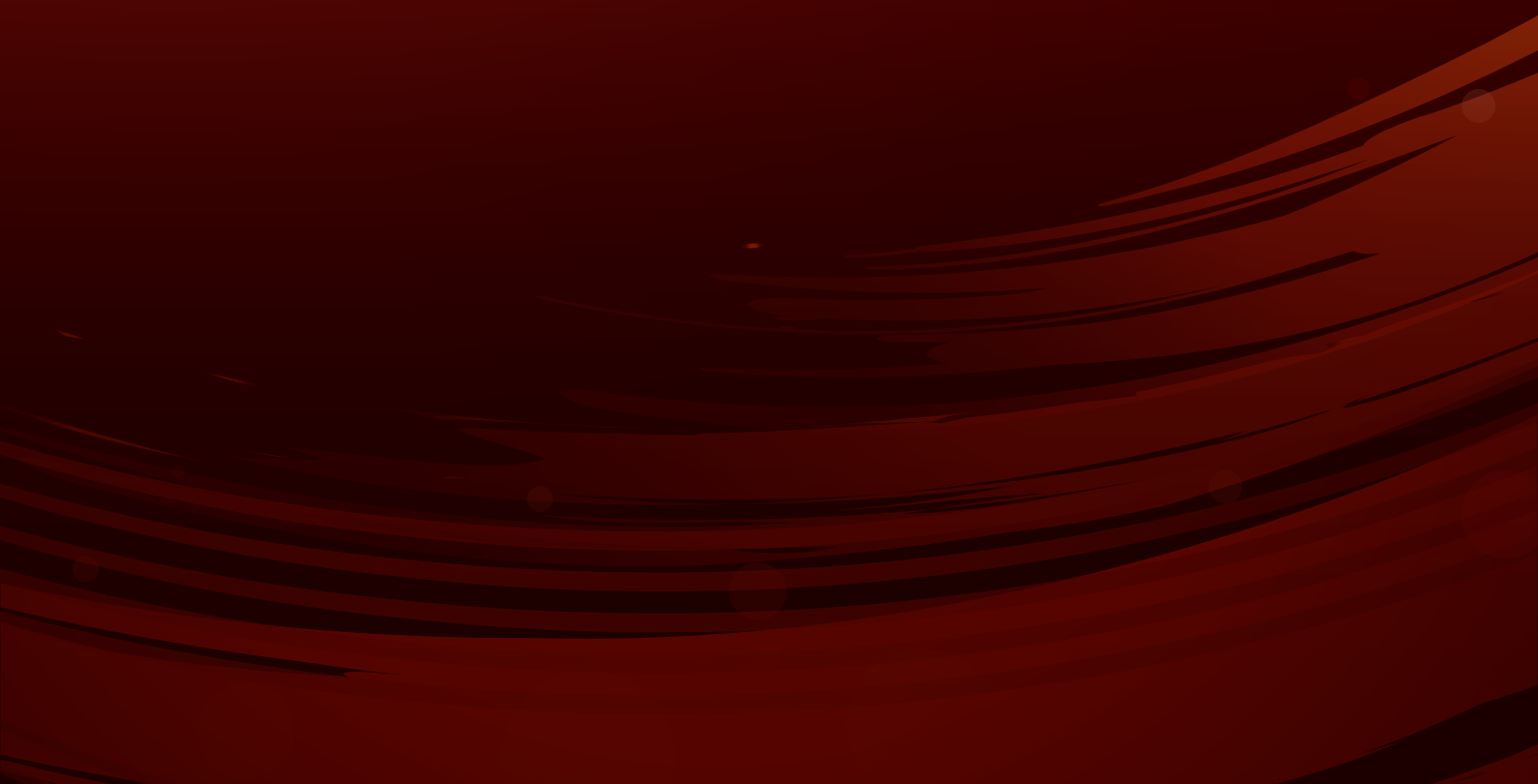
Dr. Colleen Lynch

Clinical Embryologist
United Kingdom



04 Introduction To Faculty

National Faculty



National Faculty



Dr. Chandan
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Founder and Scientific Director
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and Scientific Director -
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Jaipur



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IVF Centre,
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Medical Director Akanksha
Hospital and Research Institute,
Anand, Gujarat



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Lab Director
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Agra



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Scientific Director
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Director and Principle
Embryologist Caree Fertility,
Bengaluru



Dr. Charulata Chatterjee

Scientific Head & Consultant
Embryologist,
Ferty9 Fertility Centre,
Hyderabad



Dr. Deven Patel

Chief Embryologist,
Sunflower Women's Hospital,
Ahmedabad



Dr. Bindu Chimote

Scientist and Consultant
Clinical Embryologist



Dr. Shrikanth Yatnale

Senior Consulting
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Dr. Rajvi Mehta

Academic Consultant-
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Senior Clinical Embryologist Kshema-IVF, Mangalore



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Consultant Embryologist Neelkanth Fertility, Jaipur



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Scientific Director Hegde Fertility, Hyderabad



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Chief Scientific Officer Cellsure Biotech & Research Center, Mumbai



Dr. Shubhangi Gangal

Chief Embryologist, Counsellor & Centre Co-Ordinator Parulekar HC & Gunjotkar Fertility and IVF Centre, Mumbai



Dr. Kersi Avari

Founder Director - Embryology Academy of Research & Training



Dr. Pranay Ghosh

Director, Elixir Fertility Centre & Consultant at Double Helix Clinical Cytogenetics & Reproductive Immunology Centre, Delhi



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Clinical Director Hegde Fertility Centre, Hyderabad



Dr. Paresh Makwana

Chief Embryologist/Centre Head at WINGS group of Hospital, Ahmedabad



Dr. Prabhakar Singh

Scientific Director & Co-founder Nimaaya Women's Centre For Health, Surat



Dr. Nirmal Bhasin

Director/Clinical Embryologist and Founder of Jannee Fertility Centre and Minsk State Medical Institute, Belarus



Dr. Gaurav Kant

Senior Embryologist at Akanksha IVF Centre, Delhi

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05 Programme

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Pre-Congress Masterclass

THEME - TECHNOLOGY, QUALITY & IVF

PROGRAM COORDINATORS

Dr. Tarika Deora | Ms. Yoshita Tanwar | Dr. Nidhi Singh | Ms. Tejaswani Konari

DAY-1

Date: Saturday, 24th July 2021

Time: 02:00 pm - 07:15 pm IST

INAUGURAL PROGRAM (02:00 pm onwards)

Time	Duration	Topic	Speaker	Moderator
02:10-02:40	20 min	QC in Conventional IVF	Dr. Bindu Chimote	Dr. Deven Patel
	10 min	AUDIENCE INTERACTION		
02:40-03:10	20 min	QC in ICSI	Dr. Rajvi Mehta	Dr. Shrikant Yalnale
	10 min	AUDIENCE INTERACTION		
03:10-03:40	20 min	QC in Embryo Transfer	Dr. Priya Bhave	Dr. Prasanna Kumar Shetty K
	10 min	AUDIENCE INTERACTION		
03:40-04:10	20 min	Quality Control Standards in PGT: Time to Re-Check	Dr. Alpesh Doshi	Dr. Fiona D'souza
	10 min	AUDIENCE INTERACTION		
04:10-04:40	30 min	Quality Control Issues in Handling Culture Media (Cryo Bio System, India)	Dr. Antonio Alcaide Raya	Dr. Randhir Singh
	10 min	AUDIENCE INTERACTION		

Time	Duration	Debate	Speakers	Judges
04:40-05:10	10 min	Conventional Methods of Sperm Preparation	Dr. Sanketh Dhumal Satya	Dr. Akanksha Mishra
	10 min	Advanced Methods of Sperm Preparation	Dr. Rahul Sen	Dr. Ann Margaret Mangalaraj
	10 min	AUDIENCE INTERACTION		

Time	Duration	Panel Discussion	Moderators	Panelists
05:10-06:10	60 min	What do you mean by Quality in IVF? Technology - Results- Patient satisfaction?	Dr. Nishad Chimote Dr. Akash Agarwal	Dr. Sayali Kandhari Dr. Gaurav Kant Dr. Kersi Avari Dr. Pranay Ghosh Dr. Vandna Hegde Dr. Paresh Makwana

Time	Duration	Topic	Speaker	Moderator
06:10-06:40	20 min	QC in Vitrification (Cryo Bio System, India)	Dr. Joe Connaghan	Dr. Prabhakar Singh
	10 min	AUDIENCE INTERACTION		
06:40-07:10	20 min	How AI & Machine Learning are Revolutionizing Quality Control in IVF	Dr. Carol Lynn Curchoe	Dr. Nirmal Bhasin
	10 min	AUDIENCE INTERACTION		

