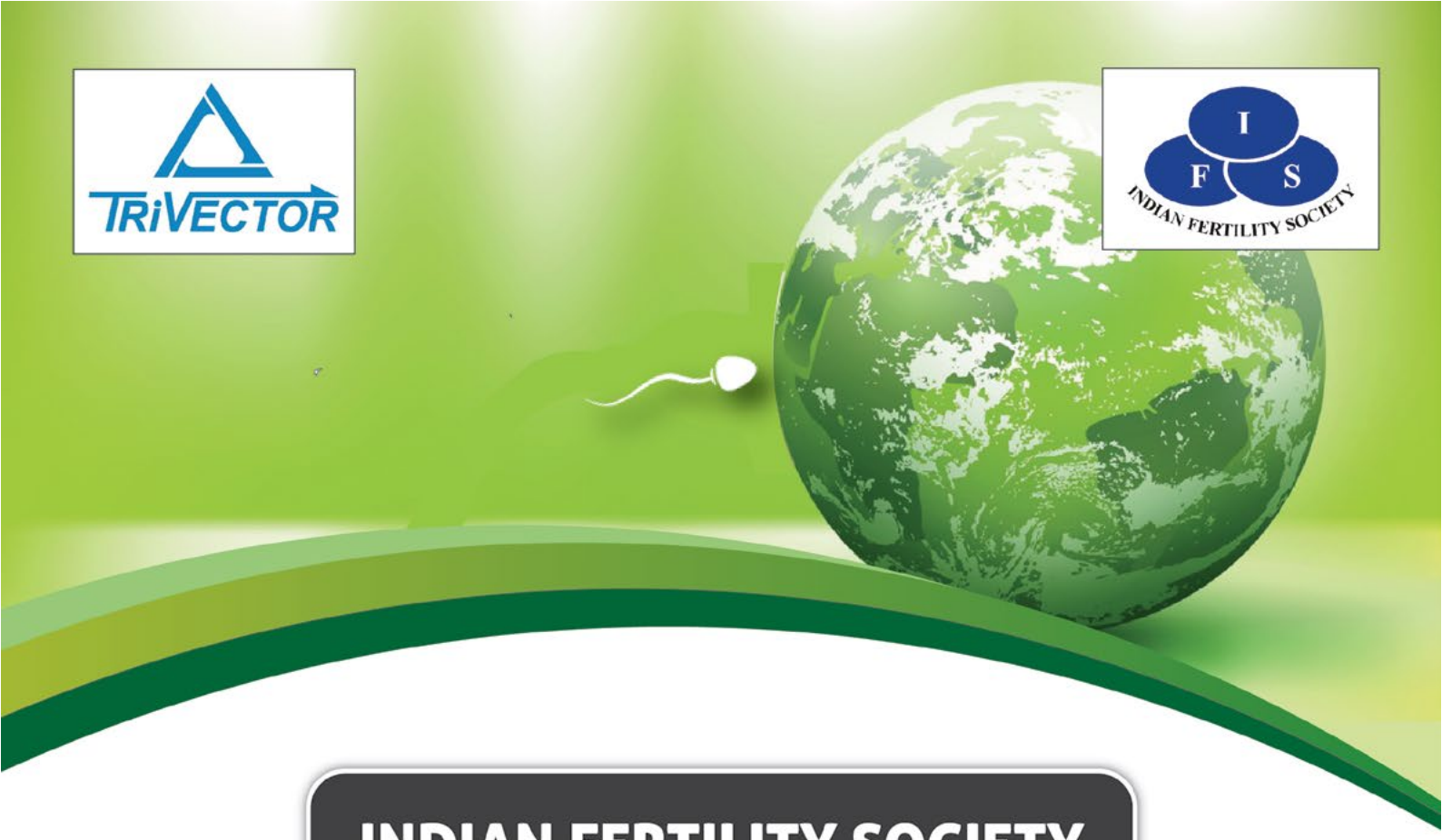


INDIAN FERTILITY SOCIETY

ENVIRONMENT & REPRODUCTION IN ART


Organised by
I F S
INDIAN FERTILITY SOCIETY
15th Annual Congress of
Indian Fertility Society
FERTIVISION
2019
6-8 December
New Delhi | India
Theme: Beyond Tomorrow
www.fertivision2019.com

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 theses 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

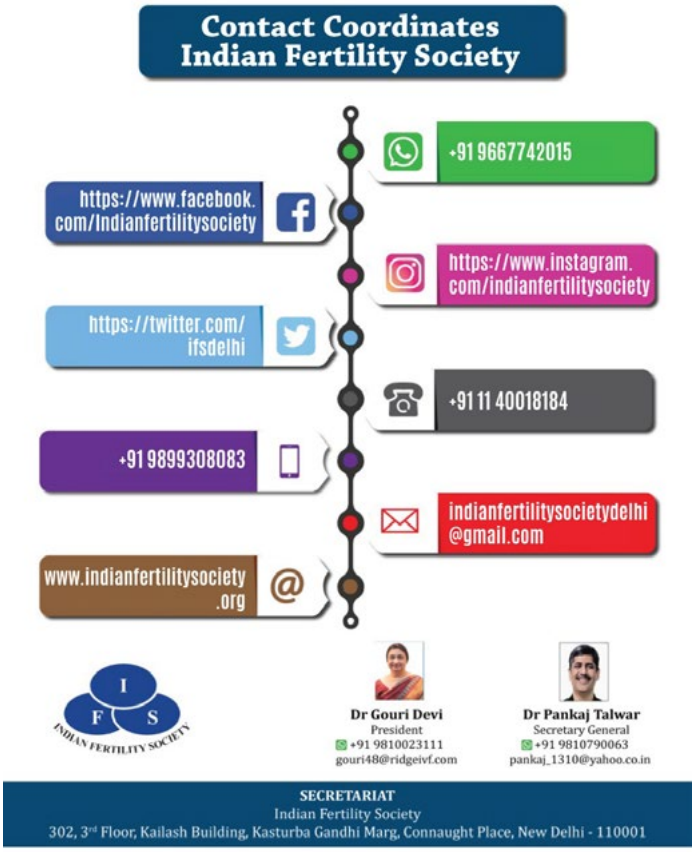
The interaction between human health and the environment has been extensively studied and environmental risks have been proven to significantly impact human health, either directly by exposing people to harmful agents, or indirectly, by disrupting life-sustaining ecosystems.

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

Dr M Gouri Devi
M.D

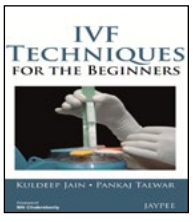
- Director, Gouri Hospitals Ltd.
- Director, Ridge IVF Group.(Runs a chain of IVF centres)
- President, Indian fertility society
- Ex-Secretary General, Indian Fertility Society
- Executive, AOGD governing council
- Member, Executive Board, NARCHI, DGES, FPSI
- Ex Vice President, NARCHI
- Chairperson, 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

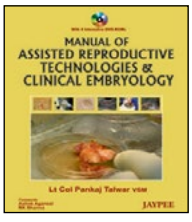
She is a keen academician, has organized many conferences, has been a speaker in many national and international conferences. Has many publications to her credit

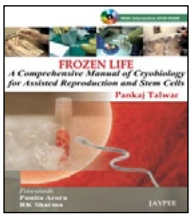
Dr Pankaj Talwar

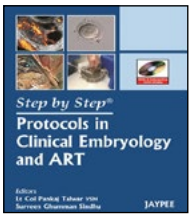
Col Pankaj Talwar, VSM
Professor and HOD
ART Centre, Army Hospital, New Delhi

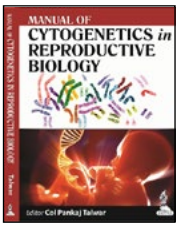
- Sec IFS.
- Secretary Fertility preservation society of India.
- Editorial board of multiple Infertility journals.
- Member Advisory committee ICMR
- Member Infertility committee FOGSI
- Editor Nexus / Artext – E bulletin of IFS
- Awarded Vishisht seva medal by the President of India for working in 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

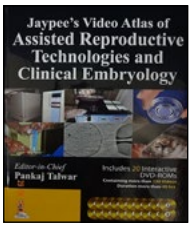




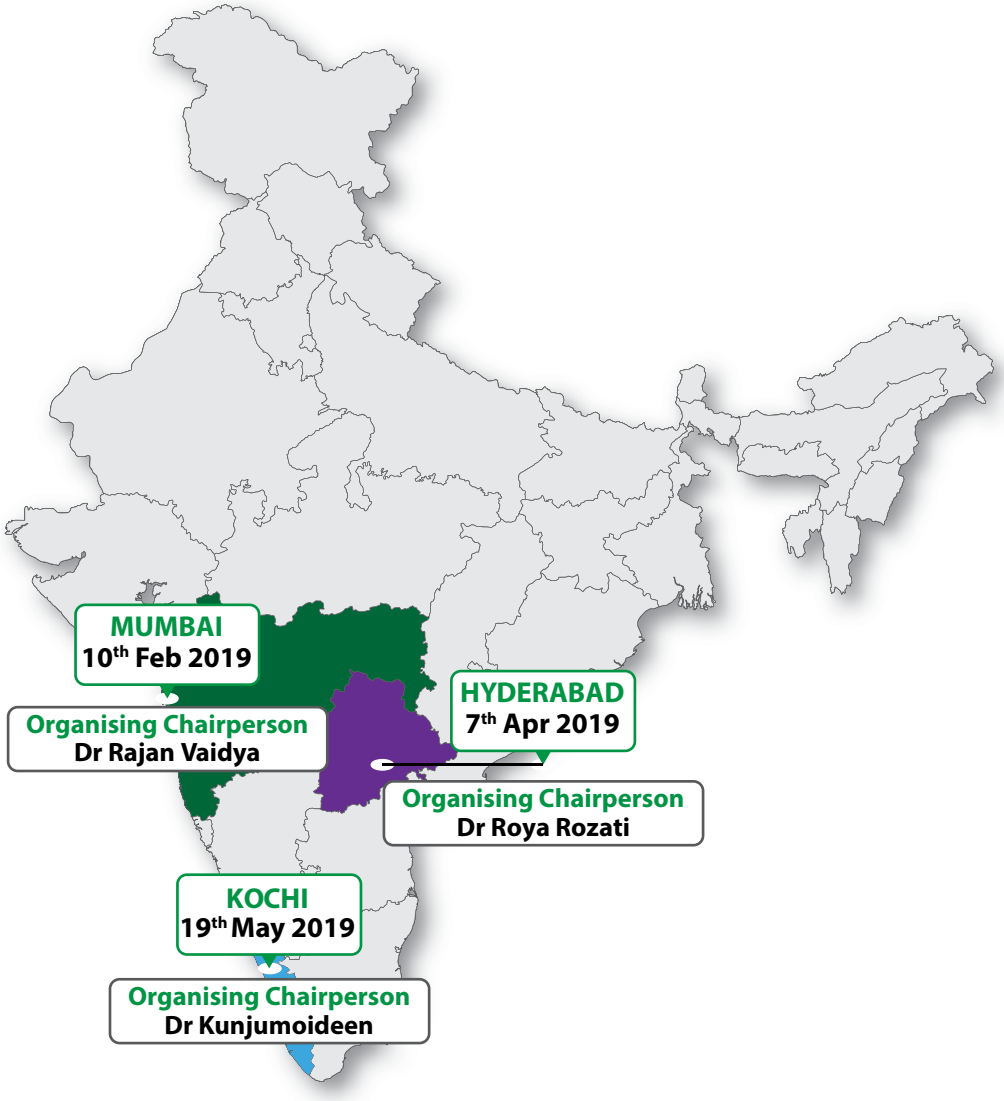








Venue and Dates



Organising Chairpersons

Dr Sweta Gupta
National Coordinator

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Dr Rajan Vaidya
Local Coordinator
Mumbai

Dr Roya Rozati
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Hyderabad

Dr Kunjumoideen
Local Coordinator
Kochi

List of contributors

Topic	Contributed by
Environment Toxicants and Male Reproduction	Dr Kunjumoideen
Environment Toxicants and Female Reproduction	Dr Roya Rozati
Interesting cases (Testicular dysgenesis Syndrome / Miscariage / Malformation etc)	Dr Nancy Kumar
Options and advances in air purification technologies	Mr. Dilip Patil
Optimizing the culture environment in the IVF lAb	Mr. Gaurav Kant
Panel Discussion : Polution (How it effects my fertility & what can be done?)	Dr Sweta Gupta

INDEX

1. Environmental Toxicants and Their Effects on Male Reproduction

Outline

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

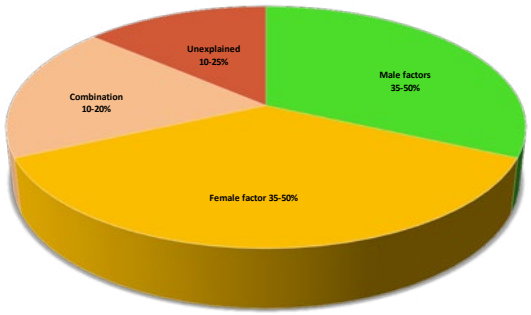
Introduction

- 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

Introduction

- 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

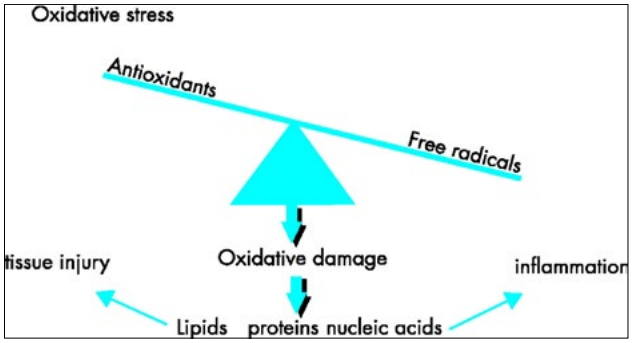
Rising infertility – a cause of concern



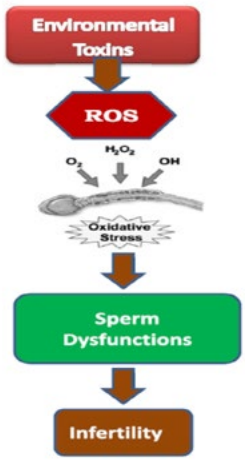
Oxidative stress

- "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"

