Dear Friends

Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after a year of regular unprotected sexual intercourse. Environment has effect on various aspects of health including reproduction. Environmental toxins and pollution have detrimental effect on both male and female fertility. In these focussed meetings we wish to discuss how these toxins can cause damage and what steps can be taken to decrease it.

I am sure you would enjoy the meetings in different parts of the country in the forthcoming months and reading the manual. I would like to sincerely thank “Trivector” for supporting us in this academic endeavour.

Dr M Gouri Devi  
President - IFS

Dear Friends

It gives me immense pleasure that IFS is organising series of meetings on environment and ART in different part of country in the forthcoming months. Environment is known to affect reproductive health and fertility in both male and female. Success rate in lab is also dependent on the environmental conditions inside the lab. We are commonly exposed to potentially toxic materials such as lead, mercury and polychlorinated biphenyls (PCBs) etc.

The purpose of these CME’s is to increase awareness and to limit the damage. In our meetings the environmental toxicants will be discussed, how it affects, what is the current evidence and what can be done to limit its harmful effects.

I would like to thank team who have worked hard to bring the program to fruition. Last but not the least, sincere thanks to Trivector team for supporting this scientific and educational initiative. I would like to thank Mr Dilip Patil for enabling the same.

Prof (Dr) Pankaj Talwar  
Secretary General -IFS
Dear Colleagues

Environment effects various aspects of health including reproductive health. There is enough robust evidence suggesting linking of toxic environmental agents to reproductive and developmental health outcomes. Reducing exposure to toxins especially in pre-conception and pre-natal period is important, as it may have profound and lasting effects. Healthcare providers should provide guidance and should act to find better alternatives. Pollution is becoming major problem especially in some metropolitan cities in India. Increased awareness and simple steps can limit toxicity.

These focussed meetings have been designed to address the above felt need of environment awareness and its effect on reproduction and ART. This handbook includes all aspects of effect of environment on both male and female reproduction, on eggs, sperms and lab.

Special thanks to whole team for their constant support to help us and organize these meetings. Centrally Dr Gauri, Dr Pankaj and Gaurav Kant for their valuable contributions, without which this initiative would have been not possible. Sincere thanks to local coordinators Dr Rajan Vaidya (Mumbai), Dr Kunjimoidee (Kochi), Dr Roya Rozati (Telangana) who had been very supportive in this educational initiative. Last not the least, sincere thanks to Mr Dilip Patil from Trivector in bringing the program to fruition. We hope that through these focussed meetings there will be increased awareness and knowledge to improve overall fertility and reproductive health.

Dr Sweta Gupta
National Coordinator

Clinical Director & Sr Consultant (Reproductive Med. & IVF)
Medicover Fertility, Delhi.

MBBS, MD(Obs & Gynae, Delhi)
MRCOG (London, UK), DFSRH (UK)
MSc (Reproduction & Development, Bristol, UK)
Fellowship in Reproductive medicine & ART (London, UK)
Certified ART expert from British Fertility Society
Executive member, governing body of Indian Fertility Society (IFS)
Member representative, RCOG North Zone, India society.
Chairperson, Infertility committee, Noida Obs and Gynae Society.
Course convener and faculty: MRCOG examination revision courses, Delhi.
RCOG(UK) International MRCOG examiner (2018), Delhi, India
Publications in International/national journals
Presenter in various international/national conferences/meetings
Founder team member of Medicover fertility chain in India.
Dear Friends

The interaction between human health and the environment has been extensively studied and environmental risks have been proven to significantly impact human health, either directly by exposing people to harmful agents, or indirectly, by disrupting life-sustaining ecosystems.

Scientists have discovered that the thousands of chemicals that have enabled many of life’s conveniences may have been robbing us, slowly but surely, of our most precious necessity for future survival: our fertility.

Everything from genetics to lifestyles to environmental exposures may play a part. And for many of these exposures, it may be impossible to determine precisely the amount that will endanger any individual at a particular stage of life.” Each of us, in essence, may have our own fertility “tipping point.”

I am ecstatic to share these focus meeting on “Environment and Reproduction” with all of you, which will be highlighting the impact of environment on fertility and lab culture conditions.

Gaurav Kant
National Co-ordinator
Co-convener (SIG Embryology IFS)
Dr M Gouri Devi

- Director, Gouri Hospitals Ltd.
- Director, Ridge IVF Group (Runs a chain of IVF centres)
- President, Indian fertility society
- Ex-Secretary General, Indian Fertility Society
- Executive, AOGD governing council
- Member, Executive Board, NARCHI, DGES, FPSI
- Ex Vice President, NARCHI
- Chairperson, Advocacy & Ethics Committee, IFS.
- State Quality Assurance Committee (SQAC) Govt of NCT of Delhi
- Member: MTP advisory committee, Govt Of NCT of Delhi
- Member Advisory committee on ethical practices in the field of obstetrics, Govt of NCT, Delhi
- Recipient of Kanak Goel Award 1995-1996 from IMA.
- Chairman’s Appreciation Award by IMA AMS – 2002
- Dr. APJ Abdul Kalam Excellence Award – 2017
- Economic Times Award one of the Most Inspiring Gynecologists of India

She is a keen academician, has organized many conferences, has been a speaker in many national and international conferences. Has many publications to her credit

Dr Pankaj Talwar

- Sec IFS.
- Secretary Fertility preservation society of India.
- Editorial board of multiple Infertility journals.
- Member Advisory committee ICMR
- Member Infertility committee FOGSI
- Editor Nexus / Artext – E bulletin of IFS
- Awarded Vishisht seva medal by the President of India for working in field of infertility
- Associate Editor FSR
- Set up four centres for Armed forces.
- Experience of 10,000 and ET cycles.
- Member International society of fertility preservation.
- Trained Human Embryonic Stem Cell Derivation – Israel
- Trained in ovarian cortex freezing (fertility preservation) - Paris
- Trained in PGD – Germany, Spain
- Trained in QA/QC-Spain
- Edited 6 books

Col Pankaj Talwar, VSM
Professor and HOD
ART Centre, Army Hospital, New Delhi
Venue and Dates

MUMBAI
10th Feb 2019
Organising Chairperson
Dr Rajan Vaidya

HYDERABAD
7th Apr 2019
Organising Chairperson
Dr Roya Rozati

KOCHI
19th May 2019
Organising Chairperson
Dr Kunjumoideen

Organising Chairpersons

Dr Sweta Gupta
National Coordinator

Gaurav Kant
National Co-Coordinator

Dr Rajan Vaidya
Local Coordinator
Mumbai

Dr Roya Rozati
Local Coordinator
Hyderabad

Dr Kunjumoideen
Local Coordinator
Kochi
### List of contributors

<table>
<thead>
<tr>
<th>Topic</th>
<th>Contributed by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment Toxicants and Male Reproduction</td>
<td>Dr Kunjumoideen</td>
</tr>
<tr>
<td>Environment Toxicants and Female Reproduction</td>
<td>Dr Roya Rozati</td>
</tr>
<tr>
<td>Interesting cases (Testicular dysgenesis Syndrome / Miscariage / Malformation etc)</td>
<td>Dr Nancy Kumar</td>
</tr>
<tr>
<td>Options and advances in air purification technologies</td>
<td>Mr. Dilip Patil</td>
</tr>
<tr>
<td>Optimizing the culture environment in the IVF Lab</td>
<td>Mr. Gaurav Kant</td>
</tr>
<tr>
<td>Panel Discussion : Pollution (How it effects my fertility &amp; what can be done?)</td>
<td>Dr Sweta Gupta</td>
</tr>
</tbody>
</table>
# INDEX

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Environment Toxicants and Male Reproduction</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Environment Toxicants and Female Reproduction</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>Interesting cases (Testicular dysgenesis Syndrome / Miscariage / Malformation etc)</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>Options and advances in air purification technologies</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>Optimizing the culture environment in the IVF Lab</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td>Panel Discussion : Polution (How it effects my fertility &amp; what can be done?)</td>
<td>84</td>
</tr>
</tbody>
</table>
1. Environmental Toxicants and Their Effects on Male Reproduction
Outline

- Introduction
- Toxicants
- Toxicants effect on male reproduction
- Summary

Introduction

- Many hazardous man-made chemicals are voluntarily or involuntarily released into the environment on a daily basis, and thus exposure to such pollutants has become inevitable.

- A growing body of evidence suggests that environmental contaminants, including natural gas, endocrine-disrupting chemicals, and airpollution, are posing major threats to human reproductive health

- Air, water, and soil pollutants adversely affect sperm function.

- Plasticizers and phthalates are common endocrine-disrupting chemicals that bind to molecular targets and disrupt hormonal milieu.

- Indiscriminate use of several compounds of heavy metals and drugs threatens the normal development of male reproductive system and spermatogenesis.

- The adverse effects and toxicity of several chemicals override their beneficial effects
Oxidative stress

“Oxidative stress (OS) is a condition that reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system’s ability to readily detoxify (antioxidant defences) the reactive intermediates or to repair the resulting damage”
Primary pathologies of male reproductive system in connection with environmental toxins, oxidative stress and infertility

Reproductive toxicity

- Reproductive toxicity is defined as adverse impacts on sexual function/fertility in adult males and females, as well as developmental toxicity in the offspring.
- Two major categories:
  1. Any impact of chemicals that would interfere with reproductive ability. This may include, but not be limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, and premature reproductive senescence.
  2. Impacts on development of the offspring: The developmental toxicity includes any impact that interferes with normal development of the conceptus, before/after birth, and resulting from exposure of either parent prior to conception.

Exogenous sources of reproductive toxicity.

(B) Endogenous sources of reproductive toxicity
Routes of human exposure to some common environmental chemicals.
DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene; DDT = dichlorodiphenyltrichloroethane; PAHs = polycyclic aromatic hydrocarbons; PCBs = polychlorinated biphenyls. (Modified from Sharpe & Irvine, BMJ, 2004).

The process of normal mammalian spermatogenesis with three major phases: (1) spermatogonial phase, (2) spermatocyte phase, and (3) spermatid phase.

Exogenous environmental toxicants and oxidative stress: impact on male reproductive health.
What are the biological mechanisms of sperm DNA fragmentation (SDF)?

- Protamination Failure Replacement of histone to protamines during spermiogenesis
- Oxidative Stress Epididymis transit Post-ejaculation: leukocytes, immature sperm, abnormal levels seminal plasma antioxidants
- Apoptosis During sperm maturation (testis & epididymis)

What are the external factors leading to increased SDF?