Concluding Remarks & Vote of Thanks

emART 2.0

Scientific Symposium

THEME - FROM BELL JARS TO BENCHTOPS

PROGRAM COORDINATORS

Dr. Rahul Sen | Dr. Sanketh Dhumal Satya | Dr. Paresh Makwana | Dr. Akash Agarwal

DAY-2

Date: Sunday, 25th July 2021

Time: 08:45 am - 07:45 pm IST

INAUGURAL PROGRAM (8.45 am onwards)

Time	Duration	Topic	Speaker	Moderator
09:00-09:30	20 min	Humidification: The Veterinary Horse of Embryology	Dr. Gabor Vajta	Dr. Chandan N
	10 min	AUDIENCE INTERACTION		
09:30-10:00	20 min	Routine Semen Analysis. Should the WHO criteria for normal sperm be the driving force for selecting the fertilization method?	Dr. George Liperis	Dr. Sushil Kumar Chopra
	10 min	AUDIENCE INTERACTION		
10:00-10:30	20 min	2PN or Not 2PN? How Genetic Fertilization Checks Answer the Question?	Dr. Colleen Lynch	Dr. Natachandra Chimote
	10 min	AUDIENCE INTERACTION		
10:30-11:00	20 min	Definition of Developmentally Incompetent Embryo	Dr. Danilo Cimadomo	Dr. Ratna Chattopadhyay
	10 min	AUDIENCE INTERACTION		
11:00-11:30	20 min	Epigenetic Risk Assessment of Assisted Reproduction Technologies	Dr. Laura Rienzi	Dr. Pankaj Talwar
	10 min	AUDIENCE INTERACTION		

Time	Duration	Panel Discussion	Moderators	Panelists
11:30-12:30	60 min	KPI: Maximize the Results: Embryo Culture- The Man, The Machine, The Tools	Dr. Sarabpreet Singh Dr. Krishna Chaitanya	Dr. Shubhangi Gangal Dr. Sanjay Shukla Dr. Varsha Samson Roy Dr. Sujata Ramkrishnan Dr. Charulata Chatterjee Dr. Sapna Srinivas

Time	Duration	ORATION	Speaker	Chairpersons
12:30-01:00	30 min	DR. SUBHASH MUKHERJEE Incubation Technology in 40 Years of IVF & Future of ART	Dr. Jayant Mehta	Dr. Ved Prakash Dr. Sanjay Shukla Dr. Charudutt Joshi

Time	Duration	Topic	Speaker	Moderator
01:00-01:30	20 min	Oocyte Cryopreservation: How to Maximize the Outcome?	Dr. Birol Aydin	Dr. Goral Gandhi
	10 min	AUDIENCE INTERACTION		
01:30-02:00	20 min	Genetic Toolkit for Male Infertility in Fertilisation Failure	Dr. Marc Torra Massana	Dr. Sunil Jindal
	10 min	AUDIENCE INTERACTION		

POWER BREAK 10 MIN

Time	Duration	Topic	Speaker	Moderator
02:10-02:40	20 min	Role of Extracellular Vesicles in Reproduction	Dr. Krishna Chaitanya Pavani	Dr. A S Ansari
	10 min	AUDIENCE INTERACTION		
02:40-03:10	20 min	Establishing a QMS in a newly constructed ART LAB	Dr. Lynne Nice	Dr. Sandeep KarunaKaran
	10 min	AUDIENCE INTERACTION		

Time	Duration	Debate	Speakers	Judges
03:10-03:40	10 min 10 min	Choo-U-Sing The Best Day For Embryo Vitrification DAY 3 DAY 5	Dr. Manika Saxena Dr. Jayalakshmi Shoraff	Dr. Kuldeep Jain Dr. Ramdoss Srinivassan
10 min		AUDIENCE INTERACTION		

Time	Duration	Topic	Speaker	Moderator
03:40-04:10	20 min	Synthetic Protein-Free chemically defined media for human ART: Compliance with safety, regulatory and cultural norms	Dr. Jaffar Ali	Dr. Brig R. K. Sharma
10 min		AUDIENCE INTERACTION		
04:10-04:40	20 min	Optimization of ICSI timing by non-invasive PLM spindle evaluation in order to minimize in vitro induced alteration of embryo development	Dr. Daniel Hlinka	Dr. Deepak Goenka
10 min		AUDIENCE INTERACTION		
04:40-05:10	20 min	Intracellular Calcium and Oocyte Quality	Dr. Omar Farhan Ammar	Dr. Geeta Goswami
10 min		AUDIENCE INTERACTION		
05:10-05:40	20 min	Double Trouble: Present Situation of eSET	Dr. Matheus Roque	Dr. K. D. Nayar
10 min		AUDIENCE INTERACTION		
05:40-06:10	20 min	The Impact of Fresh and Frozen Testicular Tissue Quality on Embryological and Clinical Outcomes	Dr. Alexia Chatziparasidou	Dr. Saroj Agarwal
10 min		AUDIENCE INTERACTION		
06:10-06:40	30 min	EXPERT PANEL: Current Scenario of Third Party Reproduction in India	Dr. Sonia Malik Dr. Nayana Patel Dr. Rajul Tyagi	Dr. Vandana Bhatia

Time	Duration	Panel Discussion	Moderators	Panelists
06:40-07:40	60 min	Incubators: From Bell Jars to Benchtops	Dr. Vijay Mangoli Dr. Keshav Malhotra	Dr. Ved Prakash Dr. Srinivas M S Dr. Charudutt Joshi Dr. Alex Verghese Dr. Suresh Kattera Dr. Sudesh Kamat Dr. Yousef Alhelou

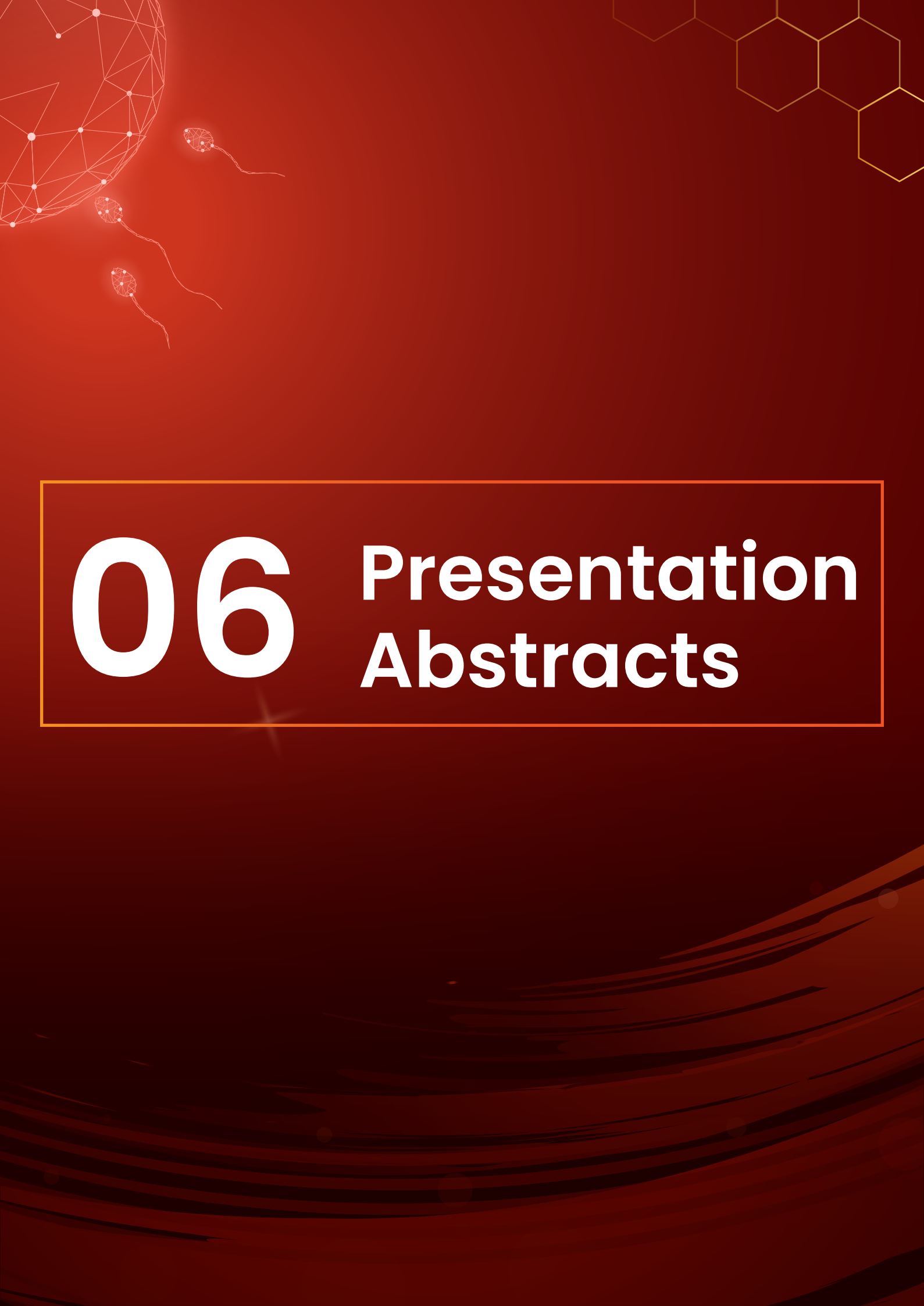
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Our website