Oxidative Stress (OS)



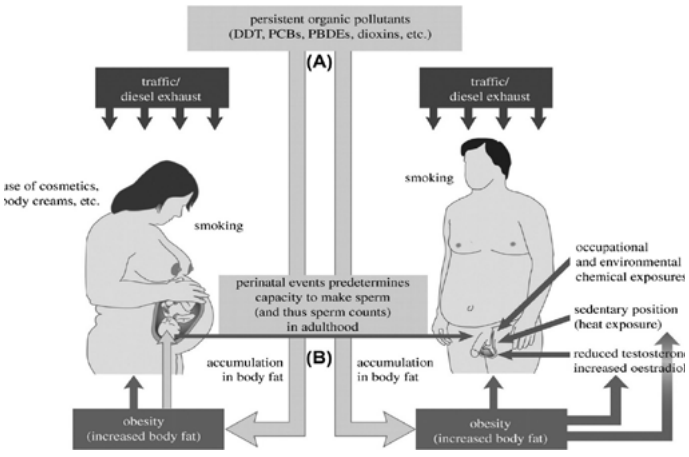
Primary pathologies of male reproductive system in connection with environmental toxins, oxidative stress and infertility

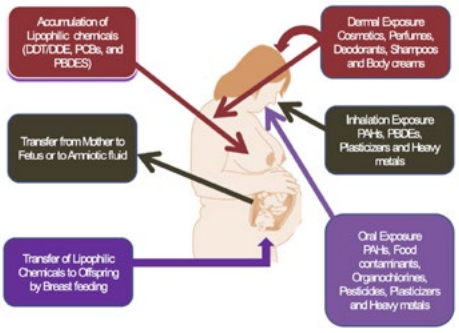


Reproductive toxicity

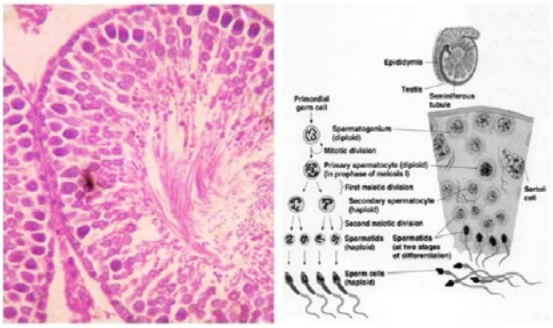
- Reproductive toxicity is defined as adverse impacts on sexual function/fertility in adult males and females, as well as developmental toxicity in the offspring.
- Two major categories:
  1. Any impact of chemicals that would interfere with reproductive ability. This may include, but not be limited 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





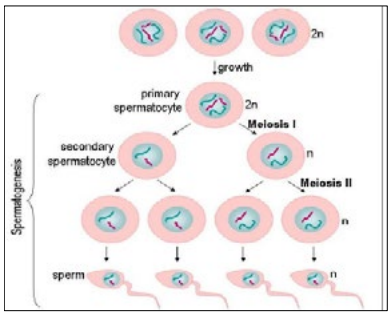
Routes of human exposure to some common environmental chemicals. DDE= 1, 1- dichloro-2, 2-bis (p- chlorophenyl) ethylene; DDT= dichlorodiphenyltrichloroethane;PAHs= polycyclic aromatic hydrocarbons; PCBs= polychlorinated biphenyls. (Modifiedfrom Sharpe & Irvine, BMJ, 2004).



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.

Sperm DNA peculiarity

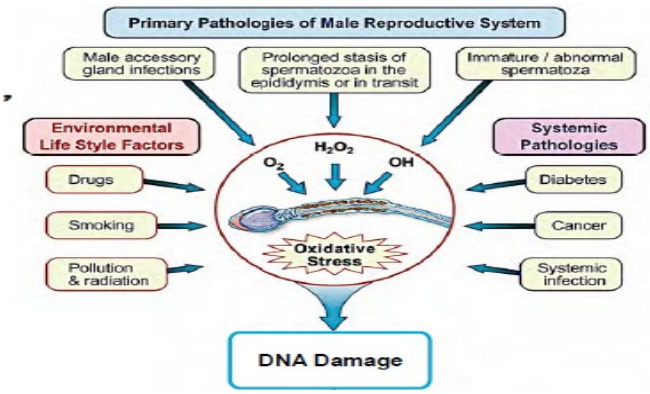


- During spermiogenesis spermatids repackage spermatids repackage their DNA with protamines, a small residue of histone-bound DNA is retained (15%).

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?



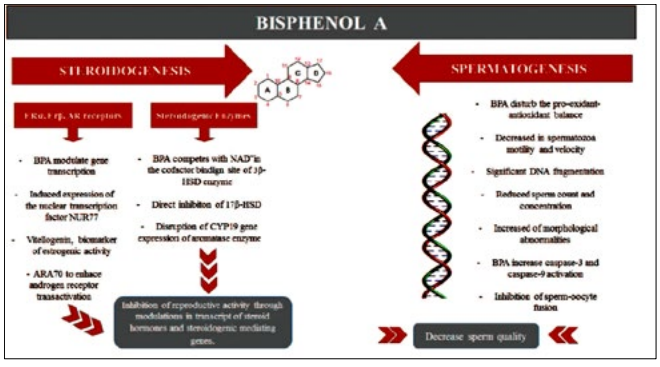
Sperm DNA damage and diminished reproductive outcomes

- 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.

Male Reproduction: One of the Primary Targets of Bisphenol A

Tomáš Jambor, Bistáková Jana, Greifová Hana, Tvrdá Eva and Lukáč Norbert

A model summary for the effects of bisphenol A (BPA) on reproductive system



Male bisphenol A (BPA) levels and reproductive outcomes in epidemiologic studies.

Author and year	Reference	Study design	Study population	Measured outcomes	Unadjusted urinary BPA concentration (ng/mL)	Main findings
Hanaoka et al. 2002	(34)	Cross-sectional	42 occupationally exposed and 42 occupationally nonexposed men	Reproductive hormones	Median (range)[a]: Exp 1.1 (0–11.2) Nonexp 0.5 (0–11.0)	Associated with lower FSH in occupationally exposed men. No differences in LH and fT.
Galloway et al. 2010	(35)	Cross-sectional	307 men from general population	Reproductive hormones	GM 4.0 (5th–95th 1.8–4.3)	No associations with S_T, SHBG, and fT. Associated with higher T
Meeker et al. 2010	(36)	Cross-sectional	190 men attending a fertility clinic	Semen parameters and DNA damage (measured as % of damage in comet tail in a subset of 132 men)	GM 1.4 (IQR 0.8–2.5)	Associated with lower sperm concentration, normal morphology, and motility. No association with total sperm count. Associated with higher sperm DNA damage.
Meeker et al. 2010	(37)	Prospective cohort	167 men attending a fertility clinic	Reproductive hormones	GM 1.3 (IQR 0.7–2.4)	Associated with lower inhibin B and LH and higher FSH. No relationship with T, SHBG, E_2, fT, T_F, T_M, and TSH.
Mendiola et al. 2010	(38)	Cross-sectional cohort	315 fertile men from prenatal clinics (302 for semen analysis)	Semen parameters and reproductive hormones	GM 1.5 (IQR 0.8–3.0)	Associated with lower FAI and FALH and higher SHBG. No association with semen parameters, FSH, LH, T, inhibin B, and fT.
Li et al. 2011	(39)	Prospective cohort	218 occupationally exposed and nonexposed men	Semen parameters	Median (IQR)[b]: Exp 38.7 (6.3–354) Nonexp 1.4 (0–17.9)	Associated with lower sperm concentration, total count, normal motility and vitality in all men. Associated with lower sperm concentration, normal motility and vitality in occupationally exposed men. Associated with lower sperm concentration in occupationally nonexposed men. No association with ejaculate volume and morphology.
Rudel-Louit et al. 2014	(40)	Prospective cohort	439 male partners of couples trying to become pregnant	Fecundability measured as time to pregnancy	GM 0.5 (5th–95th 0.4–0.6)	No association with time to pregnancy.
Knez et al. 2014	(41)	Prospective cohort	149 male partners of couples undergoing IVF treatments	Semen parameters	GM 1.6 (5th–95th 0.3–6.7)	Associated with lower total sperm count, concentration, and vitality. No association with other semen quality parameters.

Miguel-Alarcón. Bisphenol A and male reproductive health. Fertil Steril 2016.

Continued.

Author and year	Reference	Study design	Study population	Measured outcomes	Unadjusted urinary BPA concentration (ng/mL)	Main findings
Lassen et al. 2014	(42)	Cross-sectional	308 young men from general population	Semen parameters and reproductive hormones	Median 3.3 (5th–95th 0.8–14.9)	Associated with lower progressive motility. No association with other semen quality parameters. Associated with higher T, LH, E_2, and fT. No association with FSH, inhibin B, and SHBG.
Bae et al. 2015	(43)	Prospective cohort	220 singleton live births of couples trying to become pregnant	SSR defined as the ratio of male to female births	Not reported	Associated with more female births.
Dodge et al. 2015	(44)	Prospective cohort	218 male partners of couples attending a fertility clinic	Fertilization rate, embryo quality, and implantation in IVF cycles. Live birth rates in IUI and IVF cycles.	GM 1.6 (IQR 0.8–2.8)	No association with IUI or IVF outcomes.
Goldstone et al. 2015	(45)	Prospective cohort	418 male partners of couples trying to become pregnant	Semen parameters, DNA damage and fragmentation	GM 0.6 (5th–95th 0.5–0.6)[a]	Associated with lower % sperm DNA fragmentation. No association with semen quality parameters.

Note: Exp = exposed; FAI = free androgen index; fT = free testosterone; GM = geometric mean; IQR = interquartile range; IUI = intrauterine insemination; IVF = in vitro fertilization; Nonexp = nonexposed; SSR = secondary sex ratio; T_F = free testosterone; T_M = thyroxine.

[a] Did based on 473 morning periodically semen sample; however, the urine for BPA assessment was collected a 4.8 times (that study population).

[b] Creatinine-adjusted BPA concentration (μg/g).

Miguel-Alarcón. Bisphenol A and male reproductive health. Fertil Steril 2016.

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 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 azoospermia
- 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.

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).

Common environmental Toxicants	Common uses and routes of exposure	The effects on male reproductive system
Heavy Metals (Mainly cadmium, Lead and arsenic)	Population exposed to cadmium and lead via contaminations 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 metals and municipal waste incineration.	a. Testicular toxicity b. Low sperm count and motility and density. c. Reduce male fertility d. Foetal toxicity and malformation of male organs.
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
Phthalates DBP = di(n)butylphthalate DiBP =di(iso)butylphthalate	Phthalates are a group of chemicals used to impart flexibility to plastic polyvinyl chloride (PVC) products as	a. Testicular toxicity b. Reduce anogenital 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).

BBP = benzyl butyl phthalate DEHP = di(2-ethylhexyl)phthalate DPP = dipentyl phthalate DINP = diisononyl phthalate DCHP = dicyclohexyl phthalate	well as in other applications, including pharmaceuticals, and pesticides. There is widespread human exposure with reported uses in building materials, household furnishings, clothing, cosmetics, dentures, medical tubing and bags, toys, modelling clay, cars, lubricants, waxes and cleaning materials. Exposure may arise via the air, through absorption when used on the skin, and through the diet.	undescended of testes in immature male. c. Reduce male fertility d. Foetal toxicity and malformation of male organs.
Paraben	Paraben is the name given to 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 some 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

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).

Triclocarban	Triclocarban (TCC or 3,4,4'-trichlorocarbanilide) is also used as an anti- bacterial in personal care products such as soaps.	a. It has sex hormone disrupting properties.
BPA (Bisphenol A)	BPA is the building block of polycarbonate plastic used in baby bottles, CDs, motor cycle windshields etc. It is also used for the production of epoxy resins used in the coating of the food packaging.	a. Oestrogenic activities b. Altered male reproductive organs and induce early puberty c. Anti androgenic activity.
Penta-BDE (Penta-brominated diphenyl ether)	There are actually 3 commercial PBDE products, which predominantly contain deca, octa and penta-BDEs, and are therefore called by these names. PBDEs are used as flame retardants to prevent fire taking hold quickly. Penta-BDE is used in polyurethane foam, for example, in mattresses and car and aeroplane seats. Apart from exposure via dust it is possible to transfer from hand to mouth.	a. Altered male reproductive organs c. Anti androgenic properties.

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).

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 sealers, paints, fluorescent lights, plasticizers, adhesives, carbonless copying paper, flame retardants, and brake linings. Human exposure also arises due to contamination of the food chain.	a. Hormone mimicking activities b. Anti androgenic properties.
Dioxins	Dioxins are a group of chemicals which are not intentionally produced, but are emitted during incomplete or relatively low temperature combustion. They can come from industrial or domestic sources, wherever a chlorine source is present. Such sources include, for example, domestic bonfires with PVC plastic, incinerators, certain chemical and metal factories (particularly aluminium recovery sites), paper pulp production using chlorine, and coal burning in power stations and in fire-places in the home. Exposure can arise from inhalation, but mainly comes from contamination of food.	a. Sex hormone disruptor b. Testicular dysfunctions c. Low sperm count d. Sperm abnormalities

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).

Diesel fuel Exhaust	As diesel is used as a fuel in many cars and lorries, diesel exhaust is widespread.	a. Disrupts androgen action b. Prenatal exposure in animals leads to endocrine disruption after birth and suppresses testicular function in male rats.
Tobacco smoke (Polycyclic aromatic hydrocarbons (PAH)	It includes active or passive smoking	a. Blocks androgen synthesis b. Testicular dysfunctions c. Low sperm count d. Sperm abnormalities
Alkylphenols Nonylphenol Octylphenol	Nonylphenol is the breakdown product of the surface active agent, nonylphenol ethoxylate. Many uses including in domestic cleaning and industrial and institutional cleaning, and in textiles and leather processing. Octyl phenol is used in the production	a. Hormone mimicking activities b. Reduce synthesis of testosterone c. Reduce testicular size d. reduce male fertility and sperm number and quality.

