eshre

SCIENCE MOVING
PEOPLE
MOVING SCIENCE

Basic course on environment and human male reproduction

Special Interest Group Andrology

4

27 June 2010
Rome, Italy

PRE-CONGRESS COURSE 4 – Table of contents

Basic course on environment and human male reproduction

Organised by the Special Interest Group Andrology

ESHRE – European Society of Human Reproduction and Embryology

What is ESHRE?

ESHRE was founded in 1985 and its **Mission Statement** is to:

- promote interest in, and understanding of, reproductive science and medicine.
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform politicians and policy makers in Europe.
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance

Executive Committee 2009/2011

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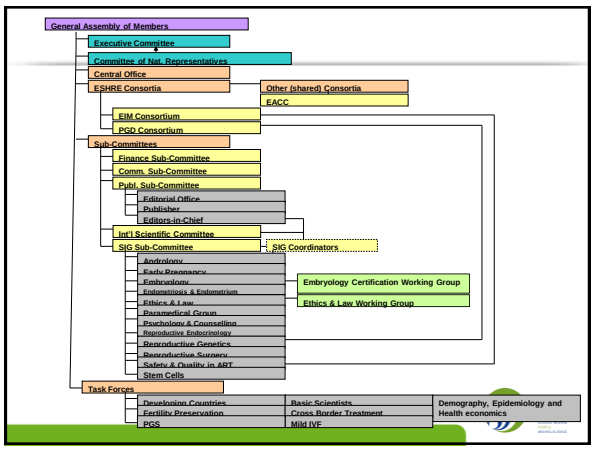

ESHRE Activities – Annual Meeting

- One of the most important events in reproductive science and medicine
- Steady increase in terms of attendance and of scientific recognition

Track record:

ESHRE 2008 – Barcelona: 7559 participants
ESHRE 2009 – Amsterdam: 8132 participants

Future meetings:

ESHRE 2010 – Rome, 27-30 June 2010
ESHRE 2011 – Stockholm, 3-6 July 2011

ESHRE Activities – Scientific Journals

Human Reproduction with impact factor 3.773

Human Reproduction Update with impact factor 7.590

Molecular Human Reproduction with impact factor 2.537

ESHRE Activities – Campus and Data Collection

- Educational Activities / Workshops
  - Meetings on dedicated topics are organised across Europe
  - Organised by the Special Interest Groups
  - Visit: www.eshre.eu under CALENDAR
- Data collection and monitoring
  - EIM data collection
  - PGD data collection
  - Cross border reproductive care survey

ESHRE Activities - Other

- Embryology Certification
- Guidelines & position papers
- News magazine “Focus on Reproduction”
- Web services:
  - RSS feeds for news in reproductive medicine / science
  - Find a member
  - ESHRE Community

ESHRE Membership (1/3)

- ESHRE represents over 5,300 members (infertility specialists, embryologists, geneticists, stem cell scientists, developmental biologists, technicians and nurses)
- Overall, the membership is distributed over 114 different countries, with 50% of members from Europe (EU). 11% come from the US, India and Australia.

ESHRE Membership (2/3)

	1 yr	3 yrs
Ordinary Member	€ 60	€ 180
Paramedical Member*	€ 30	€ 90
Student Member**	€ 30	N.A.

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.
**Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.

ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:

Annual Meeting	Ordinary	€ 480	(€ 720)
	Students/Paramedicals	€ 240	(€ 360)
Workshops	All members	€150	(€ 200)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573!)

3) ESHRE monthly e-newsletter

4) News Magazine "Focus on Reproduction" (3 issues p. a.)

5) Active participation in the Society's policy-making

Special Interest Groups (SIGs)

The SIGs reflect the scientific interests of the Society's membership and bring together members of the Society in sub-fields of common interest

- Andrology
- Early Pregnancy
- Embryology
- Endometriosis / Endometrium
- Ethics & Law
- Safety & Quality in ART
- Psychology & Counselling
- Reproductive Genetics
- Reproductive Surgery
- Stem Cells
- Reproductive Endocrinology

Task Forces

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive

Annual Meeting

Rome, Italy 27 June to 30 June 2010

Pre-congress courses (27 June):

- PCC 1: Cross-border reproductive care: information and reflection
- PCC 2: From gametes to embryo: genetics and developmental biology
- PCC 3: New developments in the diagnosis and management of early pregnancy complications
- PCC 4: Basic course on environment and human male reproduction
- PCC 5: The lost art of ovulation induction
- PCC 6: Endometriosis: How new technologies may help
- PCC 7: NOTES and single access surgery
- PCC 8: Stem cells in reproductive medicine
- PCC 9: Current developments and their impact on counselling
- PCC 10: Patient-centred fertility care
- PCC 11: Fertility preservation in cancer disease
- PCC 12: ESHRE journals course for authors

Annual Meeting – Scientific Programme (1/2)

Rome, Italy 27 June to 30 June 2010

- Molecular timing in reproduction
- Rise and decline of the male
- Pluripotency
- Preventing maternal death
- Use and abuse of sperm in ART
- Live surgery
- Emerging technologies in the ART laboratory
- Debate: *Multiple natural cycle IVF versus single stimulated cycle and freezing*

Annual Meeting – Scientific Programme (2/2)

- Fertility preservation
- Congenital malformations
- ESHRE guidelines
- Data from the PGD Consortium
- European IVF Monitoring 2007
- Debate: *Selection of male/female gametes*
- Third party reproduction in the United States
- Debate: *Alternative Medicine, patients feeling in control?*
- Historical lecture: "Catholicism and human reproduction"

Certificate of attendance

1/ Please fill out the evaluation form during the campus

2/ After the campus you can retrieve your certificate of attendance at www.eshre.eu

3/ You need to enter the results of the evaluation form online

4/ Once the results are entered, you can print the certificate of attendance from the ESHRE website

5/ After the campus you will receive an email from ESHRE with the instructions

6/ You will have TWO WEEKS to print your certificate of attendance

Contact

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PRE-CONGRESS COURSE 4 - Programme

Basic course on environment and human male reproduction

Organised by the Special Interest Group Andrology

Course coordinators: Jose A. Castilla (Spain) and Sheena Lewis (United Kingdom)

Course description: This course will present the causal links between the intrauterine exposure and occupational risk and their impact on human reproductive health. The basic principles on how to perform quality studies of human semen and toxicology will be presented. The overall role of environmental risks in the introduction of male reproductive disease (hypospadias, cryptorchism) will be covered. Finally, Influence of environment on puberty will be analyzed.

Target audience: All those with interest in the effects on human male reproduction of environment factors.