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06 Presentation Abstracts

**Dr. Antonio Alcaide Raya**

Senior Embryologist & European
Technical Applications Scientist (TAS) at
Fujifilm Irvine, Madrid

QC Issues in Handling Culture Media**Abstract**

Culture media play a central role in ART. Handling them properly is crucial to get the best performance and therefore get the best results. Manufacturers design and produce specific media for each type of cell and each clinical procedure, and embryologists are responsible of using them preserving the properties and functionality with which they were produced for as long as possible. Storage, sterility, pH and osmolality are the main aspects that can be affected by an improper handling. The focus of this presentation is to comment on those aspects of media management that can affect their properties to a greater extent.



Dr. George Liperis

Deputy Scientific Director
Westmead Fertility Centre, Australia

Routine Semen Analysis. Should the WHO Criteria for Normal Sperm Be the Driving Force for Selecting the Fertilization Method?

Study question

To evaluate fertilization outcomes by conventional IVF on ejaculates with less than WHO reference values as assessed on day of fertilisation.

Summary answers

Fertilisation outcomes originating from less than WHO reference value ejaculates assessed on day of fertilisation, suggest that these samples can be considered for conventional IVF.

What is known already

Intra-cytoplasmic sperm injection (ICSI) was initially developed for male infertility cases, however ESHRE data shows that ICSI cycles outnumber conventional in vitro fertilisation (IVF) by almost two to one. Over the past few decades, there has been a worldwide increase in the use of ICSI for all kinds of infertility with the rationale that ICSI is associated with a higher likelihood of fertilization. According to a pair of recent retrospective European studies, use of ICSI in cases without male infertility holds no advantages over conventional IVF.

Participants/materials. Setting, methods

Participants were consented patients undergoing IVF/ICSI at a single centre between Jan 2018 – Dec 2019. Sperm ejaculates were collected on the day of oocyte retrieval, where volume (balance scale), concentration (haemocytometer) and motility (slide assessment) were assessed as per were

assessed as per WHO criteria. Following gradient centrifugation, re-suspended samples with total motile sperm count of ≥ 0.1 mil/ml and $\geq 70\%$ progressive motility were used for conventional IVF by adding 0.1 mil/ml motile sperm to each insemination dish.

Main results and the role of chance

Over the designated study period, 83 couples had fertilisation by conventional IVF from ejaculates with less than WHO reference value across the following 3 categories: total motile count (< 20 mil/mL), density (< 15 mil/mL) and progressive motility ($< 32\%$). The average age of men at the time of ejaculation was 36.8 0.7 and ejaculatory values were: volume of 2.7 0.1 mL, total count of 26.7 1.6 mil/mL, total motile count of 5.2 0.4 mil/mL, density of 9.9 0.3 mil/mL and progressive motility 19.8 0.8%. Following gradient centrifugation and two wash steps, the mean volume of re-suspended sperm in fertilisation media was 0.5 0.0 mL. The number of oocytes collected per cycle was 8.0 0.6 (n=662 oocytes), with 7.2 0.5 being at the meiosis-II (MII) stage at fertilisation check (n=596). Fertilisation rate per cycle was 66.3 3.0% (n=422 fertilised oocytes) and 73.2 3.0% per MII oocytes. Two cycles resulted in failed fertilisation (2.4%) with an average of 5.0 0.2 oocytes collected from these cycles (total of 11, with 10 at the MII stage at fertilisation check). During the study time-frame at the same centre, failed fertilisation rate by conventional IVF for sperm ejaculates within the WHO norms for patients with 4 MII oocytes was 1.8% (28/1546).

Limitations, reasons for caution

The results represent the experience gained from current practice and not of a prospective controlled study. Developmental potential of embryos originating using this approach and clinical outcomes were not explored.

Wider implications of the findings

Sperm ejaculates with less than WHO reference values should not be excluded for conventional IVF, as these samples can still result in satisfactory fertilisation outcomes without increasing failed fertilisation outcomes.



Dr. Danilo Cimadomo

Science and Research
Manager of the Genera Life Centers, Rome

Definition of Developmentally Incompetent Embryo (DIPE)

“Developmentally incompetent embryos” (DIPE) is a definition largely missing in the literature and/or based on outdated and unreliable criteria (1-5). In Italy, this represents an issue since Italian clinics are not allowed to discard viable embryos (Italian Law 40/2005). But, what information can be used to define a DIPE in our daily routine? Previous definitions of non-viability failed. In fact, blastomere fragmentation, degeneration, mitotic arrest, and multinucleation are insufficient to clearly identify non-viable embryos and blastocyst culture is the only reasonable strategy to assess embryo developmental competence. Yet, also at this stage, both static and morphodynamic assessments are insufficient to define reproductive incompetence. Recently also zygotes outlined as “abnormally fertilized”, especially when not cultured in a time-lapse system, were shown to result into euploid-diploid blastocysts and healthy babies. SIERR (Italian Society of Embryology, Reproduction and Research) along with SIGU (Italian Society of Human Genetics) then drafted an official document to define DIPE based on both embryological and genetic criteria, which has been then published in the Journal of Assisted Reproduction and Genetics (6). Three categories of embryos were outlined:

- **Developmentally competent embryos (DeCE)**
 - Genetically-untested and euploid 2PN-derived viable embryos (preferably blastocysts)
 - Euploid-diploid OPN- and 1PN-derived blastocysts
 - Blastocysts affected from monogenic conditions and/or aneuploidies compatible with a pregnancy beyond the 1st trimester

- **Developmentally competent preimplantation embryos of undefined reproductive competence (DeCURC)**

- Genetically-untested OPN- and 1PN-derived blastocysts
- “Mosaic” embryos
- Embryos affected from segmental (also known as partial) aneuploidies

- **DIPE**

- ≥3PN-derived embryos
- Developmentally-arrested and degenerated embryos
- Embryos affected from:
 - (i) Constitutive complex aneuploidies;
 - (ii) Constitutive monosomies;
 - (iii) Constitutive trisomies of chromosome 1, 2, 3, 4, 5, 7, 10, 11, 12, 14, or 19;
 - (iv) Haploidy or poliploidy;
 - (v) Lethal monogenic conditions

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Dr. Laura Rienzi

Senior Clinical Embryologist and IVF Laboratory
Director of Genera Life Centre in Italy.

Definition of Developmentally Incompetent Embryo (DIPE)

Epigenetics refers to chromatin modifications that regulate gene activity and are not due to DNA sequence change. Epigenetic mechanisms entail DNA methylation, histone modification, non-coding RNA, remodeling of nucleosomes and organization of chromatin structure. The term genomic imprinting describes the expression of specific genes in a parent-of-origin specific manner (not biparentally inherited). Specifically, the epigenetic mark (e.g., methylation) placed on the allele during oogenesis silences the maternal allele in the offspring and only the paternal allele is transcribed to mRNA. Germ cell development and early embryogenesis are crucial windows in the erasure, acquisition and maintenance of genomic imprints. Indeed, in mammals, two major genome-wide epigenetic reprogramming events take place during gametogenesis and early embryogenesis. In particular, during oogenesis the acquisition of maternal DNA methylation begins at puberty in primary to antral stage follicles and it is mostly complete in MII-ovulated oocytes; conversely, during spermatogenesis, paternal DNA methylation acquisition occurs during prenatal stages of spermatogenesis and it is completed by birth. Also the epigenetic landscape is different in oocytes and sperm: in the MII oocyte, the genome possesses histones compacting chromatin, which is then further condensed into loops that are bound to spindle fibers. Oocyte chromatin is hypermethylated and carries repressive histone modifications; in the mature sperm, instead, protamines tightly compact chromatin into toroids (90–99% chromatin) that are punctuated by histone solenoids (1–10% chromatin). Sperm DNA is hypermethylated except at regions bearing active and bivalent histone modifications. Within 4 hr of fertilization the male pronucleus undergoes rapid demethylation via a mechanism mediated by oocyte DNA

methyl transferase. The maternal genome remains protected from this process and is therefore likely to undergo passive demethylation. Lastly, de novo methylation occurs at the blastocyst stage, which is lineage specific: the inner cell mass is in fact more methylated than the trophectoderm.