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).

	of phenol/ formaldehyde resins (Bakelite) and in the production of octylphenol ethoxylates, and used in the formulation of printing inks and in tyre manufacture	
DDT (break-down product DDE).	DDT is an insecticide which was used extensively on crops, but is now only used in a few countries against the malaria-bearing mosquito. DDT and DDE last in the soil for a very long time, potentially for hundreds of years. Unfortunately, due to this persistence, it is still found in some produce, such as vegetables, fish and liver. DDE is also found as a persistent contaminant in our bodies. The DDT breakdown product or metabolite, p,p'-DDE, is able to block testosterone.	a. Hormone mimicking activities b. Reduce synthesis of testosterone
Linuron Diuron	Linuron and diuron are herbicide used to control weeds on hard surfaces such as roads, railway tracks and in crops and forestry. It has been detected in tap water and as a residue in vegetables such as carrots, parsnips and spinach.	a. Anti-androgenic properties

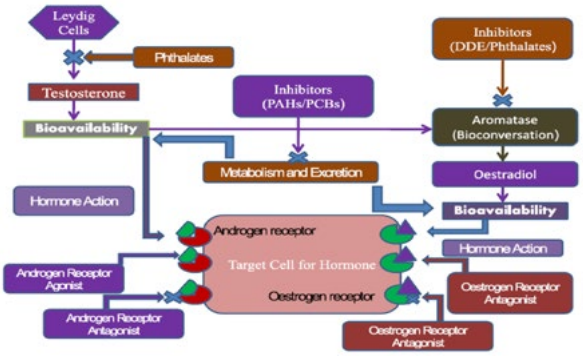
The examples of few chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are summarized below (Woodruff et al., 2008).

Vinclozolin Procymidone Iprodione Prochloroz Fenarimol	These are all fungicide used on fruits and vegetables.	a. Blocks testosterone action b. Reduce testosterone synthesis c. Anti-androgenic properties d. Feminize male offspring.
Fenarimol Fenitrothion Chlorpyrifos-methyl	These are all insecticides are used, for example, on apples, plums, barries, peas, sweet corn and cereals. Those have been found as a contaminant of fruit, such as oranges and grapes etc.	a. Blocks testosterone action b. Reduce testosterone synthesis c. Anti-androgenic properties
Ketoconazole	Ketoconazole is as an anti-fungal product in pharmaceuticals to treat fungal infections of the skin.	a. Blocks testosterone action b. Reduce testosterone synthesis
Pyrethroid pesticides Permethrin Beta-cyfluthrin Cypermethrin	Some pyrethroid pesticides such as Permethrin beta-cyfluthrin, cypermethrin, are still in use, with for example, the latter found as a residue in apples, beans, melons and oranges	a. Blocks testosterone action b. Reduce testosterone synthesis c. Anti-androgenic properties

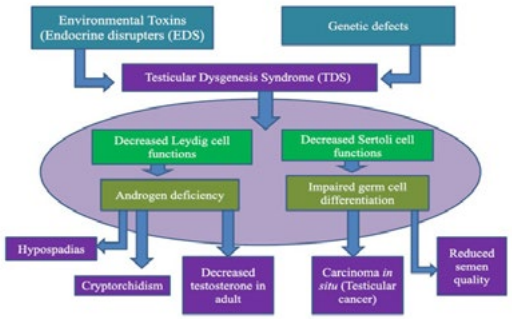
The examples of few chemicals which are reported to disrupt the sex hormones and/or damage the male in animal studies are summarized below (Woodruff et al., 2008).

Certain sun-screens 4-MBC 3-BC	A few ultraviolet (UV) filters have been found as contaminants in waste water treatment plants and rivers.	a. Estrogenic activity b. Anti-androgenic activity c. Interfere male sexual activity d. delay male puberty e. reduce reproductive organ weights in male offspring.
Heat, Ionizing radiation, Non-ionizing radiation, microwaves, electromagnetic fields	Mostly occupational exposures in home or industry as well as the mobile phone users.	a. Testicular toxicity b. Low sperm count and motility and density. c. Reduce male fertility d. Azospermia
Chemotherapeutic drugs (Cisplatin, cyclophosphamide, procarbazine, and doxirubicine, and vincristine etc.)	Anticancer treatment.	a. Testicular dysfunctions b. Low sperm count and motility and density. c. Infertility d. Azospermia and oligospermia.

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 functions. Damage of the testicular cells (Leydig cells and Sertoli cells), disrupts androgen production from Leydig cells and secretion of paracrine factors from sertoli cells, leading to birth defects (hypospadias, cryptorchidism) and impaired germ cell differentiation, apparent later as reduced semen quality or in the worst cases as carcinoma *in situ* (CIS) of the testis and consequent testicular cancer. (Modified from Skakkeback et al. Human Reproduction, 2001).



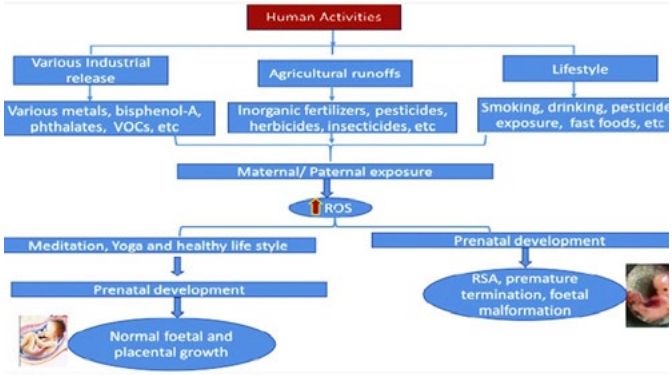
Measurement of oxidative stress

Direct Assay	Indirect Assay
Chemiluminescence assays	Myeloperoxidase test
Nitroblue tetrazolium test	Measurement of redox potential
Cytochrome *c* reduction	Lipid peroxidation levels
Flow cytometry	Chemokines Antioxidants

Lifestyle Factors Modifiable Without Risk

Lifestyle Factor	Results	Recommendations
Smoking	Strong correlation with % DFI, DFI markedly higher in infertile smokers	Cessation of smoking
POP/PCB	Positive correlation between exposure and % DFI PCB accumulate in food chain	Avoid fatty fish, particularly farmed
Organophosphorus	Marked increase in % DFI (>30%) in exposed workers	Avoid pesticide exposure
Lead	Increase in percentage of spermatozoa with DNA fragmentation	Avoid occupational exposure and smoking or exposure to cigarette smoke
Bisphenol A	Significant trend of increased DNA damage with increased urinary bisphenol A concentrations	Avoid plastic packaging, tinned foods, heating or storing foods in plastic
Testicular heat	Increase in DNA fragmentation with 2–3°C temperature increase	Avoid cycling with tight pants, avoid sauna use, avoid using laptop with legs close to each other
Mobile phone radiation	No specific studies on DNA fragmentation, increased ROS, and decreased antioxidants	Do not store mobile phone in trouser pocket
Obesity	Positive correlation of body mass index and DNA fragmentation, higher incidence in obese males	Weight loss through diet and moderate exercise

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.



Prevention

- 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

Moving forward

- 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

Primary prevention: the role of reproductive care professionals beyond the clinical setting

- 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 endocrinedisrupting 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

- 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

Critical Window of Susceptibility

- 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 Evironment Health

- Methylmercury
- Lead
- Ionizing radiations
- Polychlorinated biphenyls
- Polycyclic aromatic compounds
- Other air contaminants
- Organic solvents
- Some pesticides
- Alcohol

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

Type of compound/substance	Specific example	Evidence of reproductive health effects
Commonly used pesticides	DDT (dichlorodiphenyltrichloroethane) Organophosphates	Multiple case studies from wildlife exposures; some human evidence
Flame retardants	PBDEs (polybrominated diphenylethers)	Animal exposure models/data
Dioxin-like substances	PCBs (polychlorinated biphenyls)	-Animal exposure models/data -Wildlife exposure studies -Weak human exposure data
Phthalates	PVC (polyvinyl chloride) Di ethyl hexyl phthalate	-Animal exposure models/data - Emerging human studies (surveys, biomarker association)
Additives to consumer products (plasticizers)	BPA (bisphenol A)	- Evidence from animal exposure models/data

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

Phtalates

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 menstural cycle and Fecundability

Azziz etal Journal of Clinical Endocrinol 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 Steril2008,90:911-40)
- 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 testesterone exposure in utero
  Genetic: may be because genetic hypersecretion of testesterone
  Environmental toxin exposure may lead to elevation of prenatal testosterone

Azziz R etal 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 Menstural Cycle

- Case Study: Pesticide exposure and altered menstural cycle
- Organochlorines- decreased menstural cycle
- Non Organochlorines pesticide- increased menstural 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
- Potantally 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;85:775–9. ©2006 by American Society for Reproductive Medicine.)

Result

TABLE 1

Xenoestrogens concentration in control and different stages of endometriosis group and one-way analysis of variance between stages (ANOVA).

Congener	Control group (μg/mL)	Endometriosis group (μg/mL)				F value between groups (ANOVA)
		Stage I	Stage II	Stage III	Stage IV	
Mono-ortho substituted						
PCB-1 (co-planar)	0.04 ± 0.13	0.23 ± 0.26	0.42 ± 0.29	0.60 ± 0.27	0.84 ± 0.56	55.57[a]
PCB-5 (co-planar)	0.01 ± 0.05	0.10 ± 0.12	0.24 ± 0.22	0.62 ± 0.39	0.75 ± 0.43	87.29[a]
PCB-29 (co-planar)	0.02 ± 0.09	0.13 ± 0.15	0.02 ± 0.31	0.50 ± 0.34	0.99 ± 0.54	71.87[a]
Di-ortho substituted						
PCB-98 (Non-co-planar)	0.00 ± 0.02	0.03 ± 0.10	0.11 ± 0.19	0.37 ± 0.32	0.26 ± 0.31	30.64[a]
Phthalate esters						
Di-*n*-butyl phthalate	0.11 ± 0.21	0.19 ± 0.17	0.29 ± 0.23	0.52 ± 0.18	1.05 ± 0.44	48.88[a]
Butyl benzyl phthalate	0.14 ± 0.26	0.28 ± 0.38	0.67 ± 0.50	0.98 ± 0.59	1.27 ± 0.61	42.76[a]
DEHP	0.48 ± 0.77	1.49 ± 1.48	1.78 ± 1.68	1.51 ± 1.08	4.39 ± 3.22	28.81[a]
Di-*n*-octyl phthalate	0.03 ± 0.16	1.78 ± 1.47	2.55 ± 1.97	3.85 ± 1.86	5.35 ± 2.76	94.88[a]

Note: Control = Fertile women without endometriosis. Data are presented as mean ± SD.
[a] $P<.05$ was considered statistically significant.

Conclusion

The higher concentration of these chemicals in the plasma of subjects with endometriosis compared to fertile controls possibly 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.

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- Prospective CASE CONTROL STUDY at Mahavir Hospital and Research Cenrtre (1999-2005) by Dr Roya etal
- Fertility and Sterlity, March2006,vol85,No3
- 645 infertile women were screened, out of which 85 women were diagnosed to have endometriosis grade 1-1V (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.

www.blackwellpublishing.com/bjog

Association of phthalate esters with endometriosis in Indian women

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- 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 endometrisis was strong and statistically significant (Pvalue <0.05)
- Conclusion: Pes have an aetiological association with endometriosis

Original Article

Evaluation of the Phthalate Esters in South Indian Women with Endometriosis

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Background: To evaluate the possible association between phthalate esters (PEs) and the occurrence of endometriosis. Blood samples were collected from 99 infertile women with endometriosis (study group); 135 age-matched women without endometriosis (control group) but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility and pelvic inflammatory diseases diagnosed by laparoscopy with no evidence of endometriosis or other gynecological disorders during laparoscopic sterilization.

Materials and Methods: This is a prospective case-control study, which recruited women undergoing infertility treatment at three collaborating centers (BMMHRC: Bhagwan Mahavir Medical Hospital and Research Centre, MHRT: Maternal Health and Research Trust, and Owaisi Hospital and Research Center) of Reproductive Medicine Hyderabad, which receives cases from all over the region of Andhra Pradesh, India. The concentrations of Phthalate Esters were measured by using the High Performance Liquid Chromatography (HPLC).
Evaluation of Phthalate Esters concentrations in women with endometriosis compared with women who are free from the disease.

Results: Women with endometriosis showed significantly higher concentrations of Phthalate esters (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Di-n-butyl phthalate (DnBP), Butyl benzyl phthalate (BBP) and Bis (2-ethylhexyl) phthalate (BEHP)) compared with control group. We found that (38%) of the cases with endometriosis and (21%) of the control group. The correlation between the concentrations of Phthalate esters and different severity of endometriosis was strong and statistically significant at $p<0.05$ for all five compounds (DMP): $r=+0.57$, $p<0.0001$; DnBP $r=+0.39$, $p<0.0001$; BBP: $r=+0.89$, $p<0.0001$; DnOP: $r=+0.66$, $p<0.0001$ and BEHP: $r=+0.33$, $p<0.0014$.

Conclusion: This study for the first time from Indian subcontinent demonstrates that possibly Phthalate Esters might have a role in etiology of endometriosis.

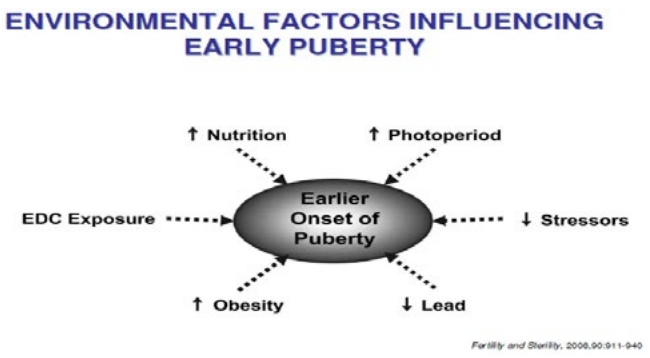
Uterine Fibroids

- 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.