Scientific programme:

09:00 – 09:30	Intrauterine exposure to environmental chemicals on human male reproduction – **Jaime Mendiola-Olivares (Spain)**
09:30 – 09:45	Discussion
09:45 – 10:15	Occupational risk and human male reproduction - **Jacques Auger (France)**
10:15 – 10:30	Discussion
10:30 – 11:00	Coffee break
11:00 – 11:30	Semen quality in European populations **- Niels Jørgensen (Denmark)**
11:30 – 11:45	Discussion
11:45 – 12:15	Endocrine disruptor and semen quality - **Marieta Fernández (Spain)**
12:15 – 12:30	Discussion
12:30 – 13:30	Lunch
13:30 – 14:00	Bisfenol A and human male reproduction – **G. Schönfelder (Germany)**
14:00 – 14:15	Discussion
14:15 – 14:45	Puberty and environment - **Anders Juul (Denmark)**
14:45 – 15:00	Discussion
15:00 – 15:30	Coffee break
15:30 – 16:00	Lifestyle factors and indications of male reproductive function - **Sally Perreault Darney (USA)**
16:00 – 16:15	Discussion
16:15 – 16:45	Hypospadias and cryptorchidism and environment - **Jorma Toppari (Finland)**
16:45 – 17:00	Discussion

INTRAUTERINE EXPOSURE TO ENVIRONMENTAL CHEMICALS ON HUMAN MALE REPRODUCTION

Basic course on environment and human male reproduction
26th ESHRE Annual Meeting, Rome, Italy, June 2010.

Dr. Jaime Mendiola-Olivares, PhD. Public Health &
Epidemiology Research Group, University of Murcia, Spain.
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Conflict of interest: Nothing to disclose

1

Learning objectives

- To understand how *animal models* can help identify environmental chemicals that can alter human male reproduction.
- To become familiar with endocrine-sensitive male *reproductive outcomes* that have been related to prenatal exposure to environmental chemicals.
- To learn about the *testicular dysgenesis syndrome* (TDS).
- To learn which *environmental chemicals* have been shown to alter human male reproduction.

2

Diethylstilbestrol (DES)

- 1941- 1971 Prescribed to 3-5 million women to prevent miscarriage
- 1971 Shown to cause vaginal cancer in adolescence among daughters exposed prenatally
- Genital malformations were three times as frequent among DES-exposed as unexposed men (sons of women in an RCT)

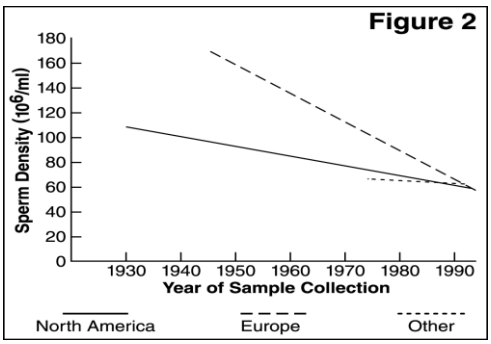
3

Sperm counts are declining

50% decline in sperm concentration in 50 years (Carlsen, et al. 1992)

Decline confirmed in two reanalyses (Swan, et al. 1997 and 2000)

4



Swan et al. (2000)

5

Testicular Dysgenesis Syndrome (TDS)

Altered *in utero* testicular development resulting in one or more of:

- Decreased semen quality
- Reduced testosterone
- Testicular cancer
- Hypospadias and cryptorchidism

Associated with exposure to fetal and perinatal EDC exposure (Sharpe and Skakkebaek, 2008)

6

Some Endocrine Disrupting Chemicals (EDCs)

- PHTHALATE ESTERS (Anti-androgens)
  - Di(2-ethylhexyl) phthalate (DEHP)
  - Di(n-butyl) phthalate (DBP)
- BISPHENOL A (BPA) (xenoestrogen)
- PESTICIDES (e.g. vinclozolin, PCBs)
- PERFLUORINATED COMPOUNDS (PFOS, PFOA)

May act cumulatively (mixture problem)

7

Exposure is nearly universal

- These are found throughout our environment:
  - Plasticizers in polyvinyl chloride
  - Solvents (lacquers, varnishes)
  - Flooring and wall coverings
  - Food contact applications (cans, baby bottles)
  - Medical devices
  - Personal-care products (perfumes, lotions, cosmetics)
  - Coatings (including used to time releases in pharmaceutical products)

8

Endocrine-Sensitive Endpoints (male)

- Physical and genital exam including:
  - Breast size (gynecomastia)
  - Location of the testis, testicular and penis size
  - Anogenital distance (AGD)
- Endocrine status (hormonal profile)
  - FSH, LH, T, E2, FT, FAI, Inhibin B and SHBG
- Male reproductive function (semen quality)
  - Sperm concentration, motility and morphology

9

Prenatal exposures (animal models)

- Fetal stage is the most sensitive period for development.
- Several environmental chemicals produce effects on reproductive development in male offspring after *in utero* exposure.
- Critical period: Altered fetal testicular hormone production at critical window for reproductive tract development (rodents gest. days 19-21).

10

Prenatal exposures (animal models)

- Phthalate esters DEHP, DBP are anti-androgens.
- Marked reduction in fetal testicular T production.
- Male reproductive tract development under androgen control.
- T decreased by changes in gene expression of enzymes involved in T biosynthesis and transport in the fetal Leydig cell.

11

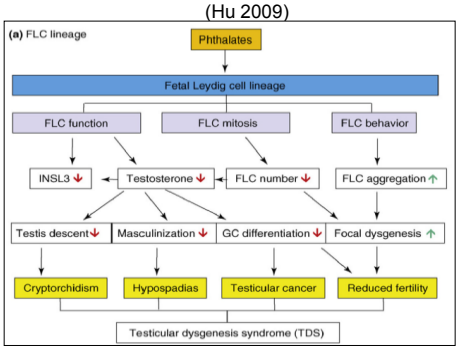
Prenatal exposures: The phthalate syndrome

- First defined in rodents: Cluster of androgen-mediated male developmental endpoints that are altered by *in utero* phthalate (DEHP and DBP) exposure.
- Characterized by malformation of the:
  - Epididymis
  - Vas deference
  - Seminal vesicles and prostate
  - External genitalia (hypospadias)
  - Cryptorchidism and retention of nipples (feminization)
  - Reduced AGD

12

The Phthalate Syndrome and TDS

(Hu 2009)



13

Relevance of animal studies to humans:

- Animals show a continuum of responses:
  - High doses: severe reproductive tract malformations
  - Low doses: changes in AGD and nipples retention
- Low doses of phthalates in rats are higher than reported exposure levels in humans….BUT
  - Alterations have been reported at very low doses
  - Enzymes involved in steroidogenesis are identical
  - Animals tested one chemical at a time (human exposure to mixtures)

14

Low dose effects (DBP)

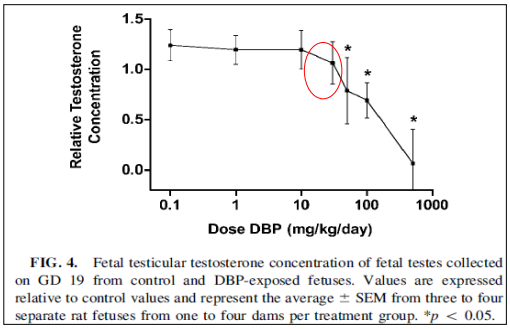


FIG. 4. Fetal testicular testosterone concentration of fetal testes collected on GD 19 from control and DBP-exposed fetuses. Values are expressed relative to control values and represent the average ± SEM from three to four separate rat fetuses from one to four dams per treatment group. *$p < 0.05$.