- During spermiogenesis spermatids repackage spermatids repackage their DNA with protamines, a small residue of histone-bound DNA is retained (15%).
There is a clear association between high SDF and decreased pregnancy rates in natural conception and IUI.

Emerging evidence suggests a negative impact of high SDF on pregnancy outcomes in IVF and ICSI cycles.
Agricultural and industrial chemicals

- Agricultural chemicals that interfere in male reproductive toxicity include DDT, chlorohydrin, ethylene dibromide, and dioxin.
- Dibromochloropropane, a nematocide extensively used in agriculture, is a testicular toxicant that induces hypogonadism.
- DDT (a commonly used pesticide) and its metabolites (as p,p'-DDT, and p,p'-DDE) have known to have estrogenic impact in males by blocking the androgen receptors.
- Polycyclic aromatic hydrocarbons are omnipresent complex mixtures in the environment because of industrial combustion and excessive use of tobacco products, which maximally impact the spermatogenesis.
### Agricultural and industrial chemicals

- Methyl chloride, an industrial chemical used in the production of gasoline antiknock additives, has been extensively studied as a reproductive toxicant, and it is reported to induce changes in semen quality and affect testicular size.
- Organochlorine exposure has been associated with human perturbations of the sperm X:Y chromosome ratio.
- These endocrine disrupters disrupt the hypothalamic–pituitary–testicular axis affecting reproductive health.
- A mixture of various endocrine disrupters present in the environment synergise the effect of their combined toxicity.

### Heavy metals

- Heavy metals (e.g., arsenic, lead, boron, mercury, cadmium, antimony, aluminum, cobalt, chromium, lithium) adversely affect reproductive function.
- Lead exposure can disrupt the hormonal feedback mechanism at the hypothalamic pituitary level.
- Boron is extensively used in the manufacture of various utensils, glass, cements, soaps, and leather products, and its exposure is attributed in oligospermia and decreased libido.
- Cadmium, is considered to be a testicular toxicant and is used extensively in various industrial plants such as electroplating, galvanizing, plastics, alloys, and paint pigments.

### Drugs and phytoestrogens

- Various pharmacological agents, phytoestrogens, and anabolic steroids affect normal endocrine functions.
  - Abuse of such steroids mainly among athletes has grown to epidemic proportions.
  - Resulted in oligozoospermia as well as decreased libido.
  - Hypogonadotropic hypogonadism due to feedback inhibition of the hypothalamus–pituitary axis is the most common cause.
**Chemotherapeutic agents**

- Mechlorethamine, extensively used as nitrogen mustard during World War II, reported to cause spermatogenic arrest
- Common cytotoxic drugs cause a dose-dependent progressive decrease in sperm count, leading to azoosperma
- Cyclophosphamide in men may affect the decondensation potential of spermatozoa because of the alkylation of nuclear proteins or nucleic acids.
- Antimicrobials such as tetracycline derivatives, sulfa drugs, nitrofurantoin, and macrolide agents, such as erythromycin, have been reported to impair spermatogenesis and sperm function

**Effect of electromagnetic radiation**

- Cell phones have become indispensable devices in our daily life. These phones operate between 400 and 2000 MHz frequency bands and emit radiofrequency electromagnetic waves (EMW).
- Aitken et al. suggested that radiofrequency EMW might have a genotoxic effect on epididymal spermatozoa, which needs further investigation.

<table>
<thead>
<tr>
<th>Common environmental toxins</th>
<th>Common uses and routes of exposure</th>
<th>The effects on male reproductive system</th>
</tr>
</thead>
</table>
| Heavy Metals (Mainly cadmium, lead and arsenic) | Population exposed to cadmium and lead via contamination found in drinking water and food, while occupational exposure takes place during mining or manufacturing of batteries and pigments or industrial activities such as smelting and refining. | a. Testicular toxicity  
b. Low sperm count and motility and density  
c. Reduce male fertility  
d. Fetal toxicity and malformation of male and female fetus  
et. Impairment of semen and sperm function  
f. Increased risk of birth defects |
| Volatile organic compounds (Toluene, benzene and xylene) | Mostly occupational exposure in industrial workers | a. Testicular toxicity  
b. Low sperm count and motility and density  
c. Reduce male fertility  
da. Testicular toxicity  
b. Reduce azoospermic distance, hypospadias and... |
The examples of few chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are summarized below (Woodruff et al., 2008).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Description</th>
<th>Potential Effects</th>
</tr>
</thead>
</table>
| Parabens | A group of chemicals used as preservatives in cosmetics and body care products, including deodorants, creams, and lotions. They are able to penetrate the skin. | a. Hormone-mimicking activities  
b. Reduce synthesis of testosterone |
| Triclosan | Triclosan is an anti-bacterial and anti-fungal chemical widely used in personal care products such as soaps, toothpaste, etc. Triclosan has also been added to plastic products such as kitchen chopping boards. | a. Hormone-mimicking activities  
b. Reduce synthesis of testosterone |
| BPA (Bisphenol A) | BPA is in the building block of polycarbonate plastic used in baby bottles, CDs, water coolers, etc. It is also used for the production of epoxy resins used in the coating of the food packaging. | a. OSterogenic activities  
b. Altered male reproductive organs and delayed puberty  
c. Anti-androgenic activity |
| Perfluorinated compounds | These are frequently used in industrial products, which predominantly contain dca, hexa and perfluorooctane sulfonate. | a. Altered male reproductive organs  
c. Anti-androgenic properties |
| PCBs | PCBs are used in a variety of applications, including electrical applications, dielectric fluids for transformers and capacitors, hydraulic and heat transfer systems, lubricants, gasket makers, paints, fluorescent light fixtures, coatings, adhesives, and dyes. Exposure occurs through inhalation, ingestion, and dermal absorption. | a. Hormone-mimicking activities  
b. Anti-androgenic properties |
| Dichlorodiphenyltrichloroethane (DDT) | Dichlorodiphenyltrichloroethylene (DDT) is a compound of diphenylmethane, dichloroethylene, and trichloroethylene. | a. Sex hormone disruption  
b. Tissue damage  
c. Skin absorption |
| Dieldrin | Dieldrin is a chlorinated pesticide that is similar in structure to DDT. Exposure occurs through inhalation, ingestion, and dermal absorption. | a. Sex hormone disruption  
b. Tissue damage  
c. Skin absorption |
| Dermal | Dermal exposure refers to exposure to a chemical through the skin. This can occur through direct contact with the skin, inhalation of vapors, or ingestion of contaminated food. | a. Sex hormone disruption  
b. Tissue damage  
c. Skin absorption |
The examples of few chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are summarized below (Woodruff et al., 2008).

**Dieldrin, Hexachlorocyclohexane and endosulfan**: These are all organic pesticides used in agriculture to control insects and weeds. They are also used in the production of certain plastics and solvents.

**Brominated flame retardants**: These are used in the production of electronic equipment and furniture. They can cause endocrine disruption and cancer.

**PCB (polychlorinated biphenyl)**: These are used in electrical equipment and hydraulic fluids. They can cause endocrine disruption and carcinogenic effects.

**Organic solvents**: These are used in the production of many products, including cosmetics and pharmaceuticals. They can cause endocrine disruption and cancer.

**Pyrethroids**: These are used in the production of insecticides and pesticides. They can cause endocrine disruption and cancer.

**Permethrin**: This is a synthetic pyrethroid used in the production of insecticides and pesticides. It can cause endocrine disruption and cancer.

**Aldehydes**: These are used in the production of adhesives and solvents. They can cause endocrine disruption and cancer.
The examples of few chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are summarized below (Woodruff et al., 2008).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE</td>
<td>A few ultraviolet (UV) filters have been found in cosmetic products and car seats.</td>
</tr>
<tr>
<td>DDT</td>
<td>A number of male health problems, including reduced sperm counts, reduced testosterone levels, and increased risk of testicular cancer.</td>
</tr>
<tr>
<td>PAHs</td>
<td>Exposure to PAHs has been linked to a range of health problems, including cancer.</td>
</tr>
<tr>
<td>PCBs</td>
<td>Polychlorinated biphenyls have been associated with a range of health problems, including reproductive and developmental effects.</td>
</tr>
</tbody>
</table>

Possible Pathways of endocrine disruption by environmental chemicals. DDE = 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene; DDT = dichlorodiphenyltrichloroethane; PAHs = polycyclic aromatic hydrocarbons; PCBs = polychlorinated biphenyls. (Modified from Sharpe & Irvine, BMJ, 2004).

- Testicular dysgenesis syndrome. Both genetic and environmental factors affect testicular development and function. Damage of the testicular cells ( Leydig cells and Sertoli cells), decreased androgen production from Leydig cells and secretion of anti-mullerian factors from Sertoli cells, leading to both defects (hypogonadism, cryptorchidism) and impaired germ cell differentiation, apparent later in reduced semen quality in the west races in cattle and in EMS of the testis and consequent testicular cancer. (Modified from Stokloska et al., Human Reproduction, 2007).
Measurement of oxidative stress

<table>
<thead>
<tr>
<th>Direct Assay</th>
<th>Indirect Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemiluminescence assays</td>
<td>Myeloperoxidase test</td>
</tr>
<tr>
<td>Nitroblue tetrazolium test</td>
<td>Measurement of redox potential</td>
</tr>
<tr>
<td>Cytochrome c reduction</td>
<td>Lipid peroxidation levels</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Chemokines, Antioxidants</td>
</tr>
</tbody>
</table>

Lifestyle Factors Modifiable Without Risk

The links between environmental toxicants from various sources, oxidative stress, and reproductive health outcomes with modifiable measures. ROS, reactive oxygen species; VOC, volatile organic compound.
The evidence that links exposure to toxic environmental agents and adverse reproductive and developmental health outcomes is sufficiently robust.

Reproductive care providers can be effective in preventing prenatal exposure to environmental threats to health.

If there are sufficient data to suggest plausibility of harm, the precautionary principle is advocated, i.e., minimizing exposures within the capabilities of those exposed.

Lack of data about a chemical's health hazard does not imply it is safe, but merely indicates that no data are available to indicate harm or not.

Scientists and health care professionals are well positioned to collaborate with other stakeholders to promote protection and to advocate for improved chemical policies.

Ultimately, evidence-based recommendations for preventing harmful environmental exposure must involve policy change.

The incorporation of the authoritative voice of health care professionals in policy arenas is critical.

In 2009, the Endocrine Society called for improved public policy to identify and regulate endocrine-disrupting chemicals and recommended that “until such time as conclusive scientific evidence exists to either prove or disprove harmful effects of substances, a precautionary approach should be taken in the formulation of EDC [endocrine-disrupting chemical] policy.”
Summary

- There are a plethora of ways in which the environmental chemicals can potentially act on the endocrine as well as male reproductive systems.