Reproductive Toxicology 2005;20:81-4

Pubertal Development



Premature Thelarche

- Environmental exposures- pesticides, flame retardants
- Case Study: In Island of Pierto Rico Linked to consumption of Soy Based product and meat product

  Environmental Health perspective 2000
- High levels of Pthalates found in 68% of women with early thelarche
- Pthalates 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 etal 1996)*

- Increased incidence of low and declining semen quality in several regions of world *(Carlsen etal 1992, Auger et al 1995, Swan etal 1997, Andersen etal,2000)*
- Increasing incidence of cryptorchidism and hypospadias *(Campbell etal, 1987; Paulozzi etal 1997)*
- Increasing incidence of testicular cancer *(Adami etal 1994, Moller 1998)*

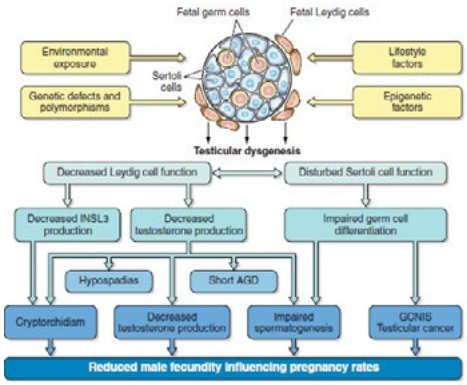


FIGURE 3. The hypothesis of testicular dysgenesis syndrome (TDS) and signs that might be linked to it: poor spermatogenesis, testicular cancer, hypospadias, cryptorchidism, and short ano-genital distance (AGD). The single symptoms and combinations thereof are risk factors for reduced fecundity. [Updated from Skakkebaek et al. (387).]

TABLE 1

Potential new endpoints of TDS in adult men.

Endpoint[a]	Evidence[a]
Compensated Leydig cell function/failure	Men with low sperm counts/infertility.
	Secular decline in adult male testosterone levels.
Reduced AGD	Evidence from rodent TDS/antiandrogen effects.
Reduced prostate volume[b]	Evidence from rodent TDS/antiandrogen effects.
	TGCC and prostate cancer incidence are inversely related.
Reduced seminal vesicle volume[b]	Evidence from rodent TDS/antiandrogen effects.
Altered "masculine behaviors"	Known role of fetal androgens in brain masculinisztion.

[a] See text for references.
[b] Such reductions might be associated with a reduction in ejaculate volume.

Sharpe. Testicular dysgenesis syndrome. Fertil Steril 2006.

- 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 etal 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 eg: 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 aetilogy of TDS

- ENVIRONMENTAL ANTIANDROGENS AS ENDOCRINE DISRUPTERS has adverse effect on male reproductive health (TOPPARI etal 1997)
- Epidemiological studies reported an increased risk of genital malformatios 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 etal 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 procymidone
2. Pthalates- 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 was associated with increased risk of cryptorchidism where as placenta levels were not *(Environ Health Perspec 115:1519-26,2007)*
- Dioxin levels in brest 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)*

Case–Control Study of Maternal Residential Atrazine Exposure and Male Genital Malformations

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Manuscript Received: 31 August 2012; Manuscript Accepted: 15 November 2012

In summary, we report on modest, but consistent, inverted U-shaped associations between estimated maternal residential exposure to atrazine and several genital malformations in male offspring. Our results add to a growing body of literature suggesting teratogenic effects of atrazine on the developing male reproductive system and on other systems of the body. However, the direction of the observed associations (i.e., inverted U-shaped) may suggest that potential teratogenic effects on male genitalia occur via a different mechanism than other types of birth defects (i.e., those with a suspected monotonic relationship with atrazine). Further research is needed to confirm our findings and to better understand the mechanisms involved.

Hypospadias

- Penile congenital malformation , in which urethra opens somewhere on the ventral side of penis instead of tip.
- Penile development regulated by dihydrotesterone that is typically produced from testesterone 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 testerone to dihydrotesterone or androgen receptor *(J Clin Endocrinol Metab 83:675-681,1998)*
- Genetic degects: androgen receptor , HOX A, HOX D, FGF 8, FGF RECEPTOR 2, *(J Med Genet 40:e492003, Net Genet 46:957-963,2014)*

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 asses 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)

BMJ 1992 Sep 12; 305(6854): 609–613 PMCID: PMC1883354
PMID: 13930172

Evidence for decreasing quality of semen during past 50 years.

E. Carlsen, A. Giwercman, N. Keiding, and N. E. Skakkebaek

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 emphasised by a concomitant increase in the incidence of genitourinary abnormalities such as testicular cancer and possibly also cryptorchidism and hypospadias, suggesting a growing impact of factors with serious effects on male gonadal function.

Environ Health Perspect. 2000 Oct; 108(10): 961–966. PMCID: PMC1240129
doi: 10.1289/ehp.00108961 PMID: 11049816
Research Article

The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996.

S.H.Swan, E.P.Elkin, and L.Fenster

FERTILITY AND STERILITY®
VOL. 78, NO. 6, DECEMBER 2002

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MALE FACTOR

Role of environmental estrogens in the deterioration of male factor fertility

Roya Rozati, M.D.,[a] P. P. Reddy, Ph.D.,[b] P. Reddanna, Ph.D.,[c] and Rubina Mujtaba, Ph.D.[a]

Design: Randomized controlled study.

Setting: Tertiary care referral infertility clinic and academic research center.

Patient(s): Twenty-one infertile men with sperm counts <20 million/mL and/or rapid progressive motility <25% and/or <30% normal forms without evidence of an obvious etiology and 32 control men with normal semen analyses and evidence of conception.

Intervention(s): Semen and blood samples were obtained as part of the treatment protocol.

Main Outcome Measure(s): Evaluation of semen parameters such as ejaculate volume, sperm count, motility, morphology, vitality, osmoregulatory capacity, sperm chromatin stability, and sperm nuclear DNA integrity.

Result(s): PCBs were detected in the seminal plasma of infertile men but not in controls, and the concentration of PEs was significantly higher in infertile men compared with controls. Ejaculate volume, sperm count, progressive motility, normal morphology, and fertilizing capacity were significantly lower in infertile men compared with controls. The highest average PCB and PE concentrations were found in urban fish eaters, followed by rural fish eaters, urban vegetarians, and rural vegetarians. The total motile sperm counts in infertile men were inversely proportional to their xenoestrogen concentrations and were significantly lower than those in the respective controls.

Conclusion(s): PCBs and PEs may be instrumental in the deterioration of semen quality in infertile men without an obvious etiology. (Fertil Steril® 2002;78:1187–94. ©2002 by American Society for Reproductive Medicine.)

J Nutr. 2016 May;146(5):1084-92. doi: 10.3945/jn.115.226563. Epub 2016 Apr 13.

Intake of Fruits and Vegetables with Low-to-Moderate Pesticide Residues Is Positively Associated with Semen-Quality Parameters among Young Healthy Men.

Chiu YH[1], Gaskins AJ[2], Williams PL[3], Mendiola J[4], Jørgensen N[5], Levine H[6], Hauser R[7], Swan SH[8], Chavarro JE[9].

RESULTS: The total intake of fruit and vegetables was unrelated to semen quality. However, the intake of fruit and vegetables with low-to-moderate pesticide residues was associated with a higher total sperm count and sperm concentration, whereas the intake of fruit and vegetables with high pesticide residues was unrelated to semen quality. On average, men in the highest quartile of low-to-moderate-pesticide fruit and vegetable intake (≥2.8 servings/d) had a 169% (95% CI: 45%, 400%) higher total sperm count and a 173% (95% CI: 57%, 375%) higher sperm concentration than did men in the lowest quartile (<1.1 servings/d; P-trend = 0.003 and 0.0005, respectively). The intake of fruit and vegetables, regardless of pesticide-residue status, was not associated with reproductive hormone concentrations.

CONCLUSIONS: The consumption of fruit and vegetables with low-to-moderate pesticide residues was positively related to sperm counts in young men unselected by fertility status. This suggests that pesticide residues may modify the beneficial effects of fruit and vegetable intake on semen quality.

Miscarraige

- 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, trichlro2,2 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)
- Laithi 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 etal* OR 1.6

- Pthalates 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

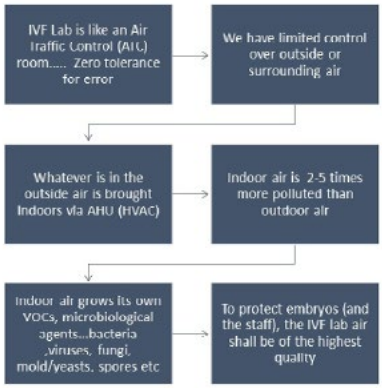
- No acknowledgement of air-borne problems
- Reluctance in acquiring knowledge and education (lack of it) about air purification process
- Some impurities can be SEEN, SMELT, but most you can not see or smell (If it is not visible – must not be a problem)
- Resistance to change or introduce new things (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 .

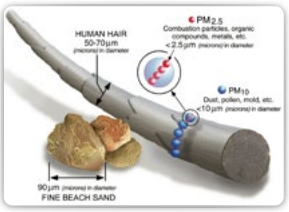
The success of the laboratory is dependent upon the embryologists' skill set, the media and ambient air

(William Schoolcraft, ESHRE 2010)



Where do the particles come From in the lab?

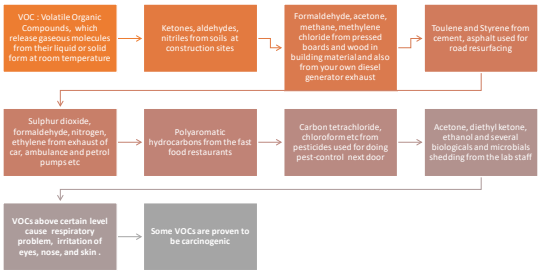
- From outside : the air leakages, openings and through inefficient filters
- Particulates and microorganisms come from People and Processes in the lab
- We (human) shed about 40,000 skin cells (35 Micron each) every minute
- Outside air contains 10-100 CFUs, while inside air has 100-1,000 CFUs per cubic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

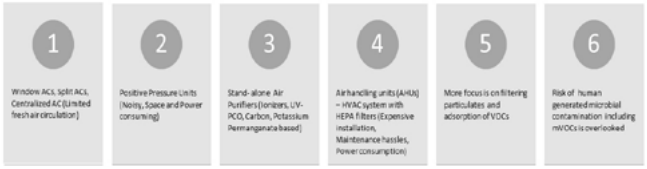
VOCs…. The curse of the Human kind!



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



THE goals of Air-purification

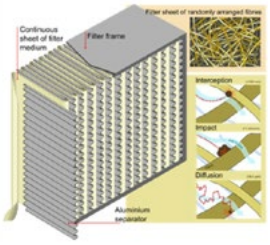
- Filtration of particulates and pollutants entering in to the lab and incubators
- Decomposition of VOCs in to inert air molecules (e.g. CO2 and H2O)
- Deactivation of DNA/RNA of the opportunistic micro organisms
- Create adequate Clean Air Delivery Rate (CADR) and Air Changes (ACH)
- Air purification 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	DEACTIVATE
HEPA/ULPA/CARBON Filtration	UV (UV+PCO)
Electrostatic Precipitation (ESP)	Pulsed Xenon
Ionizers (sometimes wrongly labeled as plasma)	Chemicals (Hydrogen Peroxide, Ozone etc)
Hybrid of all above	Cold PLASMA Air Sterilization

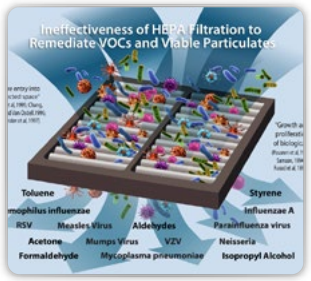
Filtration (Air Handling Units)

- HEPA filter
  - Filters particles > 0.3 microns
- ULPA filters
  - particles > 0.1 microns
- Workstations
- ?? Viruses (so small can't be filtered)
- Microbes accumulate in the filter - they are not killed (Colonization of filters)
- Filter performance is affected by humidity
- VOCs can't be filtered (VOCs are 100-1000 times smaller than the pore size of HEPA)
- High cost of maintenance and electricity (60-80% of life-cycle cost of filter is energy consumption)



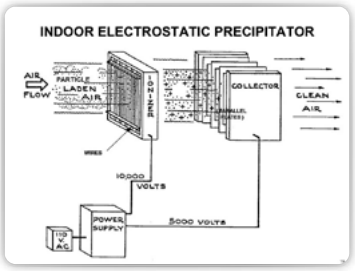
Air Handling Unit (HVAC) Can Breed

- 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)

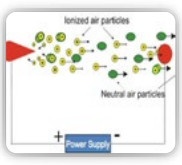
- Series of parallel alternating charged and grounded plates, which collect particles
- ESP usually preceded by ionisers
- The electric charge can be neutralised by high humidity, heat ,ionising radiation and solvents like paints making it less effective



Activated Carbon Filters

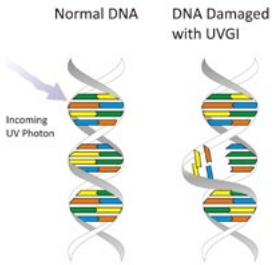
- Many gaseous contaminants (e.g. VOCs) will adsorb (adhere) to tiny internal pores of activated charcoal and be removed from the air
- Performance depends on the surface area and the air pressure being applied to these filters
- When the surface is covered, the adsorption stops, usually without warning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



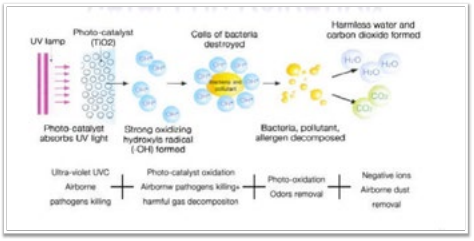
UV Irradiation (G-UV)

- 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

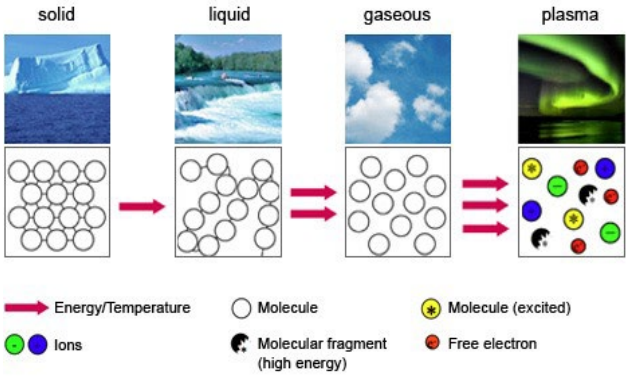
The Long-lasting effective solution is here!defend 1050 : PLASMA BASED complete air sterilizer

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 to into their constituent components
- e.g (Formaldehyde): CH2O -> CO2 + H2O
- The clean air would then passes through a 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)

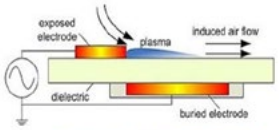
'This device brings the room to the plasma sterilizer'

What is Plasma Technology ?