15

Lehmann et al. (2004)

Prenatal exposures (animal models)

- Bisphenol A (xenoestrogen)
  - Rodents exposed to BPA during prenatal or perinatal periods show decreased epididymal weight and daily sperm production (Richter et al. 2007)
- Cumulative risk of chemicals (mixtures)
  - Risk assessment (RA) on chemical-by-chemical basis
  - In real life we are exposed to mixtures of chemicals
  - Cumulative RA of chemicals acting via similar pathways
  - Cumulative effects of anti-androgenic chemicals (combinations) behaved in a dose-additive manner

16

Dose additivity of mixtures

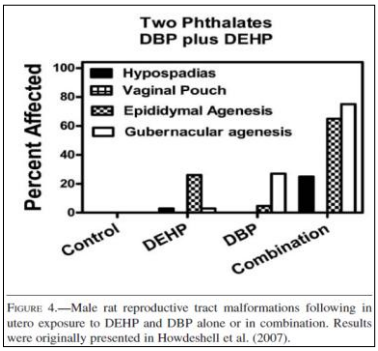


FIGURE 4.—Male rat reproductive tract malformations following in utero exposure to DEHP and DBP alone or in combination. Results were originally presented in Howdeshell et al. (2007).

Rider et al. (2009)

17

Human studies

- European men giving a semen sample and physical exam were asked to answer, in collaboration with their mother, questions on *in utero* exposures, including smoking while pregnant. (Jensen et al. 2004)

18

In *utero* exposure to Maternal smoking

- Semen quality among European men exposed to smoking *in utero* or in childhood as compared with unexposed men (Jensen et al., 2004)

Change in sperm count (%)		Change in % of motile sperms	
β	95% CI	β	95% CI
- 20.1	-33.5, -6.8	- 1.85	-3.23, -0.46

- Exposed men had a 20.1% lower sperm concentration and 1.85% fewer motile sperm cells than unexposed men.

19

Phthalates and AGD in humans

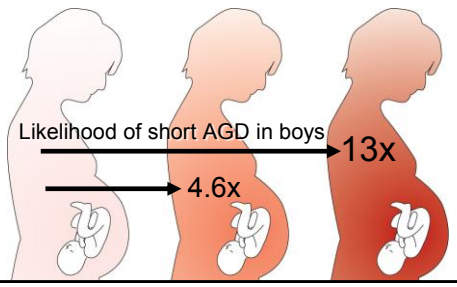
- AGD and other genital measurements in boys 2-36 months. Examined in relation to their concentrations of phthalate metabolites in prenatal urine samples (Swan et al. 2005, 2008).
- Several phthalate metabolites were inversely related to AGD, supporting the hypothesis that prenatal phthalate exposure may adversely affect male reproductive development in humans.

Swan et al. 2005, 2008

20

Swan (2008)

	Percentile (ng/mL)		
Monoester Metabolite	25th	50th	75th
MEHHP	6.0	11.4	20.1



21

Summary of results on phthalates and male genitalia

- DEHP metabolites in prenatal urine were associated in males with:
  - Shorter AGD
  - Smaller penile width
  - Incomplete testicular descent
- DBP metabolites associated with shorter AGD but not significantly with smaller penile width or testicular descent.

Swan (2008)

Xenoestrogens and male malformations

- Fernández et al. (2007) compared 50 newborns with diagnosis of cryptorchidism and/or hypospadias with 114 boys without malformations matched by gestational age, date of birth, and parity.
- The aim of the study was to determine whether the combined effect of environmental estrogens (in placenta) measured as total effective xenoestrogen burden (TEXB) is a risk factor for male urogenital malformations.

23

Xenoestrogens and male malformations

- Adjusted ORs (95% CIs) for urogenital malformations among male offspring in relation to the presence in placenta samples of specific EDCs, according to case/control status of newborn.

Variable	P-value	OR (95% CI)
DDT	0.02	2.63 (1.21-5.72)
Endosulfan I	0.03	2.19 (0.99-4.82)
Lindane	0.002	3.38 (1.36-8.38)
Mirex	0.02	2.85 (1.22-6.66)

Fernández et al. (2007)

24

Maternal beef intake and semen quality

- Swan et al. (2007) investigated possible long-term risks from anabolic steroids and other xenobiotics in beef. Authors examined mens' semen quality in relation to their mother's self-reported beef consumption during pregnancy
- Sperm concentration was inversely related to mothers' beef meals per week. In sons of 'high beef consumers' (>7 beef meals/week), sperm concentration was 24.3% lower.
- Maternal beef consumption, and possibly xenobiotics in beef, may alter a man's testicular development in utero and adversely affect his reproductive capacity.

25

Maternal beef intake and semen quality

Table 3: Regression analyses of semen parameters in relation to two measures of mother's beef consumption.

	Log_{10} sperm concentration	
	Coefficient	*P*-value
Mothers' beef servings per week		
Number	−0.0102	→ 0.041
>7 versus ≤7	−0.1208	→ 0.014

Swan et al. (2007)

26

Pesticide exposure and male malformations

- Andersen et al. (2008) investigated whether occupational pesticide exposure during pregnancy causes adverse effects on the reproductive development in the male infants.
- 113 mother–son pairs were included. The mothers were categorized as occupationally exposed (91 sons) or unexposed (22 sons) to pesticides during pregnancy.
- Testicular position and volume, penile length, and position of urethral opening were determined at 3 months of age using standardized techniques.

27

Pesticide exposure and male malformations

- Prevalence of congenital cryptorchidism at 3 months of age in sons of female greenhouse workers in Funen and boys born in the Copenhagen area.

Prevalence of congenital cryptorchidism		RR (95% CI)
Funen [% (n)]	Copenhagen area [% (n)]	3.2 (1.4 - 7.4)
6.2 (7)	1.9 (19)	

Andersen et al. (2008)

28

Occupational exposures and male malformations

- Ormond et al. (2009) assessed the risk of hypospadias associated with occupational exposure of the mother to endocrine-disruptor chemicals, between others.
- The authors designed a case–control study of 471 hypospadias cases referred to surgeons and 490 randomly selected birth controls, born 1 January 1997–30 September 1998 in southeast England.

29

Occupational exposures and male malformations

- Multiple regression models of hypospadias: adjusted ORs and 95% CIs.

Variable	P-value	OR (95%CI)
Folate during 1st trimester	0.02	0.64 (0.44-0.93)
Maternal occupational exposure to hair spray	0.004	2.93 (1.40-4.17)
Maternal occupational exposure to phthalates	0.01	3.12 (1.04-11.46)

Ormond et al. (2009)

30

Human studies: Postnatal exposure

- Concentrations phthalate metabolites in human breast milk examined in relation to serum hormones in newborn boys (n=130).
- Two phthalate metabolites (MEP, MBP) positively associated with infant serum LH/FT ratio, SHBG and LH levels.
- MBP was negatively associated with serum FT levels.