- Spermatogenesis is vulnerable to environmental pollutants and several chemicals and thus, we need to develop stringent guidelines to minimize or prevent exposure to these reproductive toxicants.
2. Environmental Toxicants and Their Effects on Female Reproduction
Overview

- Potential role of environment in etiology of female reproduction
- Mechanism of action of environmental toxicants in affecting female fertility and fecundity
- Uterine, ovarian and pubertal disorders related to environmental toxicants
- Original research articles

Critical Window of Susceptibility

- Female reproductive disorders may develop during fetal, childhood, adolescence and adult life
- Multiple causes for adverse female reproductive health have been postulated
- Recent focus is on potential environment cause
- Period during which there are numerous changing capabilities in the developing fetus
- Exposure to environmental toxins may result in permanent damage as well as adverse reproductive potential of the fetus
- Critical windows are present during pregnancy, infancy, childhood, puberty
- Maternal environmental is imp factor in development of female reproductive organs
- Adverse effects may arise during, infancy, childhood, puberty and adult life
Parental Environment Health

- Developmental toxicants' effects:
  - Spontaneous abortion
  - Stillbirth
  - Low birth weight
  - Decreased head circumference
  - Preterm delivery
  - Birth defects
  - Visual and hearing deficits
  - Chromosomal abnormalities

Chemicals Potentially Associated with Reproductive Health Effects

<table>
<thead>
<tr>
<th>Type of Chemical</th>
<th>Specific example</th>
<th>Evidence of reproductive health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commonly used pesticides</td>
<td>DDT (dichlorodiphenyltrichloroethane)</td>
<td>Multiple case studies from wildlife exposure, some human evidence</td>
</tr>
<tr>
<td>Flame retardants</td>
<td>PBDEs (polybrominated diphenyl ethers)</td>
<td>Animal exposure models/data</td>
</tr>
<tr>
<td>Dioxin like substances</td>
<td>PCBs (polychlorinated biphenyls)</td>
<td>Animal exposure models/data, Wildlife exposure studies, Viral human exposure data</td>
</tr>
<tr>
<td>Phthalates</td>
<td>PVC (polyvinyl chloride), Di (ethylhexyl) phthalate</td>
<td>Animal exposure models/data, Emerging human studies (surveys, biomarker association)</td>
</tr>
<tr>
<td>Additives to consumer products (plastics)</td>
<td>BPA (bisphenol A)</td>
<td>Evidence from animal exposure models/data</td>
</tr>
</tbody>
</table>

Endocrine disruptors

- These are exogenous agents affecting synthesis, transport, metabolism and action of endocrine hormones
- Alters estrogen, androgens, thyroid and other steroid hormones and their actions
- Examples:
  - Pesticides: DDT (dichlorodiphenyltrichloroethane), DDE (dichlorodiphenyldichloroethylene)
  - Herbicides: atrazine
  - Persistent organic pollutants (POPs): eg-dioxins
  - Phthalates
Mechanism of action

- Genetic- DNA mutation
- Epigenetic- augmentation of gene expression, without direct effect on DNA,
- Endocrine mimicking- disrupt the physiological function of naturally occurring hormones
- Neuroendocrine route
- Systemic toxicity
- Xenohormones- compounds that mimic naturally acting steroid like androgen, estrogen

Female Reproductive Disorders

- UTERINE
- OVARIAN
- PUBERTAL

Disorders of Ovary

- Poly cystic ovarian syndrome
- Premature ovarian failure
- Altered menstrual cycle and Fecundability

Azziz et al. Journal of Clinical Endocrinology and Metabolism
2004:89:2745-9
## Disorders of Ovary

- Maintenance of proper estrogen balance is essential for healthy ovarian and follicular development
- Endocrine disruptors which interfere with estrogen function can impair ovarian development (Fertil Steril 2008, 90:911-40)
- Animal studies showed female alligators exposed to estrogenic compounds eg pesticide like difocol caused poor follicular development

## Poly Cystic Ovarian Syndrome

- MC endocrine abnormality affecting reproductive age women
- Etiology – Genetic + Environment
- Potential mechanism: excessive testosterone exposure in utero
  Genetic: may be because genetic hypersecretion of testosterone
  Environmental toxin exposure may lead to elevation of prenatal testosterone

### Azziz R et al Journal of clinical endocrine and metabolism 2004;89:2745-9

## Premature Ovarian Failure

- Affects 1% of female population
- Cause: Autoimmune - thyroid, adrenal
  Genetic
  Environment:
  1. pesticide – Menozeb
  2. water disinfectants - dibromoacetic acid
  3. Polycyclic aromatic hydrocarbons
  4. Cyclophosphamide

### Journal of clinical Endocrinol Metab 2007;92:4418-26
Altered Menstrual Cycle

- Case Study: Pesticide exposure and altered menstrual cycle
- Organochlorines - decreased menstrual cycle
- Non Organochlorines pesticide - increased menstrual cycles

*Chemosphere 2004;56:813-9
American Journal of Epidemiology 2004;160;1194-204*

Uterine Disorders

- ENDOMETRIOSIS
- UTERINE FIBROIDS
- Poor uterine development: In utero Diethylstilbestrol exposure
- Occupational exposure during reproductive years

Endometriosis

- Affects 15% of women
- Estrogenic dependent disease
- Potentially linked to environmental agents affecting estrogenic pathway
- CASE STUDY: DIOXIN AND ENDOMETRIOSIS
- Dioxin, an industrial byproduct produced during waste incineration may be associated with development of endometriosis due to its estrogenic effect

*Fertil Steril 2004;82:1501-8*
High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study

The objective of this study was to detect the probable association between polychlorinated biphenyls (PCBs) and phthalate esters (PEs), and the occurrence of endometriosis in a prospective case-control study. We found that PCBs and PEs may be instrumental in the etiology of endometriosis. (Fertil Steril 2006;85:773-8. ©2006 by American Society for Reproductive Medicine)

Result

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Parameters, concentration in control and different stages of endometriosis group and one-way analysis of variance between stages (ANOVA).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (μg/mL)</td>
</tr>
<tr>
<td></td>
<td>Stage I</td>
</tr>
<tr>
<td>Mono-ortho-substituted</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>PCB-2 (mono-ortho)</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>PCB-3 (mono-ortho)</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>PCB-5 (mono-ortho)</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>PCB-4 (mono-ortho)</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Dioxin-like polyhalogenated</td>
<td>0.11 ± 0.21</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>DDE</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.06 ± 0.03</td>
</tr>
</tbody>
</table>

Note: Control = Fertile women without endometriosis. Data are presented as mean ± SD.
**P < 0.01 was considered statistically significant.

Conclusion

One higher concentration of these chemicals in the plasma of patients with endometriosis compared to fertile control patients suggests an association of PCBs and PEs with the occurrence of endometriosis. Because endometriosis is a very poorly understood disease, further studies are necessary to determine the genes and factors that play a role in its etiology.

Acknowledgments: We thank Dr. V. S. R. Ram and H. K. Ram Reddy for assistance in statistical analysis. Professor D. L. Darapu for preparing the manuscript, and Dr. Ram Reddy for statistical analysis. We thank the Centre for Reproductive Health and Women's Hospital and Research Centre for providing the samples and for their support throughout this study.

R. Babu Ram Reddy, M.B.B.S.*
Raja Ram Reddy, M.D.**
S. Basavaraj, M.B.B.S.*
Shankarappa Reddy, M.B.B.S.*
Padmashree Reddy, M.D.*
Ramesha Reddy, M.D.*
*Department of Reproductive Medicine, Nageshwar Bhagwan Medical Research Center, A.C. Ganga, Hyderabad.
†Maternal Health and Research Trust Center for Infertility Management, Rajgira Hills, Hyderabad.
‡Obstetric Hospital and Research Centre, Indian College of Medical Sciences, Karimnagar, Hyderabad, and Department of Pharmacology, Rajeev Research Foundation, J.P.T.E., Hyderabad, India.
• Prospective CASE CONTROL STUDY at Mahavir Hospital and Research Centre (1999-2005) by Dr Roya et al
• Fertility and Sterility, March 2006, vol 85, No 3
• 645 infertile women were screened, out of which 85 women were diagnosed to have endometriosis grade 1-IV (Revised ASRM criteria)
• 135 control women undergoing lap sterilization with no evidence of endometriosis were selected
• This study showed significant higher levels of PCBs and PEs in women with endometriosis than with fertile women without endometriosis suggesting an association of PCBs and PEs with occurrence of endometriosis.

Association of phthalate esters with endometriosis in Indian women

BS Reddy, R Puri, BVR Reddy, P V V S Raman

1. Department of Reproductive Medicine, Diagnostic Mahavir Research Centre, A.C. Road, Hyderabad-500 004, Andhra Pradesh, India
2. National Health and Research Trust MIBT, Centre for Infertility Management, Banjara Hills, Hyderabad-500 034, Andhra Pradesh, India
3. Department of Analytical & IT, Patna Research Foundation, A.P. lane, Hyderabad-500 004, Andhra Pradesh, India
4. Correspondence: Dr Roya Rani MD, ERCOG, National Health and Research Trust MIBT, Centre for Infertility Management, Banjara Hills, Hyderabad-500 034, Andhra Pradesh, India. Email: drroya@vsnil.com

Accepted 15 February 2006.

• CASE CONTROL STUDY at Mahavir research Centre, HYDERABAD
• Blood samples were collected from 49 infertile women with endometriosis (study group) and 38 age matched women without endometriosis (control group)
• Outcome: Evaluation of phthalate esters concentration in women with endometriosis compared with women free of disease
• Results: Correlation between the concentration of PE s and different severity of endometriosis was strong and statistically significant (P value < 0.05)
• Conclusion: PEs have an aetiological association with endometriosis
Uterine Fibroids

- High prevalence: 50% of women
- In utero exposure of estrogenic agents as well as during childhood and adulthood may be linked to its etiology
- Prenatal DES exposure is linked to uterine Leiomyoma development.
Pubertal Development

Premature Thelarche

- Environmental exposures - pesticides, flame retardants
- Case Study: In Island of Puerto Rico Linked to consumption of Soy Based product and meat product
  Environmental Health perspective 2000
- High levels of Phthalates found in 68% of women with early thelarche
- Phthalates are plasticizers with high estrogenic and antiandrogenic activities

CONCLUSION

- Strong and consistent indication that reproductive health is vulnerable to insult from the widespread environmental and occupational toxicants
- Considering the possible health effects further research and more epidemiological data is required
Thank You
3. Interesting cases (Testicular dysgenesis Syndrome / Miscariage / Malformation etc)
Testicular Dysgenesis Syndrome

Recently adverse trends in male reproductive health have been observed in many countries (Toppari et al 1996)

- Increasing incidence of cryptorchidism and hypospadias (Campbell et al, 1987; Paulozzi et al 1997)
Poor semen quality, testes cancer, undescended testes and hypospadias are symptoms of one underlying entity, the testicular dysgenesis syndrome (TDS).