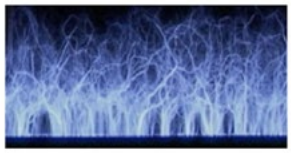
- Electrons split from the atoms.
- Ions.
- Free Radicals

Plasma Air Sterilization



Dielectric Barrier Discharge Plasma

Plasma Coil contains plasma within 2 cms



Plasma generated under surface of coil

Plasma physics to Plasma Biology

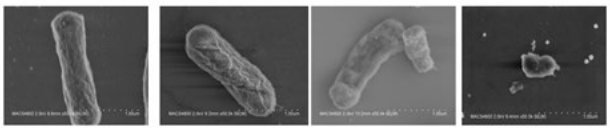
Sterilization of contaminated matter with an atmospheric pressure plasma

M. Laroussi
Dept. of Electr. & Comput. Eng., Tennessee Univ., Knoxville, TN, USA

- 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 in to CO2 and H2O

Novaerus DBD Plasma

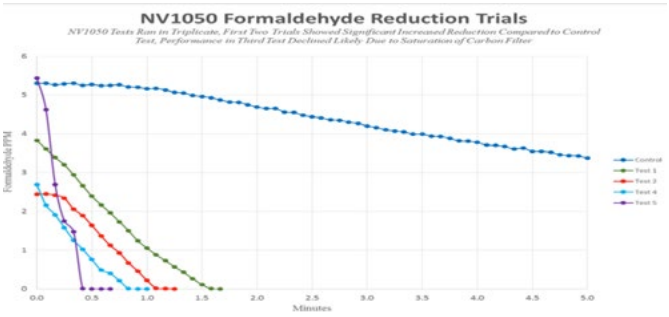
What happens to The Microorganism when subjected to different sterilization techniques?



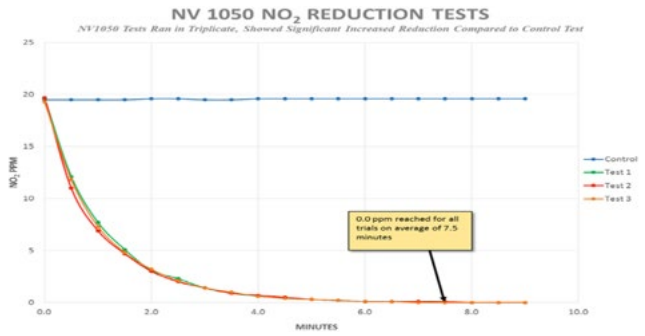
Healthy E.Coli before sterilzation	Heat Sterilization - 160°C for 2 hours	Exposed to Ozone 350000 ppb for 2 hrs	Exposed to Novaerus DBD Plasma: less than 2 Microseconds

- 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

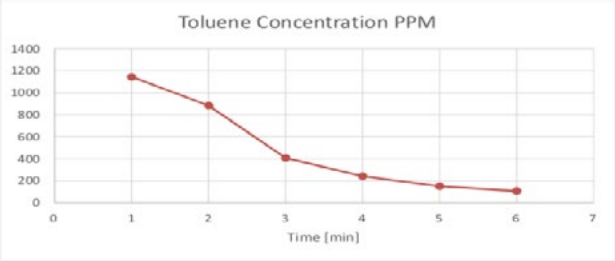


Figure 25, Toluene reduction when air cleaner is activated.

Clean Air Delivery Rate

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

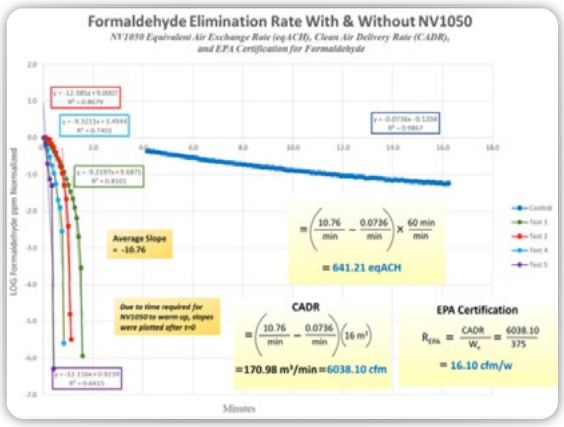
"The CADR is a measure of a portable air cleaner's delivery of contaminant-free air, expressed in cubic feet per minute. For example, if an air cleaner has a CADR of 250 for dust particles, it may reduce dust particle levels to the same concentration as would be achieved by adding 250 cubic feet of clean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

Methodology for measuring CADR for any contaminant

- With air cleaner off and missing 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 the slopes) x (room volume)

- Methodology by Stephen N. Rudnick, MS, ScD, CIH 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: 50 m³/hour
- Covers up to 20 m²
- Portable, 3.4 Kg
- 11 watts
- Virtually no maintenance
- One Plasma Coil
- Single speed fan
- Not for IVF lab use (personal unit)

NV 800

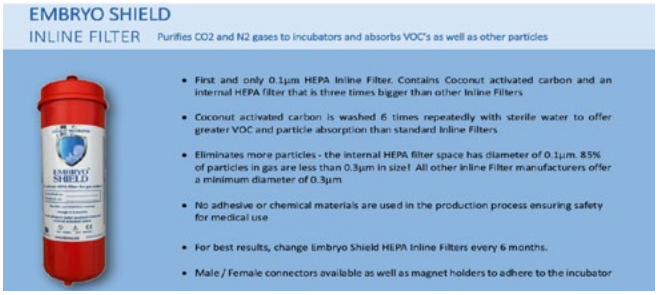
- 260 m³/hour
- Covers up to 60 m²
- Wall or stand mounted
- 4.5 kg
- 25 Watts
- Two Plasma Coils
- Two speed Fan
- Cleaning of pre-filter

Defend 1050

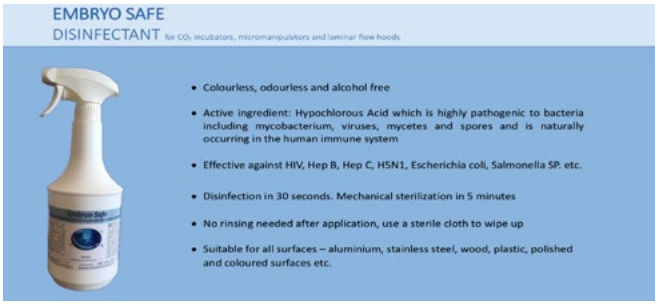
- 1000 m³/hour
- 3 Filters – Carbon, HEPA, Pre-Screen
- Portable on castor wheels
- 125-385 Watts
- Six Plasma Coils
- 5 speed adjustable fan

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)

(A systematic review by Julie Carré, Nicolas Gatimel, Jessika Moreau1, Jean Parinaud and Roger Léandri published in 'Biomed Central-Environmental Health' Open Access Journal in 2017)

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

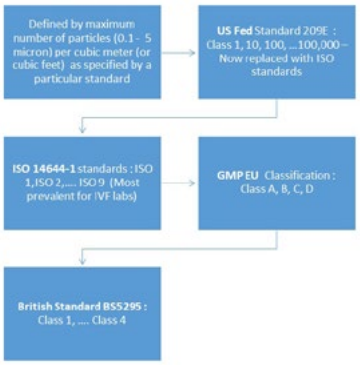
Summary of research by Sandro C Esteves, and Fabiola C Bento published in Asian Journal of Andrology, November 2015 and referred in Cairo Consensus document 2018

Table 1. Analyzed studies, overview

Reference	Year	Place	Design	Air filters	Increase of IVF success	Clinical pregnancy rates
Boone et al.[5]	1999	USA	Retrospective cohort	HEPA + VOC active carbon + $KMnO_4$	Yes	16% to 59%
Gomes et al.[6]	2003	Brazil	Prospective qualitative	HEPA + VOC active carbon	Yes	Class 1000: 32.7% Class 100: 43.1%
Jindal et al.[7]	2008	USA	Retrospective cohort	HEPA + VOC + KMnO4	Yes	46.8% vs 32.9%
Dickey et al.[8]	2010	USA	Retrospective	HEPA + VOC active carbon	Yes	63.4% vs 46.4%
Khoudja et al.[9]	2013	China	Prospective cohort	HEPA + VOC active carbon	Yes	40.6% to 54.6%
Forman et al.[10]	2014	USA	Prospective Descriptive	HEPA + VOC active carbon + KMnO4	Yes	Increase
Munch et al.[11]	2015	USA	Retrospective cohort	Active carbon	No	Unchanged

B.N. Chakraborty 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 outcome"

Air Quality / Clean room Standards



Air quality / Cleanroom Standards at a glance

(Maximum particles per cubic meter – At rest)

US FED	ISO	GMP EU	0.3 uM	0.5 uM	1 uM	5 uM	
	ISO 1		1.02	0.35	0.083	0.0029	
	ISO 2		10	3.5	0.83	0.029	
Class 1	ISO 3		102	35	8.3	0.29	
Class 10	ISO 4		1,020	352	83	2.9	
Class 100	ISO 5	Class A	10,200	3,520	832	29	IVF LAB
Class 1,000	ISO 6	Class B	1,02,000	35,200	8,320	293	IVF LAB
Class 10,000	ISO 7	Class C	10,20,000	3,52,000	83,200	2,930	IVF-OT
Class 1,00,000	ISO 8	Class D	1,02,00,000	35,20,000	8,32,000	29,300	ET Room
Room Air	ISO 9	Room Air	10,20,00,000	3,52,00,000	83,20,000	2,93,000	Room Air

Region (directive)	European Union (EU directive 2004/23/EC; 2006/86/EC)	Brazil (Anvisa RDC33/2006; RDC23/2011)
Particle filtration	Equivalent to GMP Grade A air quality in the critical areas with a background environment at least equivalent to Grade D (exceptions apply)	At least equivalent to ISO class 5 (NBR/ISO 14644–1) in the critical areas
Microbial contamination	Microbial colony counts equivalent to those of Grade A as defined in the current GMC guide with a background environment at least equivalent to Grade D	Microbiological monitoring required; specifications not defined
Volatile organic compounds filtration	Not required	Ventilation systems should be equipped with filters imbedded with activated charcoal

Reviewed by Esteves & Bento, RBM Online 2013.

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 than 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/m3 and less than two spores/ m3 'at rest'
- VOCs. Total VOCs less than 500 ug/m3 (~400–800 ppb total VOC, depending on molecular species); less than 5 ug/m3 aldehydes (1ug/m3 = 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%

Outdoor air pollution & human infertility

Products used in Home and work	Possible VOCs
Petroleum distillates: Paint thinner, oil-based paints, liquid insect pest products, furniture polishes	BTEX (benzene, toluene, ethylbenzene), hexane, cyclohexane, 1,2,4-trimeyhylbenzene
Personal care products: nail polish & remover, colognes, hair spray	Acetone, ethyl alcohol, isopropyl alcohol, methacrylates, ethyl acetate
Fabric cleaners: Dry cleaned clothes, stain removers, leather cleaners	Tetrachloroethene, perchlorcethene, trichloroethene
Adhesives: PVC cement, contact cement, model cement	Tetrahydrofuran, cyclohexane, methyl ethyl ketone, toluene, acetone, hexane, 1,1,1-trichloroethane
Refrigerant: air conditioners, freezers, dehumidifiers	Freons (trichlorofluoromethane, dichlorodimethane)
Air fresheners: Moth balls, deodorizers,	1,4-dichlorobenzene, naphthalene
Furniture: Upholstered furniture, carpets,plywood, pressed wood products	Formaldehyde

Table 3 – Odour thresholds of organic contaminants typically found in assisted reproduction technology laboratories ( ).

Organic compound	Geometric mean AIHA	Comment
Ethanol (ethyl alcohol)	18–100 ppm	Most common VOC in ART laboratories.
Isopropyl alcohol (2-propanol)	19–43 ppm	Second most commonly found VOC.
Acetone (2-propanone)	62–130 ppm	
Propene (propylene)	23–68 ppm	Plastic.
Hexamethylcyclotrisiloxane	No data	Silicone from gaskets.
Acetonitrile (methyl cyanide)	1160 ppm	Plastics.
Formaldehyde	0.03–9970 ppm	
Acetaldehyde	0.067 ppm	
d-Limonene	0.5 ppm	Scent of lemon.
α-Pinene	0.005 ppm	Scent of pine.

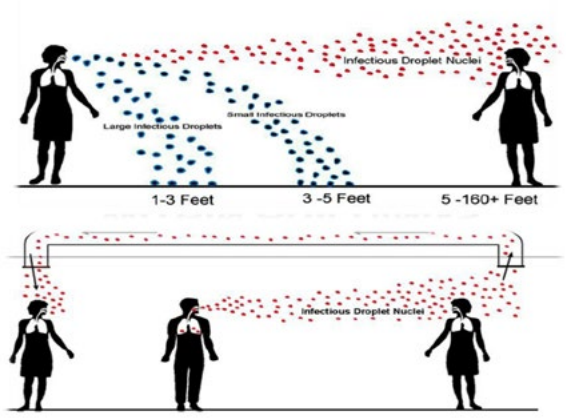
AIHA, American Industrial Hygiene Association, ART, assisted reproduction technology; VOC, volatile organic compound.

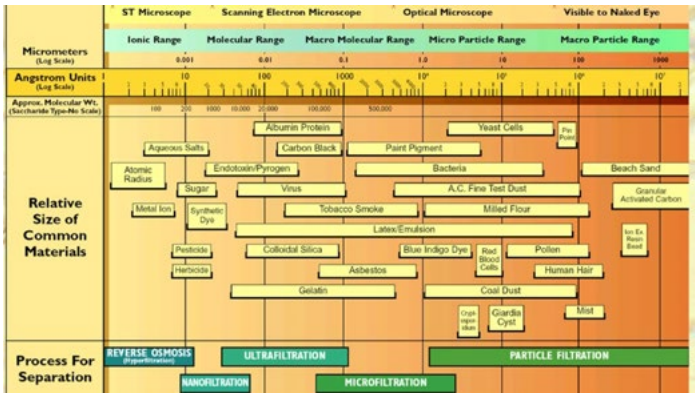
Air Quality / Clean room Standards

Table 4 – Aldehyde levels measured in four IVF laboratories that were experiencing significant decreases in outcomes (data from Alpha Environmental Inc., Emerson, NJ, USA).