Main et al. (2006)

31

Phthalates in breast milk and male hormones

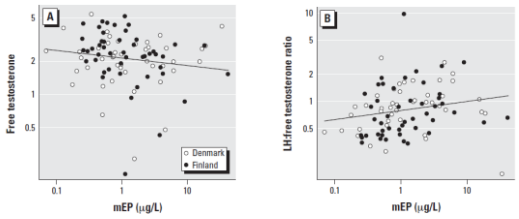


Figure 2. Regression plots of mEP levels (µg/L) in human breast milk and serum hormonal levels in boys 3 months of age (n = 96).

Main et al. (2006) 32

Conclusions

- Animal studies consistently demonstrate intrauterine exposures to environmental chemicals affect male reproductive system in adulthood.
- Environmental chemicals are ubiquitous and much more studies about chemical mixtures are needed.
- Only a few human observational studies looked into *in utero* exposures related to male reproductive outcomes.
- However, every single study found a kind of alteration related to impaired male reproductive function, consistently with the findings in rodents exposed to EDCs.

33

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38

I declare not to have commercial relationships
or other activities that might be perceived
as a potential conflict of interest

Occupational Risk and Human Male Reproduction

Jacques Auger,
Service de Biologie de la Reproduction/CECOS,
Hôpital COCHIN - PARIS - FRANCE

Learning objective:

Lifestyle and environmental factors are the main suspects to explain the increase of various anomalies of the male genital tract recently reported.
The vulnerability of the male genital tract and fertility to various chemicals has been shown notably by a number of occupational studies. Occupational hazards are by far the best documented in epidemiological research in reproductive health.
Occupational studies are necessary for assessing the reproductive risk for the workers themselves. They may also generate useful data for our understanding of the impact of various chemical and/or physical factors in the general environment as well as for risk assessment policies.
Where do we stand now? Overall, it will be shown that indisputable evidence of occupational adverse effects on male reproduction exists only for a relatively limited number of exposures or toxicants while for other exposures, the association is only suspected or suggested requiring further evaluation.
It will be explained how the study design for investigating possible associations between occupational exposure (which should be measured as precisely as possible) and various endpoints such as natural fertility, semen quality, hormone levels, etc... is of the utmost importance.
Updated knowledge in this domain should help the andrologist, gynecologist occupational physician or the general practitioner, as well, to improve counseling and management of their patients, notably those trying to have a child.

High vulnerability of the male reproductive function to a number of lifestyle / man made factors

Working and living with poison:
The DBCP (dibromochloropromane) story

- Lesions of the seminiferous tubules
- Changes in sex-ratio (↑ female)
- Irreversible azoospermia or severe oligozoospermia
- Decreased motility
- Hormonal Changes
- Embryonic/fetal injuries? few cases studied

What are the factors involved?

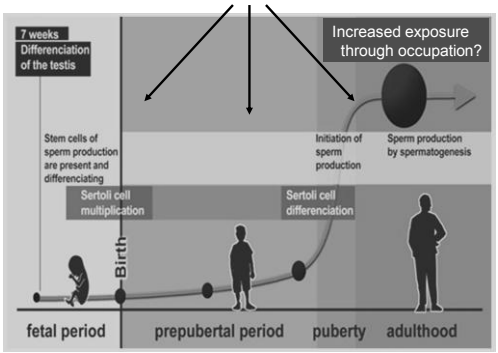
In humans, 5 categories of factors may theoretically interfere with male reproductive function:

- Genetic factors
- Physical factors: Radiation, temperature, ...
- Biological / clinical factors: STD ...
- Socio-cultural / lifestyle factors: stress, tobacco, ...
- Chemical factors: Industrial and or agricultural chemical compounds

Chemical exposures

- Constant increase of chemical compounds in use since the 2nd World War
- >10^6 compounds known
- ~10^5 products used in the industrial/agricultural world

Endocrine Disruptor Compounds (EDc)



What are the reproductive risks
in men occupationally exposed ?

The problem of identifying an association between a single factor and fertility

Reproductive health $= \alpha var1 + \beta var2 + \delta var3 + \gamma var4 + \tau var5 + \ldots + \theta var97(!), etc\ldots$

Likelihood of pregnancy within the shortest possible period of time

Varicocele

Occupational exposure
Welding
What type of solder?
what metals?
radiation effect?
duration of exposure?

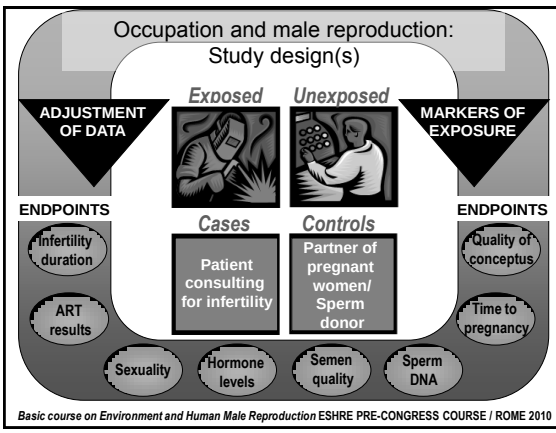
taking potentially reprotoxic medication

Ovulation quality

Semen characteristics

?

Chronic psychological stress



Occupational exposure and semen quality

Occupational lead exposure and semen quality

Table 4 Semen quantity and quality by current blood lead concentration (median (range), crude and adjusted GM)

	Current blood lead concentration (µg/dl)						
	≤10 n=149	10.1–20 n=57	20.1–30 n=90	30.1–40 n=101	40.1–50 n=63	>50.1 n=24	p Trend‡
Volume (ml)							
Adjusted GM (SE)	2.7 (3)	2.6† (3)	2.5† (3)	2.4† (2)	2.6† (3)	2.6† (4)	>0.05
Sperm concentration (x10⁶/ml)							
Adjusted GM (SE)	32 (5)	28† (5)	33† (5)	29† (5)	35† (6)	19* (4)	>0.05
Total sperm count (x10⁶)							
Adjusted GM (SE)	92 (16)	80† (15)	90† (16)	78† (14)	105† (20)	51* (12)	>0.05

*p<0.05 in an analysis of variance comparing with baseline in group (≤ 10µg/dl); †p>0.05 in an analysis of variance comparing with baseline in group (≤ 10µg/dl); ‡least square regression of semen characteristics on lead concentration in spermatozoa (continous variable); covariates with significant

Bonde et al., 2002

Occupational exposure to pesticides in Guadeloupe: Semen quality and hormones

Semen characteristics	Unexposed (n=45)	Exposed (n=42)	Difference
	m (ET)	*m (ET)*	*p* [1]
Volume séminal (ml)	3.8 (1.7)	3.4 (1.6)	0.804
Concentration (10^6/ml)	90 (81)	70 (60)	**0.123**
Numération (10^6)	308 (237)	231 (230)	**0.143**
Mobilité a + b (%)	42 (12)	43 (14)	0.960
Morphologie (%)	14 (7.0)	13 (8.0)	0.605
Vitalité (%)	54.1 (13.4)	54.5 (17.3)	0.656
Hormones	*m (ET)*	*m (ET)*	*p* [2]
Testostérone (ng/ml)	7.5 (2.5)	6.8 (1.7)	0.921
Inhibine B (pg/ml)	170 (72)	168 (67)	0.970
FSH (mIU/ml)	6.3 (4.4)	5.9 (4.1)	0.692
LH (mIU/ml)	4.7 (1.9)	5.4 (2.6)	0.359