Imprinting disorders are a group of congenital diseases affecting genomically imprinted chromosomal regions and genes, and disturbances of imprinted genes may alter their regulation (epigenetic mutation). The clinical phenotypes of imprinting disorders are diverse, but primarily involve growth or neurological development. The main diseases are Beckwith-Wiedemann syndrome (BWS) [prevalence: 1-5 /10.000], Angelman syndrome (AS) [prevalence: 1/10,000 to 1/20,000], Prader-Willi syndrome (PWS) [prevalence: 1/15,000-30,000] and Silver-Russell syndrome (SRS) [prevalence: 1/30,000 to 1/100,000]. Some reports exist of a higher prevalence especially of BWS after IVF (relative risks up to 5.2). Nevertheless, the evidence to date is insufficient to support an association between IVF and imprinting disorders. Most importantly, in the absence of a comparison group comprising couples with infertility who conceived naturally, it is difficult to be certain as to whether an effect is due to IVF or to the infertility per se.

Yet, it is clear that from ovarian stimulation, throughout fertilization, embryo culture, biopsy (if performed) and embryo transfer, we operate in a very delicate phase of the gametogenesis and embryo preimplantation development. Therefore, limiting the manipulations and constantly controlling the safety of our protocols and their medium- and long-term effects (if any), is key. Of note, also the absence of issues must be reported in peer-reviewed journal so to decrease the impact of “reporting bias”, which often causes misinterpretations of the data. Studying epigenetic effects, still, will always be complex also due to the numerous confounders that might bias the interpretation of the evidence (like lifestyle, environment, ...) as well as the definition of a proper study design, analytical protocol and control group. Therefore, a careful interpretation of the evidence is critical.

Couples should continue to be made aware of the potential associations between ART and imprinting disorders, and informed that the absolute risk of their child being affected remains low.



Dr. Marc Torra Massana

Embryologist, Researcher and Master's Degree professor at Clínica Eugin, Barcelona

A Genetic Toolkit For Male Infertility In Fertilization Failure – Summary

Intracytoplasmic sperm injection (ICSI) has a mean fertilization rate around 70-75%, but total fertilization failure (FF) happens in 1-3% of all cycles. FF is a problem usually unexpected which implies a high psychological and economic impact to the patients and, currently, there is lack of tools for its diagnosis or prediction. Fortunately, during last years, a considerable number of studies have identified more than 30 different genetic variants in infertile men responsible of FF after ICSI. Most of these genetic variants affect PLCZ1, an oocyte activation factor which can explain around 30% of male-related FF. Most of these variants are pathogenic, affect regions which are crucial for PLCZ1 protein activity and, although not fully confirmed, certain PLCZ1 variants would cause infertility when carried in heterozygosis. In addition, recent studies broadened the spectrum of genes involved in FF, including ACTL9 and DNAH17. Despite all these important advances, future research is required to develop a complete genetic toolkit for the diagnosis of FF.



Dr. Lynne Nice

Laboratory Manager,
Care Fertility, Northhampton

**Laboratory KPI and their projections on IVF
clinic and laboratory management**

The aim of this presentation is to discuss how to choose and set Key Performance Indicators (KPI) in the laboratory.

Clinical and laboratory KPIs will be reviewed. The role of communication, documentation and audit in the laboratory will be discussed. The importance of change control before the introduction of new equipment and process and the monitoring of optimal parameters, value of training and standardisation in the laboratory will be demonstrated.

A good quality management system will demonstrate that irrespective of operator or laboratory, it is possible to provide a high quality process for patients.



Dr. Alexia Chatziparasidou

Consultant Clinical Embryologist,
Co-Founder and Director of Embryolab Fertility Clinic, Greece

The Impact of Fresh and Frozen Testicular Tissue Quality on Embryological and Clinical Outcomes.

Brief Lecture's Description

Azoospermia is considered the most severe form of male infertility. Until 1993, azoospermia was synonymous to sterility and was considered untreatable. The introduction of Intracytoplasmic Sperm Injection has revolutionized the treatment of azoospermia and enabled, for the first time, azoospermic men to father their own genetic offspring. Despite the progress so far, our ability to predict the potential of testicular spermatozoa to support embryonic development is still limited.

This lecture will focus in:

The impact of testicular tissue quality in terms of spermatozoa presence, motility and morphology when used fresh or frozen on embryological and clinical outcomes.

And aims to:

Contribute to maximize the chances of conceiving in azoospermic men via the development of customized management treatment approaches.



Dr. Jaffar Ali

Retired Professor and Senior Consultant Clinical IVF
Embryologist - University of Malaya, Singapore

Synthetic protein-free chemically defined media for human ART: Compliance with safety, regulatory and cultural norms

The SYNBIOS synthetic embryo culture, handling and cryopreservation media formulations are chemically defined. Their clinical efficacy has been demonstrated / published. The main topic of discussion of the present communication is that of issues relating to compliance with safety, regulatory and cultural norms with special reference to the synthetic media formulation. The synthetic media are devoid of biological components of non-uniform composition. This eliminates batch variation in the quality of media manufactured allowing batch-to-batch chemical consistency during production month after month. The direct consequence of which is that the variation in quality of embryos generated between batches is minimised or eliminated and is maintained uniform. It follows that the treatment outcome too likewise remains uniform and does not fluctuate between batches of media. This is because the quality of embryos generated can be maintained uniform month after month. Batch consistency in the quality of media manufactured is an important consideration with possible regulatory implications.

It is well recognized donor serum proteins cannot be sterilized with absolute certainty. Donor serum proteins carry a theoretical health risk because it may harbour hazardous protein-bound pathogenic agents such as viruses and prions which could be transmitted to embryos, patients and healthcare workers. The synthetic media prevents disease transmission which is anticipated to comply with safety and regulatory norms.

More than a hundred contaminant proteins other than albumin have been identified in human serum albumin (HSA) preparations used in making conventional IVF media products. Of these, 18 are associated with the innate immune and 17 with inflammatory responses. These undeclared contaminant proteins could potentially adversely influence embryonic development, gestation age, birth weight and perhaps have subsequent effects on health of the offspring. However this issue does not arise for/with synthetic media.

Serum albumin is extracted from blood and blood products. The latter are considered impure/unclean in many cultures. However the synthetic media is anticipated to comply with the cultural norms and lifestyle of many religions and beliefs, e.g.: Caste Hindus, Muslims, Jehovah's Witnesses, (possibly other faiths as well), including vegetarians, vegans, etc.

Micro RNA and DNA strands may be present as contaminants in media containing donor serum proteins. Albumin binds RNA, and to a lower level, DNA suggesting contaminant donor RNA/DNA in protein-containing culture media carry the risk of crossover with the embryonic genome which is not permissible in some cultures in absolute terms. The synthetic media being synthetic will circumvent this issue such that the genetic purity of the lineage of the progeny (i.e. ART babies) can be ensured which is paramount in a number of cultures. This attribute complies with the norms of major cultures of the world.

In conclusion the synthetic media is anticipated to be or appears compliant with the regulatory and safety issues as well as the cultural norms and values of some communities.



Dr. Daniel Hlinka

Cofounder Prague Fertility Centre
Prague, Czechia

Optimization of ICSI timing by non-invasive PLM spindle evaluation in order to minimize in vitro induced alteration of embryo development

1. Thank you Deepak for inviting me here and for my introduction and lets start with explanation why Optimising of ICSI timing should be of our interest
2. We know there are three major contributors of embryo creation
1st one:>
Spermatozoon – bringing the half of the chromosomes, centrioles missing in the oocytes and triggering the fertilisation
2nd one:.... >
Oocyte – which is the major biological contributor of the embryo creation
But we have to consider also the third one...>
in-vitro conditions including ICSI procedure.....>
which is the ultimate unnatural intervention However,
3. it's clear that without ICSI, the treatment of infertility would be substantially less effective, therefore.....>
if ICSI is used we have to minimize potentially negative effects of it.....>
4. And the first, and essential condition for successful ICSI is to inject>
the oocytes in their real mature state.
But the question which I am opening now is...
5. is if our oocyte maturity evaluation by conventional microscopy is correct?
6. So, let's go to clarify it by polarized microscopy which can reveal the stages of meiosis in much more details by intravital non-invasive spindle detection.