Laboratory Number	Material	Concentration (μg/m³)		Observations
		IVF laboratory	Incubator	
I	Formaldehyde	29.0	N/A	Human embryos: unable to reach blastocyst stage.
	Acetaldehyde	13.0		
II	Formaldehyde	N/A	140	MEA: poor result
			81	Human embryos: unable to reach four-cell and blastocyst stages.
	Acetaldehyde		61	
	Hexaldehyde			
III	Formaldehyde	25.0	2.8	MEA: unable to pass
	Acetaldehyde	17.0	12.0	Human embryos: 'very
	Hexaldehyde	8.2	4.4	limited clinical success'
IV	Formaldehyde	21.0		Human embryos: unable to reach blastocyst stage; no pregnancies.
	Butyraldehyde	23.0		
	Benzaldehyde	8.8		
	Total with all aldehydes detected	74.1		

Air samples were taken at locations selected by the client and Alpha Environmental; aldehyde concentrations (generally ranging between 20 and 282 μg/m³) were determined using a US Environmental Protection Agency (EPA) TO-11a method used by the EPA and the US Occupational Safety and Health Administration (OSHA); see www3.epa.gov/ttn/amtic/airtox.html.
MEA, mouse embryo assay.




ST Microscope
Scanning Electron Microscope
Optical Microscope
Visible to Naked Eye
Micrometers
Ionic Range
Molecular Range
Macro Molecular Range
Micro Particle Range
Macro Particle Range
Angstrom Units
Approx. Molecular Wt.
Relative Size of Common Materials
Albumin Protein
Yeast Cells
Aqueous Salts
Carbon Black
Paint Pigment
Atomic Radius
Endotoxin/Pyrogen
Bacteria
Beach Sand
Sugar
Virus
A.C. Fine Test Dust
Granular Activated Carbon
Metal Ion
Synthetic Dye
Tobacco Smoke
Milled Flour
Latex/Emulsion
Pesticide
Colloidal Silica
Blue Indigo Dye
Red Blood Cells
Pollen
Herbicide
Asbestos
Human Hair
Gelatin
Coal Dust
Giardia Cyst
Mist
Process For Separation
REVERSE OSMOSIS
ULTRAFILTRATION
PARTICLE FILTRATION
NANOFILTRATION
MICROFILTRATION

THANK YOU

5. Optimizing the culture environment in the IVF LAb

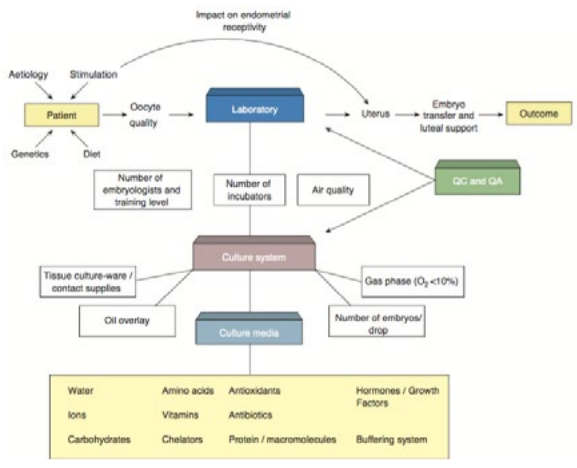
Wikipedia

- Embryo culture is a component of in vitro fertilisation where in resultant embryos are allowed to grow for some time in an artificial medium .

Embryo Culture System

- 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.

Embryo Culture System



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

Concentrations of essential media components from commercial embryo culture media as determined via various analytical methods.

			Vitrolife		Sage		Cook		In vitro care		Origio	
Variable	IVFOnline global	Irvine CSC	G1	G2	QACM	QABM	SICM	SIBM	IVC1	IVC3	ISM1	BA
Glucose (mM)	0.2	0.5	0.5	3.4	0.1	2.8	0.3	3.1	0	2.7	1.0	1.0
Citrate (mM)	0	0.01	0.08	0.08	0	0.16	0	0	0	0.16	0.02	0.003
Lactate (mM)	4.8	5.6	10.8	6.0	3.9	3.9	1.8	1.8	10.1	9.4	3.2	2.4
Pyruvate (mM)	0.20	0.17	0.30	0.07	0.52	0.07	0.36	0.31	0.08	0.09	2.0	0.17
L:P ratio	24	33	36	86	7.5	56	5.0	5.9	126	105	18.5	1.2
Amino acid concentrations (μM)												
Essential												
Arg	328	281	0	360	0	313	25	252	0	590	138	124
Cys	52	46	0	54	0	54	2	32	0	9	42	38
His	111	105	0	121	0	102	8	86	0	188	99	54
Ile	221	202	0	249	0	209	17	169	0	388	147	208
Leu	230	214	0	265	0	227	18	182	0	408	158	217
Lys	232	223	0	260	0	223	18	174	0	417	148	179
Met	51	53	0	63	0	56	4	43	0	100	89	54
Phe	112	106	0	125	0	106	8	86	0	200	90	104
Thr	216	195	0	242	0	210	18	172	0	374	81	211
Trp	28	26	0	30	0	28	2	22	0	51	100	21
Tyr	100	95	0	114	0	100	12	114	0	186	70	91
Val	233	215	0	256	0	224	17	179	0	428	356	225
Nonessential												
Ala	65	62	148	151	0	0	135	135	0	136	338	124
Asn	52	57	126	129	112	124	88	84	0	113	73	104
Asp	55	47	0	0	93	104	81	85	0	95	6	578
Glu	54	46	0	0	0	0	90	87	0	103	1	102
Gln	0	0	0	0	0	0	30	26	0	0	778	0
Gly	63	58	135	141	119	131	6647	4815	0	121	1760	701
Pro	51	48	112	113	93	103	85	80	0	99	82	96
Ser	58	55	127	130	107	123	92	89	0	109	96	113
Tau	0	0	131	0	122	120	6489	6380	0	0	296	0

Note: QACM = Quinn's Advantage Cleavage media; QABM = Quinn's Advantage Blastocyst media; SICM = Sydney IVF Cleavage media; SIBM = Sydney IVF Blastocyst media; BA = Blastassist. (Adapted from Morbeck et al. [27]).

Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

Composition

Electrolytes and metals in embryo culture media.

	IVFOnline	Irvine	Vitrolife		Sage		Cook		In Vitro Care		Origio	
Variable	Global	CSC	G1	G2	QACM	QABM	SICM	SIBM	IVC1	IVC3	ISM1	BA
Calcium (mM)	1.9	1.9	1.1	1.1	2.2	2.2	1.1	1.1	2.1	1.9	1.8	1.4
Phosphorus (mM)	0.4	0.2	0.3	0.3	0	0.3	0.2	0.4	0	0.3	0.3	1.1
Potassium (mM)	2.9	2.8	5.8	5.8	4.7	4.9	4.8	5.0	4.6	4.9	8.4	5.3
Chloride (mM)	112	112	124	127	118	113	122	121	107	108	116	114
Sodium (mM)	137	135	152	149	132	132	140	136	142	143	131	144
Magnesium (mM)	0.2	0.8	1.7	1.7	1.8	1.8	1.5	1.5	0.2	0.2	0.9	0.8
Copper (mg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Iron (µg/L)	0	4	0	3	1	2	11	9	5	5	9	57
Selenium (µg/L)	0	0	0	0	0	0	3	3	0	0	4	4
Zinc (mg/L)	0	0	0	0	0	0	0	0	0	0	0	0
Aluminum (µg/L)	1	0	1	1	18	21	1	1	2	6	5	16
Chromium (µg/L)	0.4	0.7	0.1	0.2	0.2	0.2	0.3	0.3	0.7	0.9	0.2	1.2
Cobalt (µg/L)	0.1	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.5	0.6
Manganese (µg/L)	0.4	0.8	0.5	0.6	3.6	3.6	0.5	0.5	0.6	0.6	1.4	0.8

Note: Abbreviations as in Table 1.

Morbeck. Composition of embryo culture media. Fertil Steril 2014.

Amino Acid

- 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 acids34 to maintain an endogenous pool.35 Amino acids are readily taken up and metabolized by the embryo.

Amino Acid

- 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.

Amino Acid

- 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.

Concentrations of essential media components from commercial embryo culture media as determined via various analytical methods.

			Vitrolife		Sage		Cook		In vitro care		Origio	
Variable	IVFOnline global	Irvine CSC	G1	G2	QACM	QABM	SICM	SIBM	IVC1	IVC3	ISM1	BA
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Citrate (mM)	0	0.01	0.08	0.08	0	0.16	0	0	0	0.16	0.02	0.003
Lactate (mM)	4.8	5.6	10.8	6.0	3.9	3.9	1.8	1.8	10.1	9.4	3.2	2.4
Pyruvate (mM)	0.20	0.17	0.30	0.07	0.52	0.07	0.36	0.31	0.08	0.09	2.0	0.17
L:P ratio	24	33	36	86	7.5	56	5.0	5.9	126	105	18.5	1.2
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Leu	230	214	0	265	0	227	18	182	0	408	158	217
Lys	232	223	0	260	0	223	18	174	0	417	148	179
Met	51	53	0	63	0	56	4	43	0	100	89	54
Phe	112	106	0	125	0	106	8	86	0	200	90	104
Thr	216	195	0	242	0	210	18	172	0	374	81	211
Trp	28	26	0	30	0	28	2	22	0	51	100	21
Tyr	100	95	0	114	0	100	12	114	0	186	70	91
Val	233	215	0	256	0	224	17	179	0	428	356	225
Nonessential												
Ala	65	62	148	151	0	0	135	135	0	136	338	124
Asn	52	57	126	129	112	124	88	84	0	113	73	104
Asp	55	47	0	0	93	104	81	85	0	95	6	578
Glu	54	46	0	0	0	0	90	87	0	103	1	102
Gln	0	0	0	0	0	0	30	26	0	0	778	0
Gly	63	58	135	141	119	131	6647	4815	0	121	1760	701
Pro	51	48	112	113	93	103	85	80	0	99	82	96
Ser	58	55	127	130	107	123	92	89	0	109	96	113
Tau	0	0	131	0	122	120	6489	6380	0	0	296	0

Note: QACM = Quinn's Advantage Cleavage media; QABM = Quinn's Advantage Blastocyst media; SICM = Sydney IVF Cleavage media; SIBM = Sydney IVF Blastocyst media; BA = Blastassist. (Adapted from Morbeck et al. [27]).

Swain, Optimizing IVF laboratory conditions. Fertil Steril 2016.

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 37oC
- 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, pHi regulation, and gene expression.

Human Reproduction, Vol.31, No.6 pp. 1192–1199, 2016
Advanced Access publication on April 6, 2016 doi:10.1093/humrep/dew059

human reproduction ORIGINAL ARTICLE *Embryology*

Ammonium accumulation in commercially available embryo culture media and protein supplements during storage at 2–8°C and during incubation at 37°C

Sander H.M. Kleijkers[1], Aafke P.A. van Montfoort[1], Otto Bekers[2], Edith Coonen[1], Josien G. Derhaag[1], Johannes L.H. Evers[1], and John C.M. Dumoulin[1,*]

MAIN RESULTS AND THE ROLE OF CHANCE: All ready-to-use, i.e. protein supplemented, culture media showed ammonium accumulation during storage for 6 weeks (ranging from 9.2 to 99.8 µM) and during incubation for 4 days (ranging from 8.4 to 138.6 µM), resulting in levels that might affect embryo development. The protein supplements also showed ammonium accumulation, while the culture media without protein supplementation did not. The main sources of ammonium buildup in ready-to-use culture media were unstable glutamine and the protein supplements. No additional ammonium buildup was found during incubation when using an oil overlay or with the presence of an embryo in the culture droplet.

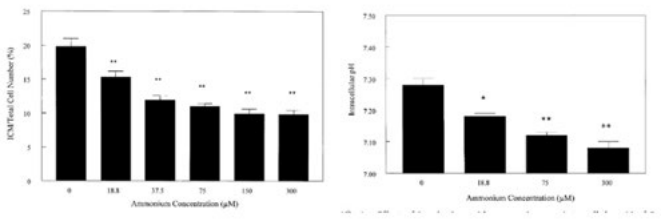
LIMITATIONS, REASONS FOR CAUTION: In addition to the unstable glutamine and the protein supplements, other free amino acids might contribute to the ammonium buildup. We did not investigate the deterioration of other components in the media.

WIDER IMPLICATIONS OF THE FINDINGS: Break-down of components into ammonium is more pronounced during incubation at 37°C, however, it is not negligible during storage at 2–8°C. This results in increasing ammonium levels in culture media over time that may affect embryo development. Therefore, it is important that the use of free L-glutamine in human embryo culture media is stopped and that

Ammonium Induces Aberrant Blastocyst Differentiation, Metabolism, pH Regulation, Gene Expression and Subsequently Alters Fetal Development in the Mouse

Michelle Lane ✉, David K. Gardner

Biology of Reproduction, Volume 69, Issue 4, 1 October 2003, Pages 1109–1117,
https://doi.org/10.1095/biolreprod.103.018093
Published: 28 May 2003 **Article history** ▾



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Published: 28 May 2003 **Article history**

TABLE 3. Effect of ammonium on the number of apoptotic cells in blastocysts.[a]

Ammonium concentration (μM)	Total cells (n)[b]	Apoptotic cells (n)[b]	Apoptotic cell index[b]
0	83.0 ± 6.6[c]	2.9 ± 0.4[c]	3.7 ± 0.5[c]
18.8	80.6 ± 5.8[c]	6.9 ± 0.9[d]	9.5 ± 1.7[d]
75	70.2 ± 7.4[d]	7.9 ± 1.3[d]	15.8 ± 3.9[e]
300	61.5 ± 5.2[d]	8.4 ± 5.2[d]	15.5 ± 2.7[e]

[a] Minimum of 20 blastocysts examined per treatment.
[b] Values are mean ± SEM.
[c–e] Different letters within a column indicate significant difference ($P < 0.05$).