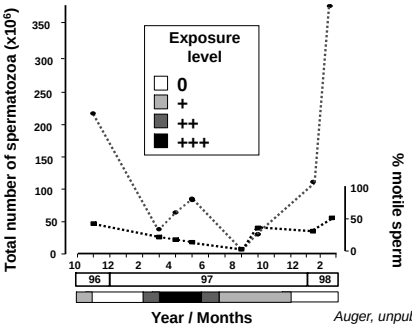
[1] ANOVA, covariables: âge, infections génitales, solvants, tabac [2] ANOVA, covariables: âge, infections génitales, solvants

Multigner et al., 2008

1/ Mr L..: arborist and infertile: pesticides used

Active compound	Reprotoxic category	Banned in
2,4-D		X
2,4-MCPA		X
Aminotriazole	**Tox dev cat 3 (UE)**	
Amitraze		2004
A. N. A.		
Benomyl		2002
Bitertanol		
Bupirimate		x
Captane		
Carbaryl		2007
Chinomethionate		x
Clopyralid		
Cyproconazole		
Difubenzaron		
Dithianon		
Diuron	**Anti-androgenic**	X
DNOC		1999
Doguadine		x
Fenarimol		x
Fenazaquin		
Fenoxycarbe	**Reprotox (US-TR)**	
Flusilazole	**Reprotox cat 2 (UE)**	
Glufosinate ammonium	**EU cat 3**	
Oligohoside		
Isoxaben		
Mancozèbe	**Reprod & dev toxicant (US-TRI)**	
Paraquat		2007
Phosmet		
Propargite		
Simazine		2004
Tebufenozide		
Tetraconazole		
Thiophanate-methyl	**Dev tox (CA & US-TRI)**	
Triadimefon		2004
Vamidothion		2003

2/ Mr L..: Intermittent exposure to pesticides and semen quality



Auger, unpublished

Occupational exposure to glycol ethers: Semen quality and hormones

	Non-exposed n = 50	Exposed n = 48		
	Mean	Mean	Mean difference (CI 95%)	Adjusted p
Seminal volume (ml)	3.7	4.1	−0.3 (−1.0 to 0.4)	0.52*
Sperm concentration (millions/ml)	119.1	74.0	45.0 (21.0 to 69.1)	<0.001*
Total sperm count (millions)	416.3	277.4	138.8 (37.7 to 240.0)	<0.001*
"a" rapid progressive motility (%)	18.4	12.8	5.5 (2.4 to 8.6)	<0.001*
Normal sperm morphology (%)	54.2	47.1	7.1 (0.8 to 13.4)	0.005*
Serum hormones				
Testosterone (ng/ml)	6.2	6.3	0.1 (−1.35 to 1.16)	0.97†
FSH (IU/l)	3.9	5.5	−1.6 (−3.3 to 0.01)	0.05†
LH (IU/l)	3.5	3.5	−0.01 (−0.74 to 0.73)	0.86†
Inhibin B (pg/ml)	219.0	215.0	−3.8 (−27.2 to 34.0)	0.71†

FSH, follicle stimulating hormone; LH, luteinising hormone.
*Calculated using analysis of variance/covariance to adjust for age, sexual abstinence, and season of semen analysis.
†Calculated using analysis of variance/covariance to adjust for age and body mass index.

Multigner et al., 2007

Self-reported occupational exposure and semen quality in men consulting for couple's infertility

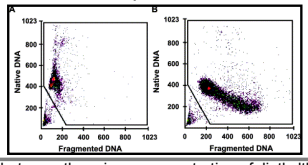
WHO99 sperm reference values

	Total (n=402)	Men with altered semen (n=314)	Men with normal semen (n=88)	Univariate analysis†	Logistic regression‡	
				p value	Adjusted OR (95% CI)	Adjusted *p* value
Chemical occupational factors (current or past exposure): n (%)						
Heavy metals	49 (12.2)	46 (14.6)	3 (3.4)	0.006	5.4 (1.6-18.1)	0.007
Pesticides	25 (6.2)	23 (7.3)	2 (2.4)	0.085	3.6 (0.8-15.8)	0.087
Solvents	150 (37.3)	131 (41.7)	19 (21.6)	<0.001	2.5 (1.4-4.4)	0.001
Fumes	136 (33.8)	115 (36.6)	21 (23.9)	0.022	1.9 (1.1-3.4)	0.016
Plastic fumes	9 (2.2)	9 (2.9)	0 (0.0)	*	*	*
Vegetable fumes	10 (2.5)	10 (3.2)	0 (0.0)	*	*	*
Welding fumes**	44 (10.9)	41 (13.1)	3 (3.4)	0.011	4.7 (1.4-15.7)	0.012
Engine fumes	92 (22.9)	75 (24.0)	17(19.3)	0.323	1.4 (0.7-2.5)	0.304
Metallurgy fumes	8 (2.0)	7 (2.2)	1 (1.1)	*	*	*
PAHs	115 (28.6)	98 (31.2)	17 (19.3)	0.012	1.9 (1.1-3.5)	0.026
Others	48 (11.9)	43 (13.7)	5 (5.7)	0.040	2.5 (0.95-6.5)	0.065
Physical occupational factors (current exposure): n (%)						
Electromagnetic fields	25 (6.2)	20 (6.4)	5 (5.7)	0.334	1.1 (0.6-1.7)	0.866
Mechanical vibrations	94 (23.4)	79 (25.2)	15 (17.0)	0.195	1.6 (0.9-2.9)	0.133
Excess heat	33 (8.2)	29 (9.3)	4 (4.4)	0.190	2.2 (0.7-6.4)	0.164
Extended periods of sitting > 20h/week	168 (41.8)	127 (40.4)	41 (46.6)	0.411	0.9 (0.5-1.4)	0.476

De Fleurian et al, 2009

Occupational exposure and sperm DNA

Occupational exposure to organophosphorus* and abnormal sperm chromatin



Relationship between the urinary concentration of diethylthiophosphate (DETP) and the level of denatured sperm DNA

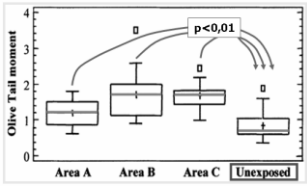
** Mexican market gardeners (n=66)*	β	p
DFI (mean)	0.477	0.026
DFI (SD)	0.1628	0.022
% DFI	0.000062	0.079

Sánchez-Peña et al., 2004

Occupational exposure to styrene and abnormalities of sperm DNA

- 44 workers exposed to styrene for at least 2 years
- 27 unexposed workers
- Exposure in 3 geographically distinct factories
- No difference in routine semen characteristics
- Differences on sperm DNA by the comet assay