7. Using conventional microscopy, we distinguish three basic stages of maturity
 - GV oocytes with a distinct germinal vesicle>
 - MI oocytes having no polar body....>
 - and MII oocytes with extruded 1st Pb which are generally considered as mature oocytes and ready for fertilization....>
8. However, under PLM we can see the different pictures....>

While GV oocytes remain GV>
9. In so called metaphase I oocytes without visible 1st Pb we can find Metaphase I, Anaphase I and early Telophase I oocytes...>
10. However, in a reality, metaphase I oocytes are very rare...>

After analysing of more than 800 of oocytes we've found MI oocytes only in two cases....>
11. But this is not surprising because metaphase I oocytes occur after GVBD what takes about 25 hrs after meiosis resumption prompted by trigger

while the oocyte punctum is performed at least 36 hrs after the trigger...>
12. This is why the absolute majority of the oocytes without the 1st Pb are at anaphase or early telophase I
13. Now we should explain what is the difference between MI, AI and early TI oocytes
14. In MI oocytes the spindle is localised centrally corresponding to the previous position of GV... >

and the chromosomes are still in the form of homologous pairs....>
15. During Anaphase the spindle moves to the periphery of the oocyte>

and the chromosomes are already segregated....>
16. And the position of the spindle and segregation of chromosomes is a crucial difference between metaphase and anaphase oocytes...>
17. In early telophase we can find the first signs of cytokinesis....>

as a very subtle protrusions of the oolemma...>
18. Taken together, we can conclude that so called MI oocytes are in fact at ...>

Anaphase I or early Telophase I and real MI oocytes are very, very rare...>
19. And now you may have a question:

Why do we need to distinguish it?

The answer is very simple>

Its about the knowledge...>

20. Because in embryology as well as in our life everything is about the timing....>
The cells are developing in the cell cycles which are very specific before and after fertilisation and therefore, the understanding of the cell cycles is a basic knowledge of embryology. The timing of embryo development is better known from time-lapse data but the oocyte maturation is still overlooked. This is why we should know it. And therefore, it doesn't matter if we call these stages MI or AI or early telophase, it is much more important that we know what's behind it and how to manage it. Now, let's go back to the oocytes because....>
21. even in so called MII oocytes we can see the different pictures under polarized and conventional microscopy...>
The 1st Pb is visible in both microscopes ...>
22. But polarized microscopy can reveal ...>
telophase I>
interkinesis and ...>
the real metaphase II oocytes...>
What's the difference between these stages?....
23. At Telophase I.....>
the cytokinesis is not finished yet and for example, if you would perform a polar body biopsy at this time> you would remove all of the chromosomes because they are still not separated from the spindle...>
24. After telophase we should have an interphase in normal mitotic cycles. However, this a meiosis and reduction of chromosomes takes place...>
Therefore, instead of the interphase leading to chromosome duplication we have a special phase called interkinesis.>
During this phase the spindle disappears in human and is not detectable for about 1-2 hrs...>
The disappearance of the spindle is a normal spindle behaviour during maturation of the oocytes and occurs before a new metaphase II spindle reappears... >
Therefore, it must be considered that the absence of the spindle is not always a sign of poor oocytes. ..>
25. And finally, after interkinesis we can reveal the real mature oocytes being at metaphase II which are arrested at this phase until fertilisation....>
26. Let's go to summarize the phases of oocyte maturation as seen under PLM in sequential recordings

27. Now we should already understand the phases of meiosis>
and we can go back to the former question...>
What is a correct time for ICSI?
28. In order to specify it we should know that the period of the
oocyte's ability....> to be fertilised and to create healthy embryos is not
the same. Here is one example for understanding it.....>
29. Maybe 20 years ago we used to do so called rescue ICSI in oocytes
that failed to fertilise by conventional IVF.
The result of this rescue ICSI....>
was high fertilisation rate but no viable embryos or pregnancies.
It means that the oocytes ability to produce healthy embryos is shorter
than ability to be fertilised.
Moreover, there is another very important factor influencing the correct
ICSI timing ...>
30. We have to distinguish between>
normal and delayed responders because in delayed responders....>
the progression of meiosis is substantially longer as it is expressed in
duration of anaphase – metaphase transition.
Pls. keep it in mind because its a crucial factor discussing latter
how to manage the ICSI timing....>
31. So let's go to find out what is the real impact of ICSI in normal responders.
Please, note that there is no clear line between normal and
delayed responders but....> for our study we considered the patients
with more than 8 oocytes retrieved and more than 80% of them being
at metaphase two as normoresponders...> We pooled the oocytes from
the same cohort and compared the effect of ICSI upon.....> fertilisation...>
blastocyst rate....> and induction of abnormal embryo development....>
32. In Metaphase II oocytes we wanted to find out...>
if ICSI shift for 3-4 hrs can influence the results...>
for the 2nd group we used telophase I oocytes with extruded 1st
polar body..... and for the third group we used the oocytes at anaphase
or early telophase it means those.....> with a visible sign of cytokinesis...>
33. Fertilisation rate was unchanged in MII and late telophase oocytes but ...
..>It was significantly decreased in anaphase and early telophase oocytes
34. Even blastocyst rate was significantly impaired only in anaphase/
early telophase oocytes

35. Abnormal 2PN fertilisation occurs when meiosis is not finished...> by extrusion of the 2nd polar body but it proceeds directly to PN formation after interkinesis....> Maternal PN was diploid as confirmed by PGT-A> This abnormal fertilisation does not have to be distinguished from normal 2 PN zygotes if you are not able to recognize a missing 2nd polar body...>
36. Another anomaly induced by ICSI of immature oocytes is 1 PN fertilisation....> An extra polar body extrusion was observed in 1 PN zygotes what could be explanation of missing PN....> Resulting embryos cleaved abnormally and were arrested very soon....>
37. Taken together we can conclude that in order to avoid in-vitro induced anomalies and keep high fertilisation and blastocyst rate in normal responders we have to keep these simple rules.....>
1. *MII oocytes having 1st Pb after OPU can be fertilised without any specific timing preferences.....>*
 2. *so called MI oocytes without 1st polar body at OPU should be fertilised at about 2 hrs after the polar body extrusion>*
 3. *use PLM to verify the spindle or time-lapse to record the time of PB extrusion*
- Now you could ask me why so many words and pictures when the situation is rather simple and clear----inject just the oocytes with the 1st polar body. Yes, its true..... >
38. but only for normoresponders....
- Because majority of our patients are delayed or poor responders having up to 5 oocytes from which only 1-2 are at MII and majority are immature oocytes...
39. And in delayed responders the situation is a bit different when compared with normoresponders.....>
- We observed a significant alteration of fertilisation and blastocyst rate even in group of telophase oocytes.....>
- Without PLM you are unable to recognise anaphase I oocytes because in conventional microscopy it already has a visible 1st polar body and....> you cannot distinguish it from real metaphase II oocytes.
- So the last question to be answered today is....>
40. How to manage ICSI timing to avoid in vitro induced anomalies in delayed responders?

We need to get a time to mature the oocytes...>

Therefore, the first step is start a trigger 2-3 hrs sooner>

and postpone the ICSI time 6-7 hrs after OPU...>

and if you have a possibility, verify it by PLM.....>

41. This a system we applied in our centre for a longer time to get at least one embryo for transfer and to avoid cancelation of the cycles what is very frequent in poor or delayed responders.....>

Finally, here is one comment from ISIDA embryologist which followed my presentation on LinkedIn last year.

In brief>

By postponing of ICSI 6-7 hrs after OPU we decreased a cancelation rate in women over 40 from 44 to 24%

And this is why we are doing it.

Thank you for your attention and if you are interested you can find this topic also on my LinkedIn profile.

Have a good day



Dr. Gábor Vajta

Freelancer, Consultant in Embryology,
and Founder of Vita Vitro Shenzhen, China

"Humidification - the Veterinary Horse of Embryology?"

The aim of this lecture is to analyse factors contributing in osmolality problems in embryo cultures, and to highlight the urgent need to resolve them. Although osmolality was always regarded as a crucial parameter of media used in assisted reproduction, its change during embryo culture was ignored until recently. During the first decades of IVF, when short-term group cultures were used in a humid atmosphere, no dramatic changes in osmolality occurred, and most embryologists supposed that the oil overlay prevented dehydration completely.