TABLE 4. Effect of ammonium on blastocyst viability and fetal outcome.[a]

Ammonium concentration (μM)	Implantation rates (%)	Fetal development rates (%)	Fetal development/ implantation (%)	Fetal weights (mg)	Crown-rump length (mm)	Abnormal fetal development (% of fetuses)[b]
0	57.1[c]	40.5[c]	70.8[c]	272 ± 10	11.5 ± 0.2	0[c]
18.8	39.3[c]	32.1[c]	81.8[c]	279 ± 23	12.4 ± 0.3[d]	0[c]
75	41.7[c]	22.2[b,c]	53.3[d]	243 ± 20	10.7 ± 0.4[e]	37.5[d]
150	61.1[c]	27.8[d]	45.5[d]	229 ± 13	10.6 ± 0.2[e]	10.0[c]
300	33.3[c]	16.7[c]	50.0[d]	207 ± 20[f]	10.7 ± 0.2[e]	33.3[d]

[a] Forty-eight blastocysts transferred per treatment. Fetal weights and crown-rump length are mean ± SEM.
[b] Abnormal fetuses included those with significant underdevelopment, abnormal cranial development, and stunted limb growth.
[c–e] Different letters within a column are significantly different ($P < 0.05$).
[f] Significantly lower than control without ammonium ($P < 0.05$).
[g] Significantly higher than control without ammonium ($P < 0.05$).

Amino Acid

- 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 37oC. Therefore, media containing this stable form of glutamine do not produce significant levels of ammonium.

Carbohydrates

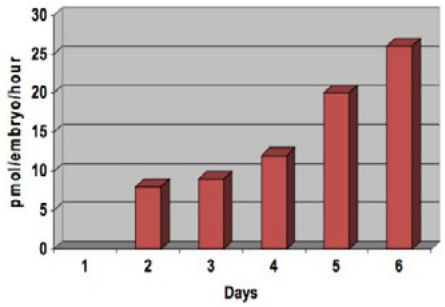
- Carbohydrates are present within the luminal fluids of the female reproductive tract. Their levels vary both between the oviduct and uterus and within the cycle
- The precise substrate requirements for the human embryo have yet to be fully elucidated. However, analysis of carbohydrate uptakes in vitro 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).

Carbohydrates

Utilisation of Glucose by human embryos



Schematically after Hardy et al., 1989

EDTA

- Ehtylenediaminetetraacetic 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)

Culture Protocol

Media

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

Pre-compaction	Post-compaction
Low biosynthetic activity	High biosynthetic activity
Low QO_2	High QO_2
Pyruvate preferred nutrient	Glucose preferred nutrient
Nonessential amino acids	Nonessential + essential amino acids
Maternal genome	Embryonic genome
Individual cells	Transporting epithelium
One cell type	Two distinct cell types: ICM and trophectoderm

QO_2, oxygen consumption; ICM, inner cell mass.

Sequential

Single

Single / Sequential

Single / 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

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

Single / Sequential

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

Component	Oviduct	Uterus
Glucose concentration	0.50mM	3.15mM
Pyruvate concentration	0.32mM	0.10mM
Lactate concentration	10.50mM	5.20mM
Oxygen concentration	8%	1.5%
Carbon dioxide concentration	12%	10%
pH	7.5	7.1
Glycine concentration	2.77	19.33
Alanine concentration	0.5	1.24
Serine concentration	0.32	0.80

Single / Sequential

Component	Mono Culture	Sequential Culture	
	KSOMaa mmol/L	G1 mmol/L	G2 mmol/L
Na pyruvate	0.2	0.32	0.1
Na lactate	10.0	10.5	5.87
Glucose	5.56	0.5	3.15

Single / Sequential

O - 056. **Single step versus sequential culture medium: effect on embryo developmente, genetic an clinical outcome**

RCT: 3652 embryos, couples undergoing ICSI

	Sequential	Single Step	P value
Blastocyst rate	43%	45%	NS
Aneuploidy rate	58,6%	61,8	NS
Ongoing implantation rate	46,4%	42,2%	NS

Single / Sequential

Human Reproduction Update, Vol.19, No.3 pp. 210–220, 2013
Advanced Access publication on February 5, 2013 doi:10.1093/humupd/dms061

human reproduction update

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

E. Mantikou[1], M.A.F.M. Youssef[1,2,3], M. van Wely[1], F. van der Veen[1], H.G. Al-Inany[2], S. Repping[1], and S. Mastenbroek[1,*]

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Submitted on February 28, 2012; resubmitted on December 11, 2012; accepted on December 17, 2012

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 treatment 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.

Factors

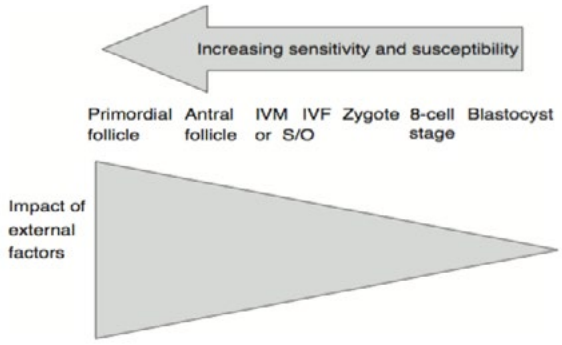


Fig 16.2 Sensitivity and susceptibility of germ cells and embryos to external factors. IVM, in vitro maturation; S/O, super ovulation.

pH

Maintenance of pH

Cell stage (human)	ipH
GV oocytes	7.04 + 0.07
MI oocytes	7.03 + 0.04
MII oocytes	6.98 + 0.02
2-8 cell	7.12 + 0.01
Morula- Blastocyst	ND

ipH could be affected by oxygen availability and substrates

Henderson–Hasselbach equation:

$$CO_2\,(gas) \leftrightarrow CO_2\,(dissolved) \leftrightarrow H_2CO_3 + H^+ + HCO_3$$

pH

- pH Media - 7.4, pH cell - 7.2
- Dependent on Bicarbobate, 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 4

Effects of reduced oxygen concentration in a predominantly blastocyst transfer program.

Endpoint	21% O_2 (%)	5% O_2 (%)
Clinical pregnancy	56/115 (48.7)[a]	74/115 (64.3)[b]
Implantation	95/267 (35.6)[b]	122/247 (49.4)[b]
Live birth	49/115 (42.6)[b]	66/115 (57.4)[b]

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 Meintjes et al. [72]).

[a,b] Different superscripts between columns represent a statistically significant difference, $P < .05$.

Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

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 com- pounds (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 leads to irreversible damage to microtubules

Temperature

TABLE 5

Impact of different culture temperature on human embryo development.

Temperature	Meta phase IIs	Fertility rate, %	Day 3 cell no.	Blast rate, %	Usable blast rate, %	Aneuploidy rate, %	Implantation rate, %
36°C	399	86.2	7.0 ± 0.1[a]	51.6[a]	41.2[a]	42.5	67.4
37°C	406	82.0	7.7 ± 0.1[b]	60.1[b]	48.4[b]	46.1	73.3

Note: A sibling embryo split design was used,and incubator type, temperature variation, and pH were accounted for as confounding variables. Data indicate that 37°C is superior to 36°C in terms of embryo development. (Adapted from Hong et al. [94]).
[a,b] Different superscripts within a column represent a statistically significant difference, $P < .05$.

Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

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

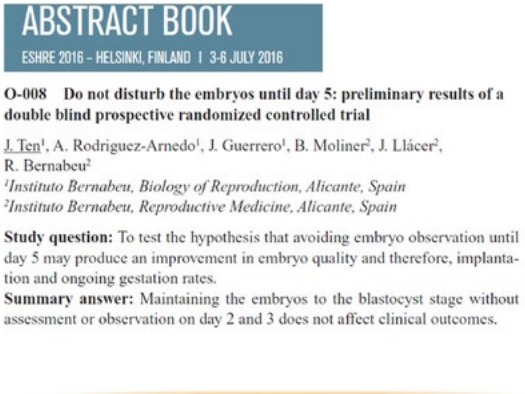
Embryo Density

- 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

ESHRE 2016 – HELSINKI, FINLAND I 3-6 JULY 2016

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

J. Ten[1], A. Rodriguez-Arnedo[1], J. Guerrero[1], B. Moliner[2], J. Llácer[2], R. Bernabeu[2]

[1]*Instituto Bernabeu, Biology of Reproduction, Alicante, Spain*

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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 6

Examples of dynamic human embryo culture aimed at replicating growth conditions embryos may experience in vivo.

Approach	Embryo source	Outcome measures	Outcome summary	Reference
Tilting	Frozen day 3 embryos	Blastocyst development High-quality blastocysts Cell no.	Equivalent blastocyst formation Improved cell no.	(151)
	Fresh embryos	Day 5 blastocyst development High-quality blastocysts Positive BhCG	Improved blastocyst development Increased pregnancy	(152)
Vibration	Fresh zygotes	Day 3 embryo quality Blastocyst development Pregnancy rate	Improved day 3 quality Increased blastocyst development Higher pregnancy rate	(153, 154)
	Fresh zygotes	Pregnancy from day 3 transfer Implantation rate Day 5 blastocyst development	Higher pregnancy rate Higher implantation rate Greater blastocyst formation	(155)
Pulsatile Flow	Fresh zygotes	Embryo fragmentation Cleavage-stage quality	Lower fragmentation on day 2 and day 3 Greater number of top-quality embryos	(156)

Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

Dynamic culture

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

- Playing music to an egg increased 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.

I used to walk along the beach,
a favorite thing to do.
Until the plastic and the trash completely spoiled my view.
The place I take my rod and reel to catch my favorite dish.
Has elevated mercury, so I can't eat the fish.

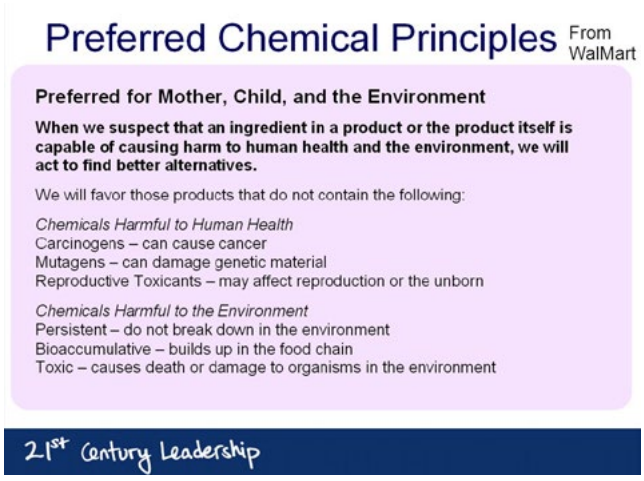
From Protect Environment by Mache Seibel

Global environment

- ACOG : The collection of studies presented here probably reflects alteration in the functionality of semen or ovarian follicles. There is, however, the potential for environmental agents to affect the systems that support pregnancy. For example, environmental estrogens may change the hormonal balance that allows sufficient endometrial growth, affects angiogenesis necessary to support a developing placenta, or causes/worsens endometriosis and tubal patency. These details are sure to be elucidated in the near future. Nevertheless, regional differences in fertility rates highlight the potential effect of the global environment on fertility

COMMITTEE OPINION

The **evidence** that links **exposure to toxic environmental agents** and **adverse reproductive and developmental health outcomes** is sufficiently **robust**, and the American College of Obstetricians and Gynecologists and the American Society for Reproductive Medicine join leading scientists and other clinical practitioners in calling **for timely action to identify and reduce exposure to toxic environmental agents** while addressing the consequences of such exposure.



Toxics & Reproduction

- 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.

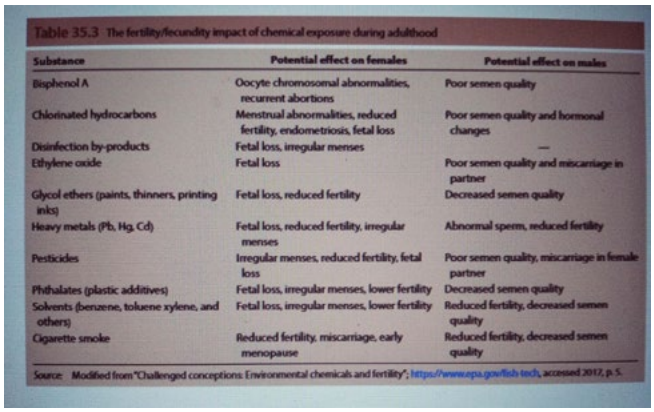
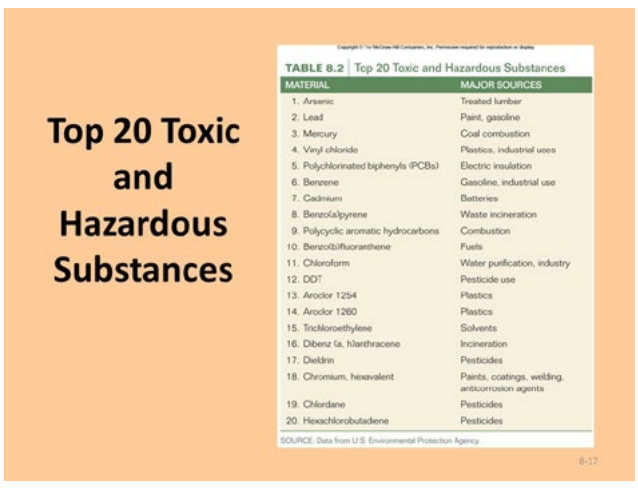


Table 35.3 The fertility/fecundity impact of chemical exposure during adulthood

Substance	Potential effect on females	Potential effect on males
Bisphenol A	Oocyte chromosomal abnormalities, recurrent abortions	Poor semen quality
Chlorinated hydrocarbons	Menstrual abnormalities, reduced fertility, endometriosis, fetal loss	Poor semen quality and hormonal changes
Disinfection by-products	Fetal loss, irregular menses	—
Ethylene oxide	Fetal loss	Poor semen quality and miscarriage in partner
Glycol ethers (paints, thinners, printing inks)	Fetal loss, reduced fertility	Decreased semen quality
Heavy metals (Pb, Hg, Cd)	Fetal loss, reduced fertility, irregular menses	Abnormal sperm, reduced fertility
Pesticides	Irregular menses, reduced fertility, fetal loss	Poor semen quality, miscarriage in female partner
Phthalates (plastic additives)	Fetal loss, irregular menses, lower fertility	Decreased semen quality
Solvents (benzene, toluene xylene, and others)	Fetal loss, irregular menses, lower fertility	Reduced fertility, decreased semen quality
Cigarette smoke	Reduced fertility, miscarriage, early menopause	Reduced fertility, decreased semen quality

Source: Modified from "Challenged conceptions: Environmental chemicals and fertility"; https://www.epa.gov/fish-tech, accessed 2017, p. 5.