	% fragmented DNA	« Olive tail moment »
Exposed	10.9 (3.0)	1.5 (0.6)
Unexposed	7.4 (2.3)	0.8 (0.4)
p	**0.001**	**0.0001**



Migliore et al, 2002

Male occupational exposure hormone levels and fertility

Occupational lead exposure and TTP

Table 3 TTP distributions for each exposure category: short term exposure model

Time period	External control (n = 236)	Internal control (n = 230)	<20 μg/dl (n = 71)	20–29 μg/dl (n = 156)	30–39 μg/dl (n = 162)	40+ μg/dl (n = 184)	Missing (n = 65)	Total (n = 1104)
1 month	66 (28)	111 (48)	35 (49)	78 (50)	84 (52)	81 (44)	21 (32)	476 (43)
2 months	43 (46)	35 (64)	14 (69)	32 (71)	17 (62)	25 (58)	12 (51)	178 (59)
3 months	32 (60)	25 (74)	9 (82)	11 (78)	20 (75)	18 (67)	8 (63)	123 (70)
4–6 months	43 (78)	27 (86)	6 (90)	12 (85)	14 (83)	30 (84)	9 (77)	141 (83)
7–9 months	10 (82)	8 (90)	1 (92)	5 (89)	5 (87)	6 (87)	2 (80)	37 (87)
10–12 months	19 (90)	9 (94)	3 (96)	11 (96)	4 (89)	7 (91)	4 (86)	57 (92)
13+ months	23 (100)	15 (100)	3 (100)	7 (100)	18 (100)	17 (100)	9 (100)	92 (100)

The values given are the number becoming pregnant in that interval (in parentheses: cumulative percentages of those pregnant up to the end of the current interval), grouped for convenience.

outcome variable, as part of the EU-funded Asclepios Project.

Methods: Exposure assessment was mainly by blood lead values, which were available from the late 1970s, supplemented by imputed values where necessary. Three exposure models were studied: (1) short term (recent) exposure; (2) total duration of work in a lead using industry; and (3) cumulative exposure. A Cox proportional hazards model with discrete ties was used for the statistical analysis, with covariates for both partners.

Results: A total of 1104 subjects took part, of whom 638 were occupationally exposed to lead at the relevant time. Blood lead levels were mainly less than 50 μg/dl. No consistent association of Time To Pregnancy with lead exposure was found in any of the exposure models, although reduced fertility was observed in one category each in models (2) and (3).

Conclusions: This basically negative result is unlikely to be due to the misclassification of key variables, to insufficient statistical power, or to bias, for example, response bias. If any impairment of male reproductive function exists at the levels of occupational lead exposure now current, it does not appear to reduce biological fertility.

Joffe et al., 2003

AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 46:614–626 (2004)

Effects of Occupational Solvent Exposure on Reproductive Hormone Concentrations and Fecundability in Men

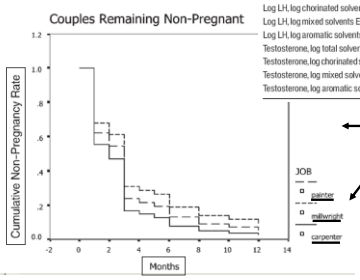
Ulrike Luderer, MD, PhD, MPH,[1] Abigail Bushley, PhD,[2,3] Bert D. Stover,[2] William J. Bremner, MD, PhD,[4] Elaine M. Faustman, PhD,[2] Timothy K. Takaro, MD, MPH,[2] Harvey Checkoway, PhD,[2] and Carl Andrew Brodkin, MD, MPH[2]

Variable	Carpenter (n = 40)	Millwright (n = 25)	Painter (n = 32)	P-value
	Mean ± SD			
Age (years)	46.8 ± 8.3	49.9 ± 9.2	42.7 ± 7.8	0.006
Height (m)	1.8 ± 0.06	1.8 ± 0.06	1.8 ± 0.05	0.911
Weight (kg)	91.2 ± 12.2	93.8 ± 13.3	88.6 ± 14.1	0.330
BMI[a] (kg/m^2)	28.8 ± 4.3	29.5 ± 4.2	28.0 ± 4.0	0.406
Years in job	22.8 ± 7.5	22.2 ± 9.0	19.5 ± 8.5	0.233
Years of college	0.82 ± 1.43	0.64 ± 1.08	0.31 ± 0.82	0.191
Years of vocational training	0.75 ± 1.48	0.78 ± 1.32	0.16 ± 0.57	0.073
Thinners, degreasers, varnishes, adhesives EI[c]	6.8 ± 10.3	12.0 ± 14.5	219 ± 17.1	<0.001
Aromatic solvent EI	1.4 ± 4.3	0.7 ± 1.8	8.1 ± 9.1	<0.001
Chlorinated solvent EI	1.0 ± 4.3	4.9 ± 8.3	2.2 ± 6.2	0.049
Total solvent EI	10.3 ± 13.5	21.1 ± 22.9	42.0 ± 32.2	<0.001
Blood lead (μg/dl)	1.1 ± 1.5	2.3 ± 1.8	1.7 ± 1.2	0.017

(Semen quality not studied)

- FSH positively associated with exposure

Model[a] (outcome variable, exposure variable)	β	95% CI	P-value
Log FSH, log total solvent EI[b]	0.07	0.005–0.14	0.035
Log FSH, log chorinated solvent EI.	0.11	0.01–0.21	0.029
Log FSH, log mixed solvents[c] EI.	0.07	−0.002–0.15	0.055
Log FSH, log aromatic solvents EI.	0.03	−0.07–0.13	0.505
Log LH, log total solvent EI.	−0.02	−0.07–0.04	0.525
Log LH, log chorinated solvent EI.	0.02	−0.07–0.10	0.690
Log LH, log mixed solvents EI.	−0.02	−0.09–0.04	0.483
Log LH, log aromatic solvents EI.	−0.08	−0.17–0.001	0.052
Testosterone, log total solvent EI.	−0.05	−3.41–3.31	0.977
Testosterone, log chorinated solvent EI.	−3.12	−8.28–2.04	0.232
Testosterone, log mixed solvents EI.	−0.38	−4.24–3.47	0.844
Testosterone, log aromatic solvents EI.	−1.00	−6.06–4.06	0.697



← Fertility unaltered whatever professional category

Luderer et al., 2004

Male occupational exposure and sexuality

Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction

- Workers from BPA-exposed and control factories
- Male sexual function ascertained by male sexual function inventory

RESULTS

Exposed workers had a significantly increased risk of:

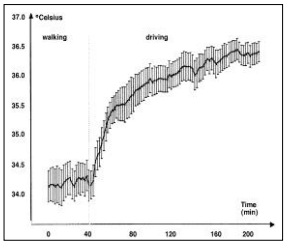
- reduced sexual desire OR = 3.9, 95% CI: 1.8–8.6
- erectile difficulty OR = 4.5, 95% CI 2.1–9.8)
- ejaculation difficulty OR = 7.1, 95% CI 2.9–17.6
- reduced satisfaction with sex life OR = 3.9, 95% CI 2.3–6.6