Rapid rate of increase of reproductive disorders suggests that environmental or life style factors, rather than an accumulation of genomic structural defects, are the most likely causes.

However, some genetic aberrations or polymorphisms may predispose to augmented effects by environmental factors.

Large fraction of male reproductive disorders including congenital disorders like hypospadias, undescended testes as well as testicular cancer is of antenatal origin (Dieckmann and Skakkebaek, 1999; Ottesen et al 1999).

Evidence also suggested that the underlying cause of male infertility is of fetal origin.

Symptoms may vary with the severity of syndrome:
- Most severe form of TDS eg in individuals with 45 X/46 XY karyotype, often include impaired spermatogenesis, undescended testes, hypospadias and or testicular neoplasia. These symptoms will develop successively. Less severe forms may have one or two symptoms.
- Mild forms may present only with slight impairment of spermatogenesis.
Causes of TDS

- GENETIC
- ENVIRONMENTAL FACTORS:
  - Epidemiological findings of geographic and temporal synchrony in the symptoms of TDS: e.g., in Finland, rates of testicular cancer, undescended testes and hypospadias are much lower than Danish men, who in return also have poor quality semen.

Endocrine disrupters in aetiology of TDS

- ENVIRONMENTAL ANTIANDROGENS AS ENDOCRINE DISRUPTERS has adverse effect on male reproductive health (TOPPARI et al. 1997)
- Epidemiological studies reported an increased risk of genital malformations in children of workers exposed occupationally to pesticides (Weinder et al. 1998)
- Clustering of cryptorchidism in areas of intensive agriculture
- Further research is needed to delineate the role of endocrine disrupters in humans and to indicate the possible actions for future protection of future generations
- Study from Denmark reported 5-6% of school boys have undescended testes, 1% have penile abnormalities, at birth and >40% of young adult men have subnormal sperm count (Andersen et al. 2000)

Cryptorchidism

- MC birth defect affecting 2-9% of boys born full term.
- Testes normally descend to bottom of scrotum before birth and if one or both testes fail to descend—congenital cryptorchidism
- Risk of cryptorchidism include infertility, testicular cancer, hypospadias suggesting that these conditions share similar causes affecting fetal testicular development.
### Causes of Cryptorchidism

Genetic:
Defect in hormone synthesis and receptors eg- mutations in AMH gene or its receptor AMHR2 Androgen and INSL3 act on gubernaculum which guides descent of testes through inguinal canal to scrotumGene defect affecting androgen production are mostly associated with cryptorchidism

### Crytorchidism

- Clustered in family- genetic, intrafamilial environmental cause
- Maternal half brother have higher risk than paternal half brother implicating maternal environment during pregnancy
- Hormonal exposure: critical male programming occurs at 7-15 weeks
  1. antiandrogen:- widely spread pesticides such as DDE(dichlorodiphenyldichloroethylene) and fungicides such as vinclozolin and procyomidone
  2. Phthalates- affect androgen synthesis
  3. Estrogenic chemicals like dioxin – inhibits production of INSL3
  4. exposure to synthetic estrogen (DES)
  - These chemicals act in a simple additive manner rendering even low dose harmful.
  - Exposures have been measured in blood, urine, placenta and breast milk that serve as a proxy to mother's loads of chemical during pregnancy

### Breast milk levels of polybrominated flame retardants

- Breast milk levels of polybrominated flame retardants was associated with increased risk of cryptorchidism where as placenta levels were not *(Environ Health Perspec 115:1519-26,2007)*
- Dioxin levels in breast milk in Danish women were associated with increased risk of cryptorchidism, where as placenta levels did not show an association *(Int J Androl 35:283-293,2012)*
- American studies of dioxin and DDT, no association was found b/w maternal serum values and cryptorchidism *(Am J Epidemiol 155:313-322,2002)*
- Greenhouse workers exposed to pesticides during pregnancy were also shown to have an increased risk *(Environ Health Perspect 116;566-572,2006)*
Hypospadias

- Penile congenital malformation, in which urethra opens somewhere on the ventral side of penis instead of tip.
- Penile development regulated by dihydrotestosterone that is typically produced from testosterone by 5 alpha reductase.
- Several genetic mutations leading to hypospadias are known, they are typically linked to disorders of testicular differentiation, testicular synthesis, conversion of testosterone to dihydrotestosterone or androgen receptor (J Clin Endocrinol Metab 83:675-681,1998).
## Role of Prenatal Exposure

- Anti androgens, Estrogen (DES) during pregnancy can cause both hypospadias and cryptorchidism. *(Environ Health Perspect 104:741-803, 1996)*
- DES causes increased risk of hypospadias even in 2nd generation reflecting epigenetic effects by DES.
- Metaanalysis reported a small increased risk of hypospadias in sons of parents were exposed to pesticides. However the studies could not assess which chemicals were behind the association as pesticides included a number of chemicals. *(J Pediatr Urol:17-24, 2009)*
- Meta analysis of 14 studies – No association between exposure to sex steroids (except DES) during first trimester and external genitalia malformation could be found. *(Obstet Gynecol 85:141-149, 1995)*

## Sperm quality

- There was a controversial study published by Carlsen etal in 1992 which showed that sperm concentration had declined 50% over previous 50 years. Limitations: poor or highly variable data, faulty statistical method.
- Detailed reanalysis in 1997 from 61 countries showed significant decline in sperm concentration in Europe and US. *(Environ Health Perspect 108:961-966, 2000)*
- Reduced spermatogenesis in adulthood can be a consequence of exposure in fetal life to environmental chemicals- endocrine disrupting chemicals such as dioxin, perfluorinated compounds (PFC), combustion products *(Environ Health Perspec, 1997)*
- Western life style factors (sedentary work/obesity, stress, sleep, maternal smoking)
by the decline in sperm density. CONCLUSIONS—There has been a genuine decline in semen quality over the past 50 years. As male fertility is to some extent correlated with sperm count the results may reflect an overall reduction in male fertility. The biological significance of these changes is emphasized by a concomitant increase in the incidence of perinatal abnormalities such as testicular cancer and possibly also cryptorchidism and hypospadias, suggesting a growing impact of factors with serious effects on male gonadal function.


Role of environmental estrogens in the deterioration of male factor fertility

Role of environmental estrogens in the deterioration of male factor fertility

Role of environmental estrogens in the deterioration of male factor fertility
Miscarriage

- Sporadic miscarriage affects 15% of all clinically recognized pregnancy
- MC cause-genetic abnormality, however, sporadic losses do occur
- Maternal age, hormonal imbalance, immunological interaction and uterine anatomic abnormalities
- CHEMICALS: endocrine disruptors, Heavy metals
- Embryonic or fetal tissues more sensitive to chemicals because of totipotent nature of embryonic cell
- Single insult at this stage can have deleterious effects on development
- Environmental toxins also affect endometrium/decidua and complex biochemical dialogue between blastocyst and decidua
Multiple industrial contaminants have the potential for endocrine disruption: Radiation exposure, heavy metals, agricultural chemicals, industrial solvent, endocrine disrupting chemicals

- DDT (1,1,1-trichloro2,2 bis(p-chlorophenyl)ethane)
  - was used first in eradicating malaria
  - persists in environment and even bioconcentrated within food chain
  - DDE, a metabolite of DDT has androgen receptor antagonist
  - causes decline in sperm count, increased time to conception, IUGR (BMJ 1992;305:609-13, Lancet 1993;341:1392-5)
  - Increased spontaneous miscarriage at higher concentration of DDE (>15 mcg/l)

### Bisphenol - A

- Affects implantation and oocyte meiosis leading to aneuploidy (Hum Reprod 2011)
- Laiithi and coworkers- significant increase in euploid and aneuploid loss (Fertil Steril 2014;102:123-8)
- Stein and coworker found increased urinal BPA leading to recurrent miscarriage
- PCBs (Polychlorinated biphenyls)
  - industrial combustion products
  - it inhibits meiotic spindle and hampers maturation of oocytes and also affects endometrium
  - increased risk of miscarriage reported by Tsukimosis et al OR 1.6

### Phthalates and phthalates metabolites

- used for plastic manufacture eg medical supplies (IV tubings and bags)
- causes developmental abnormalities of male reproductive system, miscarriage, endometriosis and low sperm counts
- it acts by inhibiting P like effect and also inhibits aromatase activity. (Environ Health Perspec 2012)
CONCLUSION

Women of reproductive age should exercise a caution in exposure to these endocrine disruptors.

Unfortunately, these compounds are ubiquitous in environment and are often difficult to avoid.

These studies could be confounded by presence of multiple chemicals.

Many of these EDC act in synergistic manner.

More prospective studies of adequate sample size and design are required to understand the full impact of these hormone like compounds on male and female reproductive potential.

Thank You
4. Options and advances in air purification technologies
Our General approach towards Air

- No acknowledgement of air-borne problems
- Reluctance in acquiring knowledge and education (lack of it) about air purification process
- Some impurities can be seen, smelt, but most you can not see or smell (If it is not visible – must not be a problem)
- Resistance to change or introduce new things (happy with status quo: normal human tendency)

IVF lab Air: Time to take it seriously

Up to 70% of the success of an IVF program is dependent upon the IVF laboratory.

The success of the laboratory is dependent upon the embryologists’ skill set, the media and ambient air

(William Schoolcraft, ESHRE 2010)
Where do the particles come From in the lab?

- From outside: the air leakages, openings and through inefficient filters
- Particulates and microorganisms come from People and Processes in the lab
- We (human) shed about 40,000 skin cells (35 Micron each) every minute
- Outside air contains 10-100 CFUs, while inside air has 100-1,000 CFUs per cubic meter

Contributing Factors to Outside Air

Traffic – all mechanisms of transport
Industrial emissions – fossil fuel combustion
Area construction
Local road repairs, resurfacing
Seasonal pollutants
Accidents, spills, weather

VOCs.... The curse of the Human kind!

VOCs: Volatile Organic Compounds, which release gaseous molecules from their liquid or solid form at room temperature.