This false assumption led to the introduction of dry benchtop topload incubators based on a number of additional unsupported arguments. Moreover, blastocyst transfer was also introduced those years. Eventually, after the application of time-lapse machines requiring single media-single embryo-uninterrupted cultures, controversial results drew attention to dehydration. Its harmful effect is widely recognised now: it may decrease the overall efficiency with 10 to 20%. Although some adjustments may slightly alleviate the problem, the ultimate way should be to restore the close-to-100% humidity. This is impossible with the highly popular and widely used dry incubators - unless we find a simple and inexpensive, promptly applicable escape route.

**Dr. Rahul Sen**

Consultant Embryologist
Neelkanth Fertility, Jaipur

Advances in Sperm Preparation Methods

A total of five million births all over the world are the result of ART. Half of the DNA contributed to the offspring is by the sperm. Numerous Advanced techniques were developed to isolate superior quality spermatozoa with intact chromatin condensation and without chromosomal abnormalities for use in ART. Currently available Conventional techniques such as Density Gradient Centrifugation (DGC), the swim-up select sperms solely based on their motility and morphology. However, the important factors that affect the fertility such as oxidative stress, physiological damage and DNA integrity cannot be assessed by any of these conventional techniques. Sperm DNA integrity has been demonstrated in plethora of publications & has shown association of sperms with poor DNA integrity with decreased implantation and pregnancy rates.

Conventional sperm separation techniques show distinct limitations in that they do not necessarily select spermatozoa according to their functional competence or genetic quality as it is achieved in the female genital tract. In view of these concerns, scientists and clinicians are increasingly urged to improve sperm separation techniques in order to select the most functional spermatozoa for fertilization. Hereby, the emphasis is rather on the health of the progeny than on achieving pregnancy or increasing the success rates of ART. To achieve selection of spermatozoa based on these natural principles, scientists and clinicians need to understand the processes of sperm selection occurring in the female genital tract in order to mimic the chemical and physical mechanisms involved i.e. the principles of sperm selection should be as close as possible to the natural selection processes in the female.

**Dr. Kersi Avari**

Founder Director -
Embryology Academy of Research & Training

**What do you mean by Quality in IVF?
Technology - Results- Patient satisfaction?**

In vitro fertilisation- a standard and one of the most dependable and sought after procedure in assisted reproduction needs no introduction. A process which has nearly conquered the challenge of infertility put forth by nature for some reason or another and which brings a smile of hope to the hopeless couple is an amalgamation of galloping technology, precise implementation and dedicated application.

Hence it will be impossible to compartmentalise the IVF procedure, its results and the patient satisfaction!! The end result is an amalgamation of all three which ultimately bears rich dividends in the form of a successful implantation resulting in the much awaited pregnancy. The endless wait and patience endured by the infertile couple is a stress stretched beyond expectations. Hence the couple undergoing any form of ART treatment undoubtedly deserves optimal care, dedicated treatment to the best of their satisfaction. Remember the infertile couple is physically, financially and emotionally stressed. An assurance, a comfort zone and a helping hand is all the patient needs.

So quality in IVF can't be the domain of any single entity. An ideal infertility laboratory professionally managed fully equipped with latest and relevant state of the art technology managed by skilful Embryologists should justify to the needs of the patient and should be worth the money spent.

The huge cost of the treatment and irony is that the results are not that towering as per the patients expectations is an another spoke in the wheel which throws us an immense challenge. Many a times treatment with a smile is enough to erase the psycho somatic stress thus propelling the patient from a state of helplessness and despair into a state of confidence and positivity a much needed catalyst!!

A patient will not care how much the doctor knows but the patient will always want to know how much the doctor cares!!



07 Oral Abstracts

Does looks matter? Impact of isolated teratozoospermia on fertilization and embryo outcome in ICSI cycles.

M.Alekhy PhD , Charulata Chatterjee*PhD, Jyothi.B MRCOG
Ferty9 Fertility Center, Secunderabad

Charulata88@gmail.com

Background -

The classification of morphologically normal sperm has been progressively redefined. Concurrently, our understanding of the significance of sperm morphology in relation to male factor infertility has evolved. In this study we will examine the impact of sperm morphology on ICSI outcomes.

Objective -

This retrospective study aimed to re-evaluate the clinical value of a 4% cut-off threshold of sperm morphology in *ICSI cycles*.

Design -

Ferty9 Fertility Center

Materials and Methods -

This study was carried out from Feb 2021 to May 2021 for a total of 294 ICSI cycles, with sperm samples classified according to WHO classification. Group 1 (Control) included 102 couples with normal sperm morphology ($\geq 4\%$ morphology). Group 2 (T; teratozoospermic) included 192 couples, with isolated teratozoospermia in the male partner (morphology, $< 4\%$).

Results -

No statistically significant difference was seen in the two groups regarding age, duration of infertility and embryos transferred. 918 oocytes were retrieved in group1 where 652 mature oocytes were injected and 613 fertilized [Fertilization rate: 94%] Where as in group; 2 a total of 192 ICSI cycles yielded 1728 oocytes and out of 1245 mature oocytes 1109 fertilized [Fertilization rate 89%] The fertilization rates were significantly lower in group 2 than in group 1. [p=.0004%] But no significant differences were found in embryo quality between groups 1 and 2.

Conclusion -

Sperm morphology assessed by WHO criteria had little prognostic value in ICSI cycle outcomes. Sperm morphology did not appear to influence embryo development or embryo morphology. Microscopic selection of sperm with "normal" morphology during the ICSI procedure allowed excellent outcomes even in samples with severe teratozoospermia.

Key words -

Teratozoospermia, ICSI, sperm morphology

A comparison of cleavage stage Vs Blastocyst embryo transfer in autologous frozen oocyte cycles.

Shalini R M.Sc ,Charulata Chatterjee*PhD, Jyothi B MRCOG
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Background -

Oocyte freezing is an established technology and empirical improvements in freezing protocols and the use of ICSI for fertilization led to an increasing number of live births. oocyte cryopreservation technology may indicated for social freezing, woman suffering with cancer or lack of partner. The other indication may include unavailability of partner at pickup time or cannot produce sample on demand and with no back up sample.

Objective -

The aim of this study is to evaluate the implantation potential and clinical pregnancy rates between the day 3 cleavage stage and Day- 5 blastocyst stage embryo transfers in autologous oocyte freezing cycles employing fresh or vitrified embryo transfer.

Setting -

Ferty9 Fertility Center

Materials and Methods -

This is a retrospective evaluation of frozen thawed oocyte and ICSI embryo transfers completed at our centre. Following fertilization, all embryos were transferred either at the cleavage or blastocyst stage. Total of 21 frozen thawed oocyte and ICSI cycles were performed. Patients were divided into two groups, Group: 1 Day-3 cleavage stage transfer (n= 11) and Group: 2 Day-5 Blastocyst transfer (n=10) Reproductive outcomes in both the groups were compared.

Results -

Our results confirmed a 50% clinical pregnancy resulting from day 3 embryo transfers, and 54.5% from day 5. This is not clinically significant.

Conclusion -

Our findings indicate that there is no significant difference in clinical pregnancy rate in cleavage stage Vs Blastocyst embryo transfer in autologous frozen oocyte cycles. To conclude further a large group study is recommended.

Key words -

cleavage stage, blastocyst stage, embryo transfer, frozen-thaw oocyte, ICSI

Is There A Role For Use of Surgically Retrieved Testicular Sperms In Individuals With Cryptozoospermia?

Shankhadeep Debnath, Krishna Mantravadi, Durga G Rao

Topic

Abstract Title -

IS THERE A ROLE FOR USE OF SURGICALLY RETRIEVED TESTICULAR SPERMS IN INDIVIDUALS WITH CRYPTOZOOSPERMIA?

What Is Known Already -

Cryptozoospermia men have very low sperm count and motility and this could be detrimental for Assisted Reproductive Outcomes (ART) outcomes. Use of testicular sperm (TESA) to optimize ART outcomes has been proposed in the past. Superiority of TESA sperm over ejaculated sperm is still a matter of debate

Study Question -

In Individuals with cryptozoospermia and undergoing ART, will surgically retrieved testicular sperm optimize reproductive outcomes?

Study Design, Size, Duration -

This was a retrospective study of couples seeking ART cycles with cryptozoospermia at our private fertility clinic between Jan 2013 to Dec 2019. Cryptozoospermia men underwent ICSI either with ejaculate sperm (n=38) or with sperm retrieved from TESA (n=45).