Li et al., 2010

Two direct/indirect factors at work:

- Heat exposure
- Chronic Stress

Heat exposure



Mean right scrotal temperature in 9 men while walking outside and driving a car

Bujan et al, 2000

Impact of heat exposure at work on semen characteristics

- Posture at work and sperm concentration
  Hjollund et al, 2002
- Posture at work and sperm morphology
  Figa-Talamanca et al, 1996
  Auger et al, 2001

Role of lifestyle factors

- Improvement of semen quality by nocturnal scrotal cooling
  Jung et al, 2001
- Sperm characteristics of endurance trained cyclists
  Gebreegziabher et al, 2004

Psychological stress at work and delayed TTP

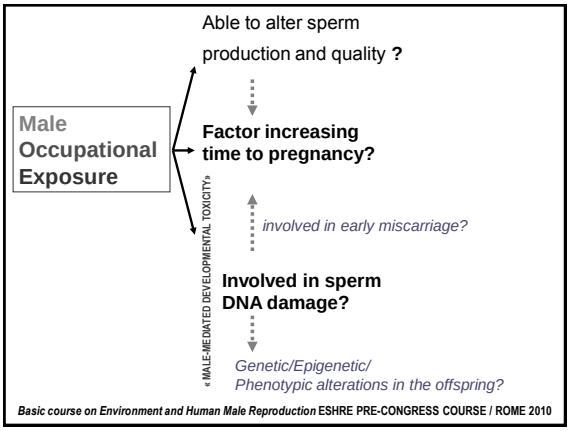
OBJECTIVE:

The aim of this study was to explore an association between psychosocial stress in married male workers of a large Korean petrochemical enterprise and TTP

RESULTS:

After adjustment for confounding effects of life-style characteristics and benzene exposure, delayed TTP was associated with one standard deviation (SD) increase of the effort-reward ratio in the chronically stressed group of married men (OR = 0.47; 95% CI = 0.22-0.99)

Lee et al., 2009



The risk of infertility depends on:

- To how many chemicals the man is exposed
- Is there any protection?
- The duration of exposure (how many hours?)
- The frequency of exposure: daily? weekly? with possible prolonged alternance periods (farmers)
- From how many years the man is exposed?

It also depends on his medical history, current health status, various lifestyle factors,...

and ... the same is true for the female partner

In practice? for the andrologist/occupational physician

For any worker potentially exposed,
living with a female partner
and having a child project

- Question +++ on the duration of infertility (evaluated similarly to a TTP)
- Check-up by andrologist recommended
- For a given exposure with recommended measures of protection, assess the degree of effective protection in order to contribute in reducing at best the potential male reproductive hazards
- Vigilance + + + for any exposure(s) suspected to provoke sperm DNA damage

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Endocrine disrupters and semen quality

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Rome, Italy
June, 2010

Overview

- Background
- EDs: Mechanisms and classes
- Human health effects of EDs
- Human exposure to EDs
- Lessons learned and implications

Background

News Release
2008-59
April 18, 2008
For immediate release

Government of Canada Takes Action on Another Chemical of Concern: Bisphenol A

OTTAWA - The Honourable Tony Clement, Minister of Health, and the Honourable John Baird, Minister of the Environment, today announced that the Government is taking action to protect the health of Canadians and the environment from another chemical of concern.

The Precautionary Principle:

....., the Government of Canada is proposing to reduce bisphenol A (BPA) exposure in infants and newborns by the following actions:

1. To ban polycarbonate baby bottles
2. To develop stringent migration targets for BPA in infant formula cans
3. To work with industry to develop alternative food packaging and develop a code of practice
4. To list BPA under Schedule 1 of the Canadian Environmental Protection Act.

Background

- Scientific community has the feeling that systems for environmental protection have failed…..
- Medical community has the feeling that things are not as good as expected…..

*Prague Declaration on Endocrine Disruption
http://www.edenresearch.info/public/PragueDeclaration.pdf

Table 2
Residues of DDTs in placenta extracts

Pesticide	Mean[a]	±SD	Median[a]	Maximum[a]	Frequency (%)
p,p′-DDT	1.02	1.47	0.50	8.66	59.00
o,p′ DDT	0.60	0.78	0.50	3.55	58.94
p,p′-DDE	2.37	2.80	1.78	28.29	96.03
o,p′-DDD	1.42	2.47	0.50	19.01	56.70
∑DDTs	5.23	5.28	3.69	31.50	99.33

[a] ng/g of placenta.

Table 3
Residues of endosulphan in placenta extracts

Pesticide	Mean[a]	±SD	Median[a]	Maximum[a]	Frequency (%)
E-I	0.67	1.37	0.28	11.16	58.95
E-II	0.40	1.30	–	12.90	24.50
E-ether	0.12	0.19	0.10	1.39	52.32
E-diol	5.11	5.23	4.46	26.23	76.86
E-lactone	0.81	2.43	–	27.31	43.30
E-sulphate	0.68	1.44	–	8.29	47.70
∑Endosulphans	8.79	8.42	7.06	49.78	98.30

E = endosulphan; – = <LOD.
[a] ng/g of placenta.

Table 4
Residues of aldrin, endrin and dieldrin in placenta extracts

Pesticide	Mean[a]	±SD	Median[a]	Maximum[a]	Frequency (%)
Aldrin	0.24	0.60	–	4.79	26.49
Endrin	0.70	1.32	–	8.83	33.11
Dieldrin	0.25	0.59	–	3.68	22.51

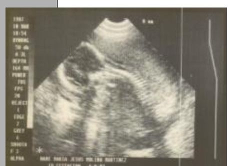
– = <LOD.
[a] ng/g of placenta.

Available online at www.sciencedirect.com
ScienceDirect
PLACENTA

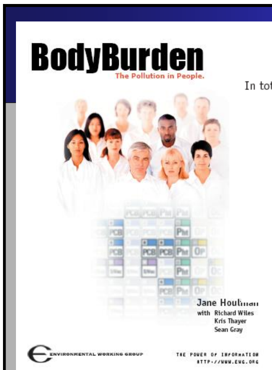
Organochlorine Pesticides in Placentas from Southern Spain and Some Related Factors☆

M.-J. Lopez-Espinosa *, A. Granada *, J. Cárreno *, M. Salvatierra *, F. Olea-Serrano *, N. Olea *,**

Inadvertent exposure to POPs



Organohalogens in placenta and in human milk



In total, the nine subjects carried:

- 76 chemicals linked to cancer in humans or animals (average of 53),
- 94 chemicals that are toxic to the brain and nervous system (average of 62),
- 86 chemicals that interfere with the hormone system (average of 58),
- 79 chemicals associated with birth defects or abnormal development (average of 55),
- 77 chemicals toxic to the reproductive system (average of 55), and
- 77 chemicals toxic to the immune system (average of 53).