- Ketones, aldehydes, nitriles from soils at construction sites
- Formaldehyde, acetone, methane, methylene chloride from pressed boards and wood in building material and also from your own diesel generator exhaust
- Toluene and Styrene from cement, asphalt used for road resurfacing
- Sulphur dioxide, formaldehyde, nitrogen, ethylene from exhaust of cars, ambulances and petrol pumps etc
- Polyaromatic hydrocarbons from the fast food restaurants
- Carbon tetrachloride, chloroform etc from pesticides used for pest control next door
- Acetone, diethyl ketone, ethanol and several biologicals and microbials shedding from the lab staff

VOCs above certain level cause respiratory problem, irritation of eyes, nose, and skin. Some VOCs are proven to be carcinogenic.
Generation of VOCs in an IVF lab

- Tissue culture Plasticware (Tubes, Dishes, Flasks)
- Isopropanol and other disinfectants
- Off-gassing of equipment, monitors etc.
- Refrigerants from Air conditioners /HVAC
- Compressed gasses
- CO2 / N2 Cylinders
- Personnel bioburden
- VOCs can enter the media (even under oil)

IVF Lab Air purification: current practices

- [Image of different practices]

THE goals of Air-purification

- Filtration of particulates and pollutants entering in to the lab and incubators
- Decomposition of VOCs in to inert air molecules (e.g. CO2 and H2O)
- Deactivation of DNA/RNA of the opportunistic microorganisms
- Create adequate Clean Air Delivery Rate (CADR) and Air Changes (ACH)
- Air purification process shall not have adverse effects on the health of embryos and the staff
- Periodic monitoring and validation of air-quality and corrective actions
Overview of air-cleaning technologies

TRAP

- HEPA/ULPA/CARBON Filtration
- Electrostatic Precipitation (ESP)
- Ionizers (sometimes wrongly labeled as plasma)
- Hybrid of all above

DEACTIVATE

- UV (UV+PCO)
- Pulsed Xenon
- Chemicals (Hydrogen Peroxide, Ozone etc)
- Cold PLASMA Air Sterilization

Filtration (Air Handling Units)

- HEPA filter
  - filters particles > 0.3 microns
- ULPA filters
  - filters > 0.1 microns
- "Viruses (too small can’t be filtered)
- Biofilm accumulates in the filter - they are not killed
- Orientation of filters
- Filter performance is affected by humidity
- VOCs can’t be filtered (VOCs are 100-2000 times smaller than the pore size of HEPA)
- High cost of maintenance and electricity (60-80% of lifecycle cost of filter is energy consumption)

Air Handling Unit (HVAC) Can Breed

- IVF laboratory room temperature, humidity and HEPA filter substrate provides an ideal environment for growth of bacterial and viral spores, mold and biologicals.
- Pathogens – viruses, bacteria, fungi
- Allergens – bacteria, mold
- Toxins – endotoxins, mycotoxins
Electrostatic Precipitation (ESP)

- Series of parallel alternating charged and grounded plates, which collect particles
- ESP usually preceded by ionisers
- The electric charge can be neutralised by high humidity, heat, ionising radiation and solvents like paints making it less effective

Activated Carbon Filters

- Many gaseous contaminants (e.g. VOCs) will adsorb (adhere) to tiny internal pores of activated charcoal and be removed from the air
- Performance depends on the surface area and the air pressure being applied to these filters
- When the surface is covered, the adsorption stops, usually without warning
- Regular replacement of Charcoal filters is necessary, else they start releasing the particles

- High voltage is applied to a needle to ionize the air which interacts with microbes & particles in the room
- Ions bind to particulates in air and drops to the floor/surfaces (Filters)
- Does not destroy all the microbes
- Generates OZONE
- Slows wound healing
- Causes significant respiratory issues
- Not advised in patient-occupied areas
- Most of the commercially available domestic purifiers use ionizing technology and are wrongly labelled as ‘Plasma,’ ‘Plasma Cluster’ etc. They may not be suitable for healthcare applications
UV Irradiation (G-UV)

- UV-C (254 nm) effective and cheaper way of killing Microbes
- UV acts on microbes which are in 'line of sight'
- Limited action on clusters
- Gradual reduction in intensity of UV lamps
- Need to be calibrated and replaced regularly
- Documented side effects of UV on Skin and Eyes
- Reflective surfaces not advisable
- Not suitable for patient-occupied rooms of less than 10 ft height.

Photocatalytic Oxidation (PCO)

- Uses UV irradiation and Titanium Dioxide surface as catalyst
- Produces hydroxyl radicals (OH+) which are extremely reactive (kills pathogens)
- May produce formaldehyde and ozone
- Delays wound healing
- Causes respiratory problems
- Lamps and cartridges need periodic replacement

Is there any sustainable and composite solution for IVF lab Air problems? YES

- Combination of Plasma air Sterilization technology with Pre-filter, HEPA and Activated Carbon is probably the most effective air-purification technology launched in recent times
- It promises to quickly eliminate VOCs and other microbial contamination in an IVF lab in a user-friendly manner.
- It would bring down the maintenance/replacement cost
- Portable green technology
- Reported improvement of embryo quality and success rates
The Long-lasting effective solution is here! defend 1050 : PLASMA BASED complete air sterilizer

How does defend 1050 work?

- Lab air is drawn inside the machine with the help of a fan with five speed control
- The air passes through a high grade Camfil pre-filter before entering a plasma zone
- Microorganisms that are destroyed by the Plasma field are broken down and returned to their original constituent components, rendering them inert
- In a plasma field, VOCs will be broken down into their constituent components
  - e.g (Formaldehyde): CH2O -> CO2 + H2O
- The clean air would then pass through a special activated carbon filter to remove any residual VOCs or ozone, if any
- The air thus filtered passes through a high grade H13 Camfil HEPA filter, before releasing back in to the lab
- This process happens continuously rendering the lab air sterile
- Novaerus Plasma is contained within the device at all times. There is no emission into the room. Safe for embryos and the staff (24X7 operation)
  "This device brings the room to the plasma sterilizer"

What is Plasma Technology?

- Electrons split from the atoms.
- Ions.
- Free Radicals
**Plasma Air Sterilization**

- Electrons bombard the cells
- Free radicals & highly reactive oxygen & nitrogen species damage lipids, proteins, DNA into inert air molecules
- Destroys cell membranes, enzymes & organelles
- Kills the microbes
- Deactivates VOCs into CO2 and H2O

**Plasma physics to Plasma Biology**

- Sterilization of contaminated matter with an atmospheric pressure plasma

**Novaerus DBD Plasma**
What happens to The Microorganism when subjected to different sterilization techniques?

- NASA AMES Research Lab, NASA, CA – studies the effect of plasma on bacteria microorganism
- Karolinska Institute, Sweden (SSI)
- Avomeen Labs USA – Formaldehyde (VOC)
- Camfil Laboratory – Toluene, NO2, Formaldehyde, PM 10, Pm2.5 and PM1 (Particulates and VOCs)
- RPS Mountain heath – mixed VOC testing
- University of Huddersfield (Smart Infection Control Solution contest)
- Microsearch - multiple organisms including bacteria, mold, spores and viruses
- ARE Labs – MS2 Influenza virus
- Indoor Biotechnologies, UK – Allergen Testing
- Qualilife Diagnostics Lab, India TB and Acinetobacter pilot study
- About 40 installations in IVF labs across India

Independent Validation of Defend 1050 for VOC Formaldehyde

Independent Validation of Defend 1050 for VOC : NO2
Independent Validation of Defend 1050 for VOC: Toluene

Figure 25: Toluene reduction when air cleaner is activated.

Clean Air Delivery Rate

The United States Environmental Protection Agency explains the number in the following way:

"The CADR is a measure of a portable air cleaner’s delivery of contaminant-free air, expressed in cubic feet per minute. For example, if an air cleaner has a CADR of 250 for dust particles, it may reduce dust particle levels to the same concentrations as would be achieved by adding 250 cubic feet of clean air each minute. - Environmental Protection Agency"

Methodology for measuring CADR for any contaminant

- With air cleaner off and testing fan on, generate the contaminant of interest and then stop the generation.
- Measure the contaminant concentration over time.
- Plot the natural logarithm of the concentration versus time.
- Repeat the procedure with the air cleaner operating.
- Equivalent air exchange rate = difference in slopes
- CADR = (difference in slopes) x (room volume)

- Methodology by Stephen N. Rudnick, MS, ScD, CIN Lecturer on Industrial Hygiene, Engineering Department of Environmental Health, Harvard School of Public Health - Airborne Infection Control Conference 2017
Calculation of Clean Air Delivery Rate (CADR) and Air Change Per Hour (ACH) with DEFEND 1050

Plasma Air Purification: options

- **NV 200**
  - Airflow: 60 m³/hour
  - Covers up to 20 m²
  - Portable: 3.4 Kg
  - 11 watts
  - Virtually no maintenance
  - One Plasma Coil
  - Simple 2-speed fan
  - Not for IVF lab use (personal use)

- **NV 800**
  - 260 m³/hour
  - Covers up to 80 m²
  - Wall or stand mounted: 4.5 Kg
  - 25 Watts
  - Two Plasma Coils
  - Two-speed Fan
  - Cleaning of pre-filter

- **Defend 1050**
  - 1000 m³/hour
  - 3 Filters: Carbon, HEPA, Pre-Filter
  - Portable on castor wheels: 105.5Kg
  - Six Plasma Coils
  - 5-speed adjustable fan

AIR quality: Supplementary information
Taking Care of impurities in Gas Cylinders

VOC free Sterilization of the Lab surfaces, Incubators and work benches

Outdoor air pollution & human infertility

- There is a significant association between air pollution and fertility rates in general population
- Subfertile population especially the one going through Infertility treatment is extremely vulnerable to air pollution which leads to increased negative outcome

(A systematic review by Miguel A. Checa Vizcaíno, Mireia Gonzalez-Comadran, M.D. and Benedicte Jacquemin, Published in Fertility and Sterility, Vol.106, No. 4, September 2016)

Air quality control in the ART laboratory is the major determinant of IVF success