Participants/Materials, Settings, Methods -

This retrospective data analysis of cryptozoospermia ART cycles was done from general population. All couples with cryptozoospermia underwent ICSI either with ejaculated sperm or sperm retrieved using TESA. Offering TESA to cryptozoospermia individuals was a departmental policy and necessary consents were obtained with prior counselling. Only couples who had a successful frozen embryo transfer with one or two blastocysts, created from autologous gametes were considered for this study. Live birth rate (LBR) and Miscarriage Rate (MR) were the primary outcomes.

Results -

Reproductive Outcomes of TESA Vs Ejaculated sperm were:

Clinical Pregnancy rate (CPR) – 19/45 (42%) Vs 20/38 (53%)
(OR 0.66 CI- 0.28 to 1.57) (P=0.34)

Implantation rate - 22/80 (28%) Vs 19/74 (26%) (OR 1.09 CI - 0.54 to 2.25) (P = 0.80)

Miscarriage per ET - 2/45 (4.4%) Vs 2/38 (5.3%)
(OR 0.84 CI - 0.11 to 6.2) (P=0.86)

LBR - 17/45 (38%) Vs 18/38 (47%)
(OR -0.67 CI 0.28 to 1.62) (P=0.38)

Though statistically not significant Ejaculate sperm group couples seem to have better LBR than TESA group.

Data from this study doesn't seem to show superiority of one intervention over the other

Considering the invasiveness of TESA procedure and lesser LBR (which was statistically not significant) in TESA group, there seems a need for a RCT to look for role of TESA for cryptozoospermia men as an active intervention to optimize reproductive outcomes.

Limitations, Reasons For Caution -

Small sample size, Retrospective data

Wider Implications Of The Findings -

Use of surgically retrieved sperm for cryptozoospermia men to optimize reproductive outcomes still needs further research.

CONCLUSION -

Use of Testicular Sperm Aspiration (TESA) to optimize reproductive outcomes for cryptozoospermia men doesn't seem to be a superior intervention over ejaculated sperm.

Can Microfluidic Sperm Sorting Help Separation of Sperms With Good Quality Dna?

Rakhi.r, Krishna Mantravadi, Durga G Rao, Sandeep Karunakaran

What Is Known Already -

It is evident that raised sperm DNA Fragmentation (SDF) negatively affects the reproductive outcomes. Management for raised sperm DFI to optimize reproductive outcomes is still elusive. Microfluidic sperm sorting and Magnetic cell sorting are few of the newer methods employed to obtain sperms with good DNA and optimize reproductive outcomes. However, there is further need for clinical validation of these newer interventions.

Study Question -

In Individuals with raised SDF, will sperm sorting with Microfluidics principle help in obtaining sperms with good quality DNA?

Study Design, Size, Duration -

This was an observational pilot study performed at our private fertility unit from August 2020 to March 2021. Couples with raised SDF (>15%) were recruited and offered microfluidic sorting (n=34).

Participants/materials, Settings, Methods -

Couples with history of one failed IVF cycle were offered testing for SDF. Individuals with SDF > 15% were included in the study. SDF testing was done with SCSA flow cytometer method. On the day of oocyte retrieval male partners were advised to come with 48hrs abstinence and semen sample was obtained by masturbation. Small aliquot of ejaculated neat sample was sent for SDF testing. Zymot multi ZMH0850 Microfluidic chamber was used for this study. Protocol for microfluidic sorting was done as per the manufacturer's instruction. Post microfluidic processing the sample was sent to SDF testing again. SDF values of neat and processed samples were compared. Appropriate consents were obtained from the couples that were recruited in this study after thorough counseling.

Main Results -

Mean of SDF of neat samples was 24%.

Mean of SDF of microfluidic-sorted samples was 3.1%.

SDF values of all samples after microfluidic sorting were less than 10%.

Paired “t” test was done and the outcomes were statistically significant.

There seemed a significant reduction in SDF values with microfluidic sperm sorting.

Microfluidics Sperm Sorting seems to be a promising intervention to obtain sperms with good DNA quality.

Limitations, Reasons For Caution -

Smaller sample size. Further research is warranted to find an optimal method for sperm sorting, which would further help improving the reproductive outcomes.

Conclusion -

Microfluidics Sperm Sorting seems to be a beneficial intervention to optimize sperm selection with good DNA quality for Individuals with raised sperm DFI.

Recurrent Implantation Failure – Role of PGT and ERA to Optimize Reproductive Outcomes

Dr Gaurav Mittal, Dr Krishna Mantravadi, Dr Durga G Rao

Objective -

To assess the role of Pre-Implantation Genetic Testing for Aneuploidy (PGT-A) and Endometrial Receptivity Assay (ERA) in couples with Recurrent Implantation Failure (RIF) to optimize reproductive outcomes.

Design -

This is retrospective data of couples from our private fertility teaching clinic with RIF during January 2014 to July 2019.

All the patients recruited for this study had a history of RIF & the study population was divided into three cohorts;

Cohort A – Both PGT-A and ERA done (n=79)

Cohort B – only PGT-A done, no ERA done – (n=54)

Cohort C – No PGT-A / No ERA done (n=189) (CONTROL GROUP)

Inclusion Criteria -

Women with at least two fresh/frozen embryos transfers with minimum 4 blastocysts transferred in total and never conceived were considered as RIF.

Only women with one euploid embryo, who underwent frozen embryo transfers (FET) were recruited in this study in cohorts A & B.

Women of all age groups who had Blastocysts available for transfer were included in Cohort- C.

Only self-gamete cycles were considered in this study.

Materials And Methods -

All the women with RIF underwent controlled ovarian stimulation and oocyte retrieval as per our clinic's standard operating protocol (SOP). ICSI was the choice of insemination considering history of failed implantation, fertilized oocytes were cultured till blastocysts and freeze-all policy was adopted. Blastocysts were biopsied and trophectoderm tissue was subjected to genetic testing through Next-Generation Sequencing (NGS).

ERA was done as per our institutes SOP. The biopsy was done using an endometrial biopsy pipette supplied by the company at 120 hours (day 5) of the start of the Progesterone supplementation. This procedure was repeated at 144 hours (day 6) as well. This was done to cater for the reduction in the error that could occur due to the extrapolation of results in cases of pre-receptive samples. The collected samples were stored and transported in the as per the company's prescribed method. The same conditions were replicated in the (FET) transfer cycle.

Cohort- A women underwent elective Single Euploid Blastocyst transfer (eSET) in an artificial cycle as per the ERA timing. Cohort B underwent eSET of a Euploid Blastocyst in a FET cycle on 6th day post progesterone support, no ERA was done. Cohort- C women underwent transfer with un-screened double Blastocyst Transfer (DET) in a FET cycle. Primary Outcome was Implantation Rate (IR).

Results -

Mean of IR of all subjects were calculated as follows:

Cohort A - 53%

Cohort B - 47%

Cohort C - 42%

Odds ratio and p-value was calculated for IR between the groups and no statistically significant difference was noted.

Though Cohort A had the best implantation rates, there was no statistical significance with the other two cohorts. Role of PGT-A and ERA as an intervention to improve reproductive outcomes is still debatable and needs well designed RCTs to further infer.

Conclusions -

PGT-A and ERA for RIF couples do not seem to offer beneficial reproductive outcomes that are statistically significant. Couples need to be counselled appropriately while offering treatment options for RIF.

Impact Statement -

Considering the data of this study and the existing literature, interventions in RIF patients to optimize reproductive outcomes still needs further research.

Optimizing Sperm Selection by Magnetic Cell Sorting (MACS) for Couple with Raised DFI and the Reproductive Outcome

Gayathri. G, Dr. Krishna Mantravadi, Dr. Durga G Rao

Aim -

To assess if MACS intervention has helped improve the reproductive outcome of couples with raised DFI.

What is Already Known -

Many studies have shown that male factor plays a major role in infertility. Sperm DNA fragmentation has recently become the most widely studied complementary test. Studies have demonstrated that sperm with genetic defects are directly associated with infertility. As we already know, MACS is a method that reduces apoptotic sperm and improves post process sperm and embryo quality. Several recent studies have recommended MACS selection regardless of DNA fragmentation results because apoptotic sperm is not exclusively associated with sperm DNA fragmentation.