Exposure monitoring (biomonitoring)

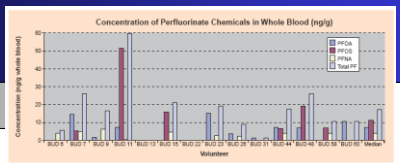
EL PAIS

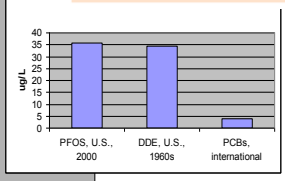
a sangre tóxica de la comisaria de la UE

Table 1: Ministers tested

Constantina Akkelidou	Cyprus	Minister of Health
Libor Ambrozek	Czech Republic	Minister of Environment
Hans Christian Schmidt	Denmark	Minister of Environment
Olavi Tammemäe	Estonia	Vice minister Environment
Jan-Erik Enestam	Finland	Minister of Environment
Serge Lepeltier	France	Minister of Environment
Miklós Persányi	Hungary	Minister of Environment
Mihaly Kokeny	Hungary	Minister of Health
Roberto Tortoli	Italy	Vice Minister Environment
Juozas Olekas	Lithuania	Minister of Health
Laszlo Miklos	Slovakia	Minister of Environment
Cristina Narbona	Spain	Minister of Environment
Lena Sommestad	Sweden	Minister of Environment
Alun Michael	UK	Minister of Environment

Chemical	Percentage of Ministers Contaminated
22 separate PCB congeners	100
p,p'-DDE (OC Pesticide)	100
Hexachlorobenzene (HCB) (OC Pesticide)	100
BDE-153 (Brominated Flame Retardant)	100
ß-HCH (OC Pesticide)	93
PFOA, PFNA (Perfluorinated Chemical)	75 (of the 12 tested)
DEHP (Di Ethyl Hexy Phthalate)	79





Definition

Endocrine Disrupter:

An exogenous substance that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

Mechanisms of Action of EDs

- Mimic or block hormone-receptor binding
- Alter hormone production, metabolism, excretion
- Modify hormonal transport or carrier protein
- Change hormone/receptor gene transcription activity
- Alter receptor levels in specific tissues

Mechanisms of action EDs

- Receptor binding
  - ER, AR, AhR, GR, (agonists and antagonists)
- Enzyme Inhibition
  - Steroidogenesis, thyroid peroxidase
- Enzyme induction
  - CYP450s, thyroxine conjugation
- Signal transduction pathways
  - Phosphatases/kinases, transcrip factors

Classes of EDs

Fungicides	Vinclozolin, Ketaconazole
Herbicides	Atrazine
Insecticides	DDT, Methoxychlor
Metals	Tributyltin, Cadmium
Pharmaceuticals	Ethynyl Estradiol
Phenols	Bisphenol A
Plasticizers	Phthalates
Polyaromatic Hydrocarbons	PCBs, dioxin
Soy Products	Genistein
Surfactants	Alkylphenol Ethoxylates, PFOS
Flame retardants	PBDEs

Sources of Exposure

- Farming, livestock and forestry practices
- Industrial chemicals
- Waste incineration
- Sewage discharge
- Human and industrial waste
- Consumer products
- Food
- Pharmaceuticals

Exposure scenario in ED

How are humans exposed to EDs?

Exposure occurs mainly through diet but also in an occupational setting

•Food, Water, Indoor air, Dust, Soil

Timing and duration of exposure

•Children: Pregnancy and Lactation

Table 1
Residues of organochlorine pesticides in adipose tissue

	Mean ng/g lipid	SD ng/g lipid	Maximum ng/g lipid	% frequency
Aldrin	25.56	24.66	137.20	40
Endrin	47.43	36.74	148.13	7
Dieldrin	17.01	16.75	84.05	28.5
Endosulfan-ether	1.04	0.78	3.98	68
Endosulfan-lactone	2.02	1.14	4.23	10.5
Endosulfan-diol	9.23	12.31	64.99	26
Endosulfan-sulfate	12.17	13.04	48.50	13.5
Endosulfan I	6.02	6.69	23.07	17
Endosulfan II	73.36	103.73	414.15	14
Total endosulfans	21.37	54.63	417.59	78
Lindane	17.44	17.84	113.31	55
o,p'-DDT	13.46	12.93	57.07	12
p,p'-DDT	61.01	51.20	246.51	39
p,p'-DDD	95.66	75.18	297.43	10.5
p,p'-DDE	508.83	410.54	2637.67	100
Total DDTs	543.25	432.51	2806.22	100
Methoxychlor	29.86	43.71	155.58	5.5

SD, standard deviation.

CHEMOSPHERE

Environmental and lifestyle factors for organochlorine exposure among women living in Southern Spain

I. Cerrillo [a], M.F. Olea-Serrano [b], J. Ibarluzea [c], J. Exposito [d], P. Torne [e], J. Laguna [f], V. Pedraza [a], N. Olea [b,*]

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Environmental Research

) 34–40

http://www.elsevier.com/locate/envres

Exposure of women to organochlorine pesticides in Southern Spain☆

Begoña Botella, Jorge Crespo, Ana Rivas, Isabel Cerrillo, Maria Fátima Olea-Serrano, and Nicolás Olea*

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Received 5 June 2003; received in revised form 30 September 2003; accepted 9 October 2003

Table 3
Residues of organochlorine pesticides in serum samples ($N = 220$)

	Mean (ng/mL)	SD (ng/mL)	Median (ng/mL)	Maximum (ng/mL)	Frequency (%)
Endosulfan I	2.10	2.81	1.47	19.39	80.40
Endosulfan II	1.31	0.88	1.00	6.85	34.40
Endosulfan-diol	15.39	14.87	9.56	76.86	92.00
Endosulfan-sulfate	2.17	5.92	0.50	53.32	45.10
Total endosulfans	25.76	21.79	18.66	145.55	100.00
o,p'-DDT	0.71	0.70	0.50	6.26	19.2
p,p'-DDT	3.64	4.91	1.85	40.96	57.6
o,p'-DDD	3.24	4.14	2.06	36.58	65.60
p,p'-DDE	5.18	4.07	4.15	25.88	96.00
2 DDTs	12.77	8.55	10.77	52.34	99.10

p,p'-DDT/*p,p'*-DDE ratio = 0.7.

Table 4
Residues of organochlorine pesticides in serum samples ($N = 220$)

	Mean (ng/mL)	SD (ng/mL)	Median (ng/mL)	Maximum (ng/mL)	Frequency (%)
Aldrin	3.75	4.32	2.62	33.76	79.0
Endrin	5.04	9.23	1.50	64.04	60.7
Dieldrin	1.85	2.74	0.50	29.42	40.7
Lindane	1.84	2.27	1.19	17.72	64.70
Methoxychlor	2.84	5.09	1.47	53.80	60.70
Hexachlorobenzene	3.88	4.50	2.31	30.29	79.9

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Environmental Research

www.elsevier.com/locate/envres

Exposure of young men to organochlorine pesticides in Southern Spain

Javier Carreño[a], Ana Rivas[a], Alicia Granada[a], Maria Jose Lopez-Espinosa[b], Miguel Mariscal[a], Nicolas Olea[b], Fatima Olea-Serrano