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Place</th>
<th>Design</th>
<th>Air filters</th>
<th>Increase of TP success</th>
<th>Clinical practice rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greene et al.</td>
<td>1999</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>HAP/ VOC active carbon + HEPA</td>
<td>Yes</td>
<td>Up to 15% to 20%</td>
</tr>
<tr>
<td>Gomes et al.</td>
<td>2003</td>
<td>Brazil</td>
<td>Prospective qualitative</td>
<td>HAP/ VOC active carbon</td>
<td>Yes</td>
<td>Class 1000/ 500/ 75</td>
</tr>
<tr>
<td>Jongst et al.</td>
<td>2008</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>HAP/ VOC + HPA + HPA</td>
<td>Yes</td>
<td>Class 1000/ 500/ 75</td>
</tr>
<tr>
<td>Cottey et al.</td>
<td>2013</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>HAP/ VOC/ active carbon</td>
<td>Yes</td>
<td>Class 1000/ 500/ 75</td>
</tr>
<tr>
<td>Koo et al.</td>
<td>2013</td>
<td>China</td>
<td>Prospective cohort</td>
<td>HAP + VOC + Active carbon</td>
<td>Yes</td>
<td>Class 1000/ 500/ 75</td>
</tr>
<tr>
<td>Rawan et al.</td>
<td>2014</td>
<td>USA</td>
<td>Prospective cohort</td>
<td>HAP/ VOC urban carbon + HPA</td>
<td>Yes</td>
<td>Increase</td>
</tr>
<tr>
<td>Martin et al.</td>
<td>2015</td>
<td>USA</td>
<td>Prospective cohort</td>
<td>HEPA/ VOC urban carbon</td>
<td>No</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

D.N. Chennavathy et al. in Journal of Assisted Reproduction and Genetics. May 2017 “volatile organic compounds and good laboratory practices in the in vitro fertilization laboratory: the important parameters for successful outcomes”

Air Quality / Cleanroom Standards

ISO 14644-1 (Standards ISO 1 4644-1: 1999 for clean rooms)

GMP EU (Classification: Class A, B, C, D)

British Standard 6553-2: Class A, B, C, D, E, F

Air quality / Cleanroom Standards at a glance

(Maximum particles per cubic meter – At rest)

<table>
<thead>
<tr>
<th>ISO 3581</th>
<th>BS 6553</th>
<th>BS 6553</th>
<th>ISO 14644-1</th>
<th>ISO 14644-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>Class 1</td>
<td>Class 1</td>
<td>Class 1</td>
<td>Class 1</td>
</tr>
<tr>
<td>Class 2</td>
<td>Class 2</td>
<td>Class 2</td>
<td>Class 2</td>
<td>Class 2</td>
</tr>
<tr>
<td>Class 3</td>
<td>Class 3</td>
<td>Class 3</td>
<td>Class 3</td>
<td>Class 3</td>
</tr>
<tr>
<td>Class 4</td>
<td>Class 4</td>
<td>Class 4</td>
<td>Class 4</td>
<td>Class 4</td>
</tr>
<tr>
<td>Class 5</td>
<td>Class 5</td>
<td>Class 5</td>
<td>Class 5</td>
<td>Class 5</td>
</tr>
<tr>
<td>Class 6</td>
<td>Class 6</td>
<td>Class 6</td>
<td>Class 6</td>
<td>Class 6</td>
</tr>
<tr>
<td>Class 7</td>
<td>Class 7</td>
<td>Class 7</td>
<td>Class 7</td>
<td>Class 7</td>
</tr>
<tr>
<td>Class 8</td>
<td>Class 8</td>
<td>Class 8</td>
<td>Class 8</td>
<td>Class 8</td>
</tr>
</tbody>
</table>

(Values may vary based on specific laboratory requirements)
Cairo Consensus on IVF Laboratory environment and air quality (2018) : Consensus points

- Fair evidence derived from both animal and human studies indicates that controlling laboratory contamination positively impacts in vitro fertilization outcomes. Great effort should be taken to ensure that IVF lab has clean air.
- For IVF lab, air quality of ISO Class 7 (GMP Grade B) air ‘in operation’ and Grade C ‘at rest’ is recommended i.e. Less than 352,000 particles larger than 0.5 um to 10 um per cubic metre (equivalent to <10,000 such particles per cubic foot).
- Micro-organisms: Less than 10 cfu/m^3 and less than two spores/ m^3 ‘at rest’
- VOCs. Total VOCs less than 500 ug/m^3 (~400–800 ppb total VOC, depending on molecular species); less than 5 ug/m^3 aldehydes (1ug/m^3 = 1ppb)
- HVAC HEPA Filters (if used) shall achieve 10—15 air changes per hour (ACH) – 20% intake of outside air.
- For VOC+HEPA filtration, manufacturers calculations of equivalent ACH should be considered.
- Positive pressure differential between 30-50 pascals in the IVF lab is recommended.
- IVF lab Temperature range shall be between 20-24 deg C with relative humidity between 40–45%
Table 2 - Odour thresholds of organic contaminants typically found in assisted reproduction technology (ART) laboratories

<table>
<thead>
<tr>
<th>Organic compound</th>
<th>Geometric mean AHA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (isopropyl alcohol)</td>
<td>18–100 ppm</td>
<td>Most common VOC in ART laboratories.</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>19–42 ppm</td>
<td>Second most commonly found VOC.</td>
</tr>
<tr>
<td>Acetone (2-propanone)</td>
<td>42–130 ppm</td>
<td>Plastic.</td>
</tr>
<tr>
<td>Propene (propylene)</td>
<td>23–88 ppm</td>
<td>Silicone from gaskets.</td>
</tr>
<tr>
<td>Hexa-methyl-3,4-dicyclosiloxane</td>
<td>Ne data</td>
<td>Plastics.</td>
</tr>
<tr>
<td>Acetone (trifluoromethyl cyanide)</td>
<td>1140 ppm</td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>0.03–9970 ppm</td>
<td>Scent of lemon.</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>6.867 ppm</td>
<td>Scent of pine.</td>
</tr>
<tr>
<td>Di-limonene</td>
<td>6.5 ppm</td>
<td></td>
</tr>
<tr>
<td>p-Xylene</td>
<td>6.905 ppm</td>
<td></td>
</tr>
</tbody>
</table>


Air Quality / Clean room Standards

- Laboratory standards are measured in the ART laboratory to ensure significant decreases in odours. Standards are set by the American Industrial Hygiene Association (AIHA).

- Standards are set for specific contaminants, such as formaldehyde and acetone, to ensure a clean and safe environment for patients and staff.

- The table below provides a summary of the standards and the contaminants they are measured against.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Contaminant</th>
<th>Concentration (ppm)</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab 1</td>
<td>Formaldehyde</td>
<td>0.03</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Lab 2</td>
<td>Acetone</td>
<td>0.04</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Lab 3</td>
<td>Acetaldehyde</td>
<td>0.05</td>
<td>≤0.05</td>
</tr>
</tbody>
</table>

- Laboratories must maintain concentrations below the standard values to ensure a clean and safe environment for patients and staff.

- Laboratories are monitored regularly to ensure compliance with the standards set by the AIHA.

- Laboratories that exceed the standards may be penalized or subject to fines.

- Laboratories that consistently exceed the standards may be required to take additional measures to improve air quality.

- Laboratories that consistently meet the standards are more likely to receive positive feedback from patients and staff.
THANK YOU
5. Optimizing the culture environment in the IVF Lab
• Embryo culture is a component of in vitro fertilisation where resultant embryos are allowed to grow for some time in an artificial medium.

• Optimizing procedures within the IVF laboratory to minimize the stress imposed on the embryo is an ongoing endeavour.
• The IVF laboratory must not only grow competent embryos but must ensure this competency is maintained after various manipulations.
Introduction

- Media
- Air
- pH
- Light
- Temperature
- Equipments

Media

- Osmolality: 275 - 305 mosmoles/kg
- pH: 7.2 - 7.5
- Bicarbonate
- EDTA
- Antioxidant
- Chelator
- Antibiotic
- Vitamins

Composition

<table>
<thead>
<tr>
<th>Variable</th>
<th>1BF/1 2BF</th>
<th>1GC/2GC</th>
<th>1G/2G</th>
<th>1A/2A</th>
<th>1H/2H</th>
<th>1Y/2Y</th>
<th>1E/2E</th>
<th>1S/2S</th>
<th>1O/2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol)</td>
<td>3.2</td>
<td>3.5</td>
<td>3.4</td>
<td>3.1</td>
<td>3.3</td>
<td>3.4</td>
<td>3.5</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>180</td>
<td>190</td>
<td>180</td>
<td>170</td>
<td>180</td>
<td>190</td>
<td>180</td>
<td>170</td>
<td>180</td>
</tr>
<tr>
<td>Sucrose (mmol)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Sucrose (mg/dl)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Glutamine (mmol)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Glutamine (mg/dl)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Glutathione</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cystine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Histidine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asparticacid</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cystine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Histidine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Methionine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
It is certainly the case that the human embryo can grow in the absence of amino acids.

Oviduct and uterine fluids contain significant levels of free amino acids.

While both oocytes and embryos possess specific transport systems for amino acids to maintain an endogenous pool. Amino acids are readily taken up and metabolized by the embryo.

Studies on the embryos of several mammalian species, such as mouse, hamster, sheep, cow, and human, have all demonstrated that the inclusion of amino acids in the culture medium enhances embryo development to the blastocyst stage.
**Amino Acid**

- Transient exposure (about 5 minutes) of mouse zygotes to medium lacking amino acids impairs subsequent developmental potential.
- During this 5-minute period in a simple medium the zygote loses its entire endogenous pool of amino acids, which takes several hours of transport to replenish after returning the embryo to medium with amino acids.
- This, therefore, has implications for the collection of oocytes, and more importantly the manipulation of denuded oocytes during intracytoplasmic sperm injection (ICSI), where plausibly the inclusion of amino acids in the holding medium will decrease or prevent intracellular stress.

- It has been demonstrated that the preimplantation embryo exhibits a switch in amino acid requirements as development proceeds.
- Up to the 8-cell stage: Nonessential amino acids and glutamine increase cleavage rates
- After compaction, nonessential amino acids and glutamine increase blastocoel formation and hatching, while the essential amino acids stimulate cleavage rates and increase development of the inner cell mass (ICM) in the blastocyst.
Amino Acid

• Ammonium by both embryo metabolism of amino acids and by the spontaneous breakdown of amino acids in the culture medium once incubated at 37°C.
• Ammonium build-up in culture medium can not only have negative effects on embryo development and differentiation in culture,39,45,57 but can affect subsequent fetal growth rates and normality at a concentration of around 300 μmol/l.19,58 Furthermore, it has been shown that ammonium affects embryo metabolism, pH regulation, and gene expression.
• The immediate answer is to renew the culture medium, thereby bringing the ammonium concentration under control.
• A second solution is to replace the most labile amino acid, glutamine, with a dipeptide form such as alanylglutamine. This dipeptide is just as effective as glutamine and has the advantage of not breaking down at 37°C. Therefore, media containing this stable form of glutamine do not produce significant levels of ammonium.