Study Design -

This is a retrospective study of couples that underwent fertility treatments at our center in the years 2018-2020. A total of 90 patients were included in this study. Couples with DFI of >20 % were only included in this study. (n=90). All women were under the age of 35years and underwent controlled ovarian stimulation as per our clinic's standard operating procedures. Semen sample on the day of oocyte retrieval was subjected to MACS and oocytes were injected with MACS separated sperm by ICSI. Embryos were cultured till Blastocyst Stage and then vitrified. In a frozen embryo transfer (FET) cycle the blastocysts, which showed 100% survival, were transferred. Implantation rates (IR), Clinical Pregnancy rates (CPR) and Miscarriage rates (MR) were calculated.

Results -

Couples with >20 % raised DFI showed a CPR of 72.13 %, IR of 43.58 % and MR of 1 %. MACS intervention seems like a beneficial intervention to optimize sperm selection at ICSI and in-turn ensuring an optimal reproductive outcome in couples with raised DNA Fragmentation.

Limitation -

Retrospective data, Small sample size

Conclusion -

MACS seems to be an effective intervention for optimizing sperm selection criterion in specific defined groups and its role in routine use for all couples undergoing fertility treatments needs further evaluation. It is also a beneficial intervention in terms of optimizing the sperm selection, and helps improve the reproductive outcome with couples having raised DFI.

Sperm Selection with Microfluidics Can Improve The Reproductive Outcomes In Women with Previous Failed IVF Cycles.

Ramya Sai Rayapati, Dr. Krishna Mantravadi, Dr. Durga G Rao

Objective -

Will sperm selection by Microfluidics optimize the reproductive outcomes for women with history of previous Failed IVF cycle?

What Is Known Already -

Reasons for failed IVF cycle are still elusive. Sperm selection criterion at Intra-Cytoplasmic Sperm Injection (ICSI) is shown to influence reproductive outcomes. In spite of decades of research we have not yet found the best method to optimize sperm selection. Microfluidics technique has shown to sort sperms with relatively normal sperm DNA fragmentation Index without adding any additional factors while processing the sample through centrifugation and there by improving the reproductive outcomes.

Study Design, Size, Duration -

This is a retrospective study of couples that underwent fertility treatments at our center in the year 2020 to 2021. 100 women with previous failed IVF cycles were offered Microfluidics and recruited in this study (n=100).

Participants/Materials, Settings, Methods -

Women with history of un-explained previous failed IVF cycles were only included in this study (n=100). Reasons that could lead to previous failed IVF cycles are advanced maternal age, uterine anomalies, high sperm DNA fragmentation. Severe male factor infertility was excluded from this study. All women were under the age of 35 years and underwent controlled ovarian stimulation as per our clinic's standard operating procedures. Semen sample on the day of oocyte retrieval was subjected to Microfluidics by using Zymot Multi (850µl) chip and oocytes were injected with Microfluidics separated sperm at

ICSI. Embryos were subjected to extended culture till blastocyst stage and vitrified. Single or double embryo transfers were done with embryos that showed 100% survival rate post thaw. Implantation rates (IR), Clinical Pregnancy rates (CPR) and Miscarriage rates (MR) were calculated.

Main Results And Role Of Chance -

This group of women had a previous failed IVF cycle and the usage of Microfluidics separated sperm in this cycle at ICSI showed Implantation rates (IR) of 59%, Clinical Pregnancy rates (CPR) of 73% and Miscarriage rates (MR) of 9%.

Microfluidics seems like a beneficial intervention to optimize sperm selection at ICSI and in-turn ensuring an optimal reproductive outcome in couples with history of previous failed IVF cycles.

Limitations, Reasons For Caution -

Retrospective data, Small sample size

Wider Implications of The Findings -

Microfluidics seems to be an active intervention for optimizing sperm selection criterion in specific defined groups and role in routine use of Microfluidics for all couples undergoing fertility treatments needs further evaluation.

Does Trophoctoderm Biopsy In Preimplantation Genetic Testing Affect Serum Beta Hcg Levels?

Dr. Swetha Kandru, Dr. Krishna Chaitanya. M, Dr. Durga. G. Rao

Objective -

To assess whether trophoctoderm (TE) biopsy has any impact on the level of Serum Beta Human Chorionic Gonadotropin (β -HCG) in early pregnancies.

Materials And Methods -

This is a retrospective cohort study conducted at a tertiary level fertility clinic. The study population comprised 100 pregnant women who underwent the transfer of single Euploid vitrified-warmed blastocysts after trophoctoderm biopsy and PGT (Preimplantation Genetic Testing) between January 2017 to July 2018. The control group had 100 women undergoing FET cycles with un-screened single good-grade blastocyst. All women having positive serum β -HCG results 14th day after blastocyst transfer were included in the study. Main outcome measure(s): Serum β -HCG levels on the 14th day after blastocyst transfer.

Results -

The mean serum β -HCG concentration of the PGT group was 1427 ± 1230.71 mIU/ml and that of the control group was 1608.07 ± 967.76 mIU/ml. After log transformation of β -HCG values to normalise distribution, mean difference in β -HCG was not found to be statistically significant ($p = 0.108$).

Conclusion(S) -

Trophoctoderm biopsy of blastocysts for PGT did not affect the serum Beta HCG level 14 days after transfer.

Impact Statement -

Data from this study shows that Trophectoderm Biopsy does not seem to affect the process of implantation and early pregnancy events. Further research is required to validate this finding.

In silico analysis of CatSper4 gene regulation in Male infertility

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Abstract -

The CatSper genes are the novel family of 4 sperm-specific Ca^{2+} -permeable channel which is pH $\square$ dependent. These genes play an important role in sperm motility, acrosome reaction, sperm and oocyte fusion. CatSper1, CatSper2 and CatSper3 are very well studied genes for their role in Asthenozoospermia. However, the role of CatSper4 is still needed to be investigated. In this study, we have used the In silico approach to analyse the role of CatSper4 gene in sperm tail defect. STRING analyses was done to understand the CatSper4 Protein-Protein Interaction (PPI) network. The interacting proteins with a confidence score of ≥ 0.900 were chosen for PPI network visualization construction, where it was observed that CatSper4 protein is not only associated with Sperm Flagellum but also has its role in Spermatogenesis. This analysis showed strong interaction with CATSPER1, DNAH1, CFAP251, TEX40 and HSPA2, these proteins which are present in this network show strong relation with Flagellated sperm motility, Sperm motility and taxes, Fertilization, Spermatogenesis and Asthenozoospermia. Similarly, analysis through Gene Ontology showed the association of CatSper4 with Sperm Flagellum. Elsevier Pathway Collection showed its role in Sperm motility impairment and proteins involved in male infertility. Therefore, we report the role of CatSper4 gene in sperm tail function at genetic level as it is expressed in

spermatogenesis and sperm flagellum. Understanding the molecular mechanism(s) of regulations of CatSper4 will help us for the development of therapeutic approach in infertile male.

Keywords -

CatSper genes, Sperm motility, Sperm flagellum, Spermatogenesis, STRING, Enrichr, Elsevier Pathway, Gene Ontology, Asthenozoospermia, Male infertility

Context -

ICSI for patients with severe OAT is a challenge with respect to finding sperm, and then choosing those that are motile as well as normal. Procedures like MACS, PICSI, or microfluidics often cannot be offered to these patients due both the sperm count and motility being much below 1 mill/ml and 1% respectively. This method uses PvP channels to obtain relatively normal motile sperm for ICSI for OAT patients for whom flushing media is usually used. FM compromises ICSI by affecting pipette suction and control.

Aim -

To visualize and catch motile sperm from severe OAT sample and use the principles of microfluidics to select normal sperm.

Methods

Materials - Spermwash media, 7% PvP

Add 1 ml spermwash to the liquefied semen sample, and centrifuge it at 1600 RPM for 15 minutes. Discard the supernatant and concentrate the pellet, and incubate at 37°C for 30 minutes. On an ICSI dish, make a thick wide rectangular streak of PvP and add droplets of flushing media to keep oocytes. Overlay with oil. Purposely overload the bottom of the PvP streak with washed semen taken from the mid of the sample tube. Incubate this dish at 37°C for 5 minutes. Once the dish is taken out, pull the thick PvP streak sideways in 3-4 areas to form channels. Incubate the dish at 37°C for a further 10 minutes after which it can be placed on the micromanipulator for ICSI.

Results and Conclusion - The incubation post addition of washed overloaded semen allows motile sperm to escape the debris filled semen streak and swim to corners of the pvp streak where no semen was deposited. The normal, good quality sperm will swim inside the channels using the principles of microfluidics movement and sperm present INSIDE the channel can be immobilized and used for ICSI. This improves fertilization and blastulation rates.