Top 20 Toxic and Hazardous Substances

TABLE 8.2 Top 20 Toxic and Hazardous Substances

MATERIAL	MAJOR SOURCES
1. Arsenic	Treated lumber
2. Lead	Paint, gasoline
3. Mercury	Coal combustion
4. Vinyl chloride	Plastics, industriel uses
5. Polychlorinated biphenyls (PCBs)	Electric insulation
6. Benzene	Gasoline, industrial use
7. Cadmium	Batteries
8. Benzo(a)pyrene	Waste incineration
9. Polycyclic aromatic hydrocarbons	Combustion
10. Benzo(b)fluoranthene	Fuels
11. Chloroform	Water purification, industry
12. DDT	Pesticide use
13. Aroclor 1254	Plastics
14. Aroclor 1260	Plastics
15. Trichloroethylene	Solvents
16. Dibenz (a, h)anthracene	Incineration
17. Dieldrin	Pesticides
18. Chromium, hexavalent	Paints, coatings, welding, anticorrosion agents
19. Chlordane	Pesticides
20. Hexachlorobutadiene	Pesticides

SOURCE: Data from U.S. Environmental Protection Agency.

8-17

CHEMICALS POTENTIALLY ASSOCIATED WITH REPRODUCTIVE HEALTH EFFECTS

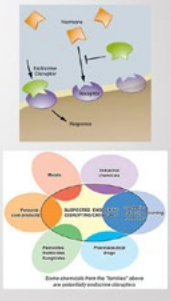
Type of compound/substance	Specific example	Evidence of reproductive health effects
Commonly used pesticides	DDT (dichlorodiphenyltrichloroethane) Organophosphates	Multiple case studies from wildlife exposures; some human evidence
Flame retardants	PBDEs (polybrominated diphenylethers)	Animal exposure models/data
Dioxin-like substances	PCBs (polychlorinated biphenyls)	-Animal exposure models/data -Wildlife exposure studies -Weak human exposure data
Phthalates	PVC (polyvinyl chloride) Di ethyl hexyl phthalate	-Animal exposure models/data - Emerging human studies (surveys, biomarker association)
Additives to consumer products (plasticizers)	BPA (bisphenol A)	- Evidence from animal exposure models/data

Mechanism of action (AIR pollution)

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

Endocrine disruptors

- Endocrine-disrupting chemicals (EDCs) are thought to affect reproduction by directly or indirectly mimicking, stimulating, antagonizing, altering, or displacing natural hormones (10) Exposure to such agents at critical stages of development can have a significant impact upon cellular, and ultimately fetal, development. Incomplete development of DNA repair mechanisms, detoxification enzymes, and the blood–brain barrier can exacerbate a chemical's effect on the developing fetus.



Reactive Oxygen Species (ROS)

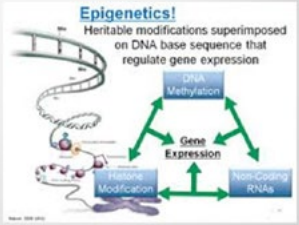
- Most air pollutants such as NO2 are ROS are capable of generating them, such as O3 or PM, through the heavy metals and the PAHs they contain. They can be transformed by CYP450 dihydro-dehydrogenase, which produces quinolone redox, catalysing electron transfer reactions and thus stimulating ROS production.

Cell dna alteration

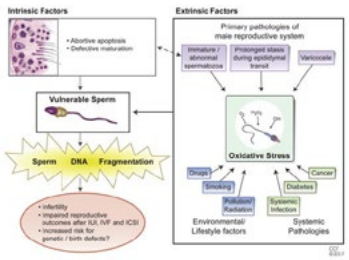
Fertility alteration caused by air pollution in the induction of alteration in the cell DNA.
Inflammation process due to ROS can alter DNA as reported in a study of taxi drivers. Telomere length has been reported to increase with increasing annual exposures to NO2, MM.
Some molecules are able to bind 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
- bnormalities of reproductive systems
  - PCO, POF, impair ovarian development
  - Poor uterine development, fibroids, endometriosis

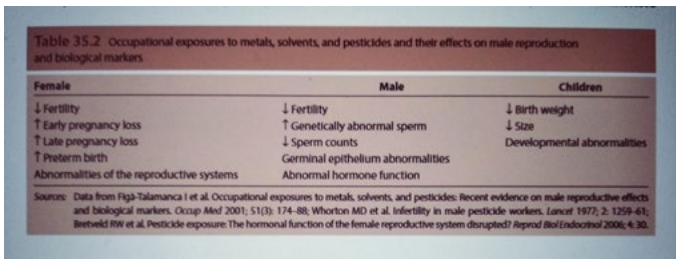
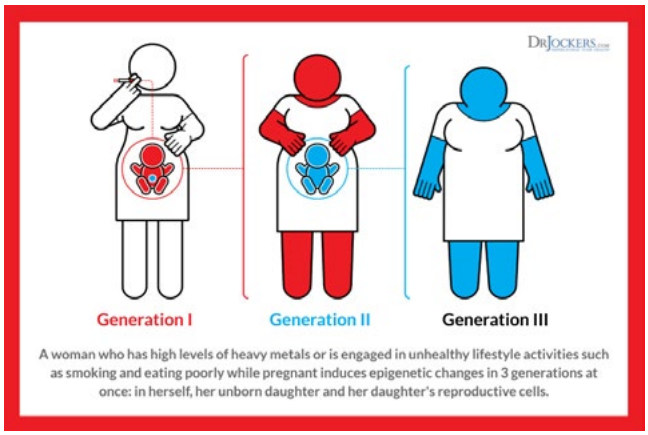


Table 35.2 Occupational exposures to metals, solvents, and pesticides and their effects on male reproduction and biological markers

Female	Male	Children
↓ Fertility	↓ Fertility	↓ Birth weight
↑ Early pregnancy loss	↑ Genetically abnormal sperm	↓ Size
↑ Late pregnancy loss	↓ Sperm counts	Developmental abnormalities
↑ Preterm birth	Germinal epithelium abnormalities	
Abnormalities of the reproductive systems	Abnormal hormone function	

Sources: Data from Figà-Talamanca I et al. Occupational exposures to metals, solvents, and pesticides: Recent evidence on male reproductive effects and biological markers. *Occup Med* 2001; 51(3): 174-88; Whorton MD et al. Infertility in male pesticide workers. *Lancet* 1977; 2: 1259-63; Bretveld RW et al. Pesticide exposure: The hormonal function of the female reproductive system disrupted? *Reprod Biol Endocrinol* 2006; 4: 30.



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. In1998 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)

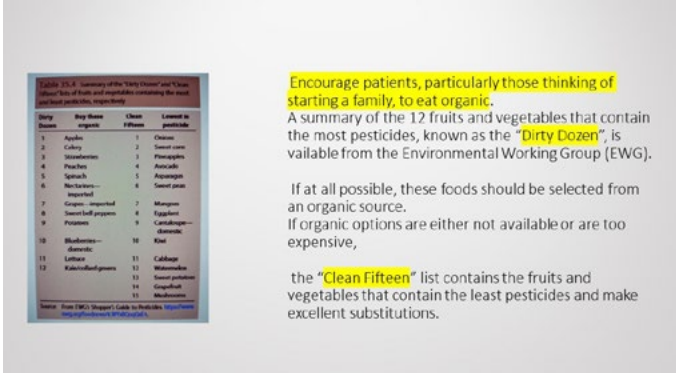
What can be done ?

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
- bnormalities 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.


Mascara
Lipstick
Toothpaste
Body Lotion & Fake Tan
Moisturiser
Shampoo & Conditioner
Hair Styling Product
Eye Shadow & Face Powder
Deodorant
Shower Gel
Up to 60% of what you put on your skin may be absorbed into your bloodstream
High-street brands may contain up to 90% of non active ingredients
Reasons to avoid:


Sustainable Shopper's Guide to a Dirty Dozen
Ingredients to Avoid in your Cosmetics
What's inside?
Are there toxic chemicals in your cosmetics?
www.DavidSuzuki.org/whatsinside
www.DavidSuzuki.org/whatsinside


10 Most Common Toxic Chemicals in Products to Avoid
Phthalates
Paraben
Lead
BPA/BPS
1,4 Dioxane
SLS
PVC
Triclosan
Oxybenzone
Diazolidinyl or Imidazolidinyl Urea


Cleaning Products You Need to Avoid

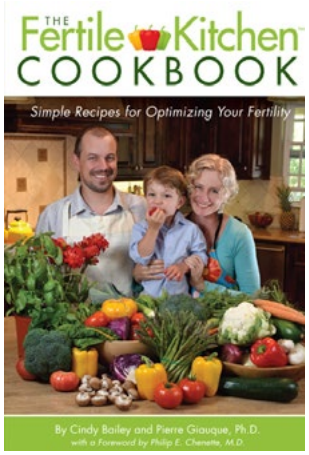
Limit use

Limit use

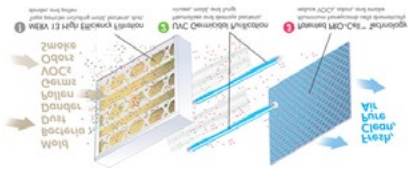
Organic food

Repost @gmoinside
Know anyone who is on the fence about choosing organic food over "conventional"? Share this with them. tomatoes tomato pesticides naturedoctor holistichealth holisticfitness holistichealing holisticnutrition holisticnutritionist organicfoods organicfood organicproducts organicproduce organicfarming organicgardening organicgardener organicgarden organicfarm urbangardening urbangardenersrepublic vegandinner veganmeal veganism herbivore gmoinside reproductivehealth fertility antioxidants

Fertility Food



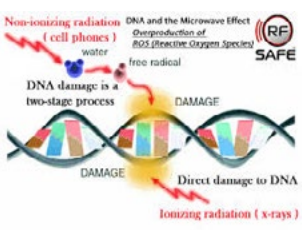
Fertility Food



Air Purifier

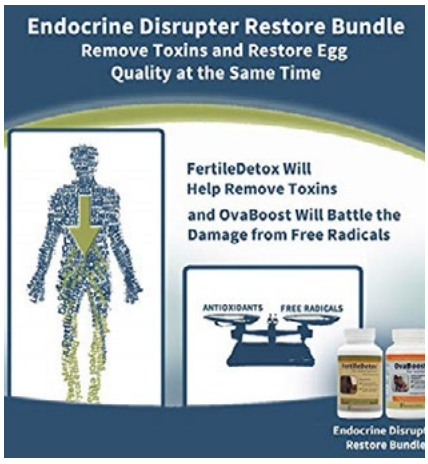
Precautions for fish

- 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.

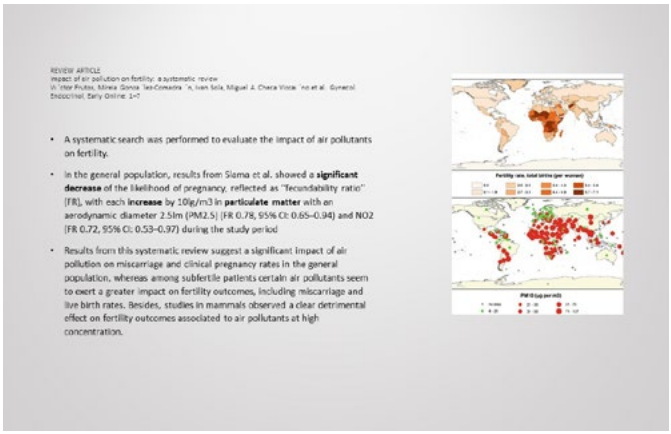


Microwave safe?


healingspotlight
An Ayurvedic Recipe for a Vibrant, Sexy Life
Detoxification, Food and Relaxation


Endocrine Disrupter Restore Bundle
Remove Toxins and Restore Egg Quality at the Same Time
FertileDetox Will Help Remove Toxins
and OvaBoost Will Battle the Damage from Free Radicals
ANTIOXIDANTS
FREE RADICALS

Detox


REVIEW ARTICLE
Impact of air pollution on fertility: a systematic review
• A systematic search was performed to evaluate the impact of air pollutants on fertility.
• In the general population, results from Sioma et al. showed a significant decrease of the likelihood of pregnancy, reflected as "fecundability ratio" (FR), with each increase by 10μg/m3 in particulate matter with an aerodynamic diameter 2.5lm (PM2.5) (FR 0.78, 95% CI: 0.65–0.94) and NO2 (FR 0.72, 95% CI: 0.53–0.97) during the study period
• Results from this systematic review suggest a significant impact of air pollution on miscarriage and clinical pregnancy rates in the general population, whereas among subfertile patients certain air pollutants seem to exert a greater impact on fertility outcomes, including miscarriage and live birth rates. Besides, studies in mammals observed a clear detrimental effect on fertility outcomes associated to air pollutants at high concentration.

Thank You

INDIAN FERTILITY SOCIETY

IFS JANUARY - JUNE 2019 CALENDAR

Dr Gouri Devi
President

Dr Pankaj Talwar
Secretary General

IFS Secretariat

302, 3rd Floor, Kailash Building
Kasturba Gandhi Marg, C.P, New Delhi-110001
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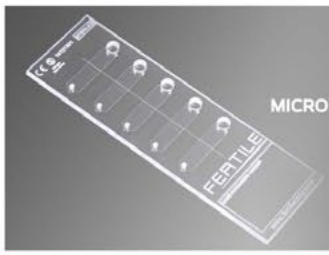

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Formerly known as FERTILE

+

ZyMot-Multi (850µl)
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- No sample preparation
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