Carbohydrates
• Carbohydrates are present within the luminal fluids of the female reproductive tract. Their levels vary both between the oviduct and uterus and within the cycle.
• The precise substrate requirements for the human embryo have yet to be fully elucidated. However, analysis of carbohydrate uptakes in vitro has revealed that the human embryo has an initial preference for pyruvate, whilst glucose uptake increases with development.
Carbohydrates

- Oviduct,
  - high concentrations of pyruvate (0.32 mmol/l) and lactate (10.5 mmol/l),
  - relatively low concentration of glucose (0.5 mmol/l).
- In contrast, uterine fluid
  - Relatively low levels of pyruvate (0.1 mmol/l) and lactate (5.87 mmol/l), and
  - higher concentration of glucose (3.15 mmol/l).

Edthlenediaminetetraacetic acid (EDTA) is a chelating agent, 0.01 - 0.1mmol/L.
Beneficial for the development of the embryo from zygote through cleavage stage, overcome 2 cells block
Exposure of post compaction stage reduces ICM number
Inhibit glycolysis through impairing 3-phosphoglycerate kinase activity
Prevents capacitation and acrosome reaction (chelates ca)

![Utilisation of Glucose by human embryos](image)
Culture Protocol

Media

Table 16.1 Differences in embryo physiology pre- and post-compaction

<table>
<thead>
<tr>
<th>Pre-compaction</th>
<th>Post-compaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low biosynthetic activity</td>
<td>High biosynthetic activity</td>
</tr>
<tr>
<td>Low QO₂</td>
<td>High QO₂</td>
</tr>
<tr>
<td>Pyruvate preferred nutrient</td>
<td>Glucose preferred nutrient</td>
</tr>
<tr>
<td>Nonessential amino acids</td>
<td>Nonessential + essential amino acids</td>
</tr>
<tr>
<td>Maternal genome</td>
<td>Embryonic genome</td>
</tr>
<tr>
<td>Individual cells</td>
<td>Transporting epithelium</td>
</tr>
<tr>
<td>One cell type</td>
<td>Two distinct cell types:</td>
</tr>
<tr>
<td></td>
<td>ICM and trophoderm</td>
</tr>
</tbody>
</table>

QO₂, oxygen consumption; ICM, inner cell mass.

Sequential
Culture of preimplantation embryos are influenced by two concepts:

"Let the embryo choose": Single culture media (with or without refreshing)

"Back to nature": Sequential culture media
### Single / Sequential

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single Medium Non-renewed</th>
<th>Single Medium Renewed</th>
<th>Sequential Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo undisturbed</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Accumulation of autocrine/paracrine factors</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Replenishment of essential nutrients</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Accumulation of toxins</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Stress levels due to embryo manipulation</td>
<td>Low</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Labour intensity</td>
<td>Low</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
</tr>
</tbody>
</table>

---

### Differences between oviduct and uterus in mammalian embryos (Lane et al, 2007)

<table>
<thead>
<tr>
<th>Component</th>
<th>Oviduct</th>
<th>Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration</td>
<td>0.50mM</td>
<td>3.15mM</td>
</tr>
<tr>
<td>Pyruvate concentration</td>
<td>0.32mM</td>
<td>0.10mM</td>
</tr>
<tr>
<td>Lactate concentration</td>
<td>10.50mM</td>
<td>5.20mM</td>
</tr>
<tr>
<td>Oxygen concentration</td>
<td>8%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Carbon dioxide concentration</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Glycine concentration</td>
<td>2.77</td>
<td>19.33</td>
</tr>
<tr>
<td>Alanine concentration</td>
<td>0.5</td>
<td>1.24</td>
</tr>
<tr>
<td>Serine concentration</td>
<td>0.32</td>
<td>0.80</td>
</tr>
</tbody>
</table>

---

### Single / Sequential

<table>
<thead>
<tr>
<th>Component</th>
<th>Mono Culture</th>
<th>Sequential Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSOMaa mmol/L</td>
<td>G1 mmol/L</td>
<td>G2 mmol/L</td>
</tr>
<tr>
<td>Na pyruvate</td>
<td>0.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Na lactate</td>
<td>10.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.56</td>
<td>0.5</td>
</tr>
</tbody>
</table>
### Single / Sequential

#### RCT: 3652 embryos, couples undergoing ICSI

<table>
<thead>
<tr>
<th></th>
<th>Sequential</th>
<th>Single Step</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocyst rate</td>
<td>45%</td>
<td>45%</td>
<td>NS</td>
</tr>
<tr>
<td>Aneuploidy rate</td>
<td>58.8%</td>
<td>61.8%</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing implantation rate</td>
<td>46.4%</td>
<td>42.2%</td>
<td>NS</td>
</tr>
</tbody>
</table>

---

### Single / Sequential

#### Embryo culture media and IVF/ICSI success rates: a systematic review


1. Faculty of Pure and Applied Sciences, Khartoum University, Khartoum, Sudan. S. Mesthene {#2}

**Conclusions:** It is yet unknown what culture medium leads to the best success rates in IVF/ICSI. Given the potential importance of culture media for IVF outcome, rigorously designed RCTs are needed for currently available, as well as newly introduced culture media.

**Key words:** culture medium / IVF / ICSI / live birth / randomized controlled trial / meta-analysis

---

### Factors Impacting
Factors

- Optimization of embryo development in vitro is not only dependent upon the composition of the culture medium or media used, but is also affected by physical parameters, such as the incubation environment, gas phase, Light and handling.

**Fig. 16.2** Sensitivity and susceptibility of germ cells and embryos to external factors. IVM, in vitro maturation; S/O, super ovulation.

**pH**

<table>
<thead>
<tr>
<th>Cell stage (human)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleavage</td>
<td>7.04 ± 0.07</td>
</tr>
<tr>
<td>Mitotic</td>
<td>7.03 ± 0.04</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>5.68 ± 0.02</td>
</tr>
<tr>
<td>2-8 cell</td>
<td>7.12 ± 0.01</td>
</tr>
<tr>
<td>Morula-Blastosomat</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Henderson–Hasselbach equation:**

\[ \text{CO}_2(\text{gas}) \leftrightarrow \text{CO}_2(\text{dissolved}) \leftrightarrow \text{H}_2\text{CO}_3 + \text{H}^+ + \text{HCO}_3^- \]
pH

- pH Media - 7.4, pH cell - 7.2
- Dependent on Bicarbonate, Amino acid, Lactate
- Fyrite
- Co2 analysers - IR sensors
- Blood Gas analyser
- CO2 dissolves < higher temperature
- CO2 dissolves > higher atmospheric pressure

Reduced O2

- 5% or 20%
- Improved human embryo development, implantation, and pregnancy rates when culturing embryos in reduced oxygen concentrations
- Difficult to identify a study that demonstrates a detriment of using low oxygen for human embryo culture.

Reduced O2

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of reduced oxygen concentration in a predominantly blastocyst transfer program.</strong></td>
</tr>
<tr>
<td>Endpoint</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
</tr>
<tr>
<td>Implantation</td>
</tr>
<tr>
<td>Live birth</td>
</tr>
</tbody>
</table>

Note: When examining all patients in a prospective randomized trial, extended culture in low oxygen significantly improved clinical pregnancy, implantation, and live birth. Adapted from Meehne et al. [76].

* A different superscript between columns represent a statistically significant difference, P < .05.

**Reduced O2**

- Although the exact mechanism of the benefit of low oxygen use for embryo culture is unknown, possibilities include reduced generation of reactive oxygen species, improved air quality/reduced volatile organic compounds (VOCs) due to filtered nitrogen gas, and perhaps other potential mechanisms that may impact gene expression, metabolism, or other cellular processes.

---

**Temperature**

- Optimal temperature to culture human embryos remains unknown. While 37°C is commonly used and is based on the estimate of human core body temperature
- Improves fertilisation and embryo development rate
- < 33°C leads to irreversible damage to microtubules

---

**Temperature**

---

**TABLE 5**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mature flh %</th>
<th>Fertil. %</th>
<th>Zyg %</th>
<th>3-cell flh %</th>
<th>5-cell flh %</th>
<th>Inv. flh %</th>
<th>Abort flh %</th>
<th>Implant. flh %</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>490</td>
<td>60±2</td>
<td>7.7±0.3</td>
<td>43±7</td>
<td>48±7</td>
<td>46±1</td>
<td>46±1</td>
<td>32±3</td>
</tr>
</tbody>
</table>

Note: Abbott et al. (1974) used in human in vitro systems. Where necessary, data from this study have been normalised and in vitro data have been normalised. Data obtained by the author's team at the IVF Centre of the Department of Obstetrics & Gynaecology, AIIMS, New Delhi, India.
Temperature

- Small tube of OPU needle
- Don't fill tube till top
- Reduced distance to lab
- Pre warmed everything
- Warm palm
- Shorter dish travelling area

Embryo Density

- Group or Single embryo culture
- Improved culture with increased embryo density
- Autocrine/ paracrine/ juxtacrine communication
- Limits diffusion of positive factors away from embryo
- Limitation: Tracking of embryo

Embryo Density

- 1:6.25 micro litre or not more than 4 embryos per 25 micro litre
- optimal: 1:12.5 micro litre
- Without heating and airflow
Embryo Density

- The WOW dish (LinKIDTM culture dish; DNP, Japan) has 25 microwells that allows group culture under a single drop of medium. Through its design, it is possible to manage embryos separately whilst in group culture. Due to paracrine effects associated with group culture, embryo culture results have been reported to be improved.

Light

- Wavelength <300nm are absorbed by plastics
- >400nm Apoptotic
- Vitamins and Oil are light-sensitive and therefore care should be taken to minimize exposure to light by storing the culture media in dark bottles or wrapping them in foil.

Temperature

ABSTRACT BOOK
EUGENE 2010 - HELSINKI, FINLAND | 3-6 JULY 2010

O-008  Do not disturb the embryos until day 5: preliminary results of a double blind prospective randomized controlled trial

J. Tei, A. Rodriguez-Arnedo, J. Guerrero, B. Moliner, J. Llacer
Instituto Bernabeu, Biology of Reproduction, Alicante, Spain
Instituto Bernabeu, Reproductive Medicine, Alicante, Spain

Study question: To test the hypothesis that avoiding embryo observation until day 5 may produce an improvement in embryo quality and therefore, implantation and ongoing gestation rates.

Summary answer: Maintaining the embryos to the blastocyst stage without assessment or observation on day 2 and 3 does not affect clinical outcomes.
Oil

- Reduces evaporation of media
- 2mm layer is enough to avoid evaporation
- Mineral oil has more unsaturated bonds making it prone for photo oxidation
- Use paraffin oil

Dynamic culture

<table>
<thead>
<tr>
<th>Approach</th>
<th>Embryo source</th>
<th>Outcome measures</th>
<th>Outcome summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thing</td>
<td>hatching time</td>
<td>Morphology, viability, development, cell integrity</td>
<td>Improved cell integrity and development</td>
<td>(31)</td>
</tr>
<tr>
<td>Vibration</td>
<td>fresh embryos</td>
<td>Day 3 embryo quality, development</td>
<td>Increased day 3 quality, development</td>
<td>(153, 154)</td>
</tr>
<tr>
<td>Fresh zygotes</td>
<td>Day 3 embryo quality</td>
<td>Embryo survival, development</td>
<td>Improved survival and development</td>
<td>(155)</td>
</tr>
<tr>
<td>Pulsed flow</td>
<td>Fresh zygotes</td>
<td>Development, viability</td>
<td>Improved viability and development</td>
<td>(155)</td>
</tr>
</tbody>
</table>

Dynamic culture

How music gives IVF eggs good vibrations by making them more likely to get fertilised

- Playing music to an egg increases chances of fertilisation by 5 per cent
- Tiny vibrations produced by music give fertilisation a helping hand
- Scientists played music by Michael Jackson and Madonna
THANK YOU
6. Panel Discussion: Polution (How it effects my fertility & what can be done?)
**What is Pollution?**

- the presence in or introduction into the environment of a substance which has harmful or poisonous effects.
- Pollution can take the form of chemical substances or energy, such as noise, heat or light. Pollutants, the components of pollution, can be either foreign substances/energies or naturally occurring contaminants.
Toxic and environmental hazards can affect reproduction at any point in the process. They can affect fertility, conception, pregnancy, and/or delivery. And, of course, they can affect the male and the female.
How pollution effects?
What are important day to day toxicants?

- Xenoestrogens, alkylphenolic chemicals (bisphenol A [BPA] and PCBs), phthalates, dioxins, lead, mercury, and pesticides are ubiquitous in the global environment.
Mechanism of action (AIR pollution)

- Action as endocrine disruptors (EDCs)
- Induction of reactive oxygen species (ROS)
- Cell DNA alteration
- Epigenetic modifications

**CHEMICALS POTENTIALLY ASSOCIATED WITH REPRODUCTIVE HEALTH EFFECTS**

<table>
<thead>
<tr>
<th>Type of compound/substrate</th>
<th>Specific example</th>
<th>Evidence of reproductive health effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commonly used pesticides</td>
<td>DDT ( dichlorodiphenyltrichloroethane) Organophosphates</td>
<td>Multiple case studies from wildlife exposure; some human evidence</td>
</tr>
<tr>
<td>Flame retardants</td>
<td>PBDEs (polybrominated diphenyl ethers)</td>
<td>Animal exposure models/data</td>
</tr>
<tr>
<td>Dioxin-like substances</td>
<td>PCBs (polychlorinated biphenyls)</td>
<td>Animal exposure models/data; Wildlife studies Weak human exposure data</td>
</tr>
<tr>
<td>Phthalates</td>
<td>PVC (polyvinyl chloride) Di (2ethylhexyl) phthalate</td>
<td>Animal exposure models/data Emerging human studies (surveys, biomarker association)</td>
</tr>
<tr>
<td>Additives to consumer products (plastics)</td>
<td>BPA (Bisphenol A)</td>
<td>Evidence from animal exposure models/data</td>
</tr>
</tbody>
</table>

Endocrine disruptors

Endocrine-disrupting chemicals (EDCs) are thought to affect reproduction by directly or indirectly interfering, stimulating, antagonizing, altering, or disrupting natural hormone (s). Exposure to such agents at critical stages of development can have a significant impact upon fertility and ultimately, fetal development. Incomplete development of DNA repair mechanisms, fertilization defects, and the broad-brain barrier can exist in a chemical's effect on the developing fetus.
**Reactive Oxygen Species (ROS)**

- Most air pollutants such as NO2 are ROS capable of generating them, such as O3 or PM, through the heavy metals and the PAHs they contain. They can be transformed by CYP450 dihydro-dehydrogenase, which produces quinolone redox, catalysing electron transfer reactions and thus stimulating ROS production.

**Cell dna alteration**

Fertility alteration caused by air pollution in the induction of alteration in the cell DNA.

Inflammation process due to ROS can alter DNA as reported in a study of taxi drivers. Telomere length has been reported to increase with increasing annual exposures to NO2, MM.

Some molecules are able to bind to a DNA base through covalent bonding, thus modifying gene expression.

**Epigenetic modifications**

- Epigenetic modifications notably DNA methylation can lead to abnormal gene expression. These can effect methylation.
- These changes can effect mitochondria.
- Air pollutants have shown to effect microRNA
How is it affecting male reproduction?

- Reduced fertility
- Genetically abnormal sperms
- Reduced sperm counts
- Germinal epithelium abnormalities
- Hormonal dysfunction

How is it affecting male reproduction?

- Reduce fertility
- Pregnancy loss
- Abnormalities of reproductive systems
  - PCO, POF, impair ovarian development
  - Poor uterine development, fibroids, endometriosis
Effect of Environment on ART

- Follicular microenvironment pesticides are present in follicular fluid at the time of resumption of meiosis when chromosome susceptibility is at its highest. For the most part, follicular toxicant concentrations are lower than serum levels. Mercury is a common toxicant. In 1998 study, children exposed to PCBs in utero were contacted and sperm analysis was performed. They found abnormal sperm motility and morphology and decreased ability to penetrate hamster eggs. (Guoa et al)

What can be done?
What can be done?

- Eat organic
- Avoid cosmetics/household products with less toxicity
- Air purifier
- Detox? Microwave safe?
- Migrate to less polluted place??
- Timely trying for conception & awareness

How is it affecting male reproduction?

- Reduce fertility
- Pregnancy loss
- Abnormalities of reproductive systems
  - PCO, POF, impair ovarian development
  - Poor uterine development, fibroids, endometriosis
Simple steps: to limit toxicity

1. Read labels: if you cannot pronounce it, do not buy it. There is an extensive list on the EWG’s website.
2. Go organic. Although it costs more, so does eating pesticides and other harmful substances. The less distance one’s food travels, the less exposure to chemicals it likely has.
3. Avoid chemicals. Cosmetics and water are common harbingers of toxins, but so are canned goods, scented perfumes, air fresheners, and household cleaners. You can create your own cleaners with lemon juice and vinegar and use essential oils as air fresheners.
4. Drink filtered water from bottles that do not have BPA. Metal containers and glass bottles are far safer than plastic ones.
5. Do not microwave in plastics or unmarked containers. If you do microwave in plastic, it must say “microwave safe”. This includes leftover Chinese food or other takeout plastic containers.
What is Maca Root?

Maca root is a tuberous root that is known for its energy-boosting properties. The native Peruvians have used maca for centuries for its nutritional and medicinal value. Maca was first cultivated in the Inca era and has been used in traditional medicine for thousands of years.

Super Nutrient Dense Food

Maca root is a superfood with high amounts of vitamins, minerals, and antioxidants. It is rich in iron, calcium, and magnesium, which are essential for energy and overall health. It is also high in antioxidants, which can help protect the body against free radicals and reduce the risk of chronic diseases.

Is it Safe?

While some people may fear the effects of maca root, it is not. Maca root is a natural source of vitamins and minerals. It is safe for most people, but as with any supplement, it is important to consult with a healthcare professional before starting a new regimen.

Fertility Food
Precautions for fish

- Current FDA recommendations are for women of childbearing age to avoid fish that are likely to contain high levels of methyl mercury (>1 µg/g), including swordfish, shark, tilefish, and king mackerel. More recently, a 2014 update from the FDA recommended women and children follow three safety tips for eating fish and shellfish:
  1. Do not eat shark, swordfish, king mackerel, and tilefish because of high mercury levels.
  2. Eat up to 12 oz (two average meals) weekly of fish and shellfish low in mercury such as shrimp, canned light tuna, salmon, pollock, and catfish. Albacore (“white”) tuna has more mercury than canned light tuna.
  3. Check local advisories about the safety of fish caught by family and friends.
Thank You
THE FUTURE OF FERTILITY IS SIMPLICITY

MICROFLUIDIC

MICROFLUIDIC BASED ‘LAB-ON-A-CHIP’ DEVICES MIMICK NATURE TO SORT AND SELECT THE BEST QUALITY SPERMS ... IN A SIMPLISTIC NATURAL WAY.

Sperm damaging procedures like Centrifugation and mixing could be a story of the past.

ZyMot-ICSII
MICROFLUIDIC SPERM SORTING CHIPS
Formerly known as FERTILE

+ ZyMot-Multi (850µl)
MICROFLUIDIC SPERM SORTING CHIPS
Formerly known as FERTILE PLUS

Features & Benefits

- No sample preparation
- No Centrifugation
- No Extensive Training
- No Expensive Equipment.
- Low ROS & DNA Fragmentation
- Compared to other techniques
- Sterile, Single-Use Chips
- Sperm sorting based on motility within a micro-environment created by micro-channels or a micro-porous Filter.

AIR STERILIZER

Improve your Embryo quality by continuously eradicating VOCs, Odour, Mold, Spores, Bacteria and Viruses.

CRIYOSTORAGE

INNOVATIVE SOLUTIONS FOR CRYOGENIC STORAGE, RECOVERY AND DELIVERY

Conventional 47-11 Dewar
with standard 11 containers

192 Patients Stored

Two Conventional 47L Containers
equals One VitroStash

384 Patients Stored

VitroStash Dewar

960 Patients Stored

Five Conventional 47L Containers
equals One VitroStash MAX

INLINE FILTER & DISINFECTANTS

Embryo Shield
Inline Filter: Inline filter with 0.1µm pore size Hepe and coconut activated Carbon with Shelf life of 6 months.

Embryo Safe Disinfectants:
Active Ingredient is Hypochlorous acid which is naturally occurring in human immune system and highly pathogenic to bacteria including mycobacterium, viruses, mycoplasma and spores without releasing VOCs and odour.

For more info & demo contact:

Trivector BioMed LLP
111-115, Marathon Max, LBS-Link Road Junction, Mulund (W), Mumbai - 400080.
Tel: +91-22-25839996 / 97 / 98 / 1111
Email: info@trivectorbiomed.com • trivector@vsnl.com

Our associate:

BabyQuest Cryobank Pvt. Ltd.
www.babyquest.in

www.trivectorbiomed.com
Your Trusted Partner in A.R.T. since 1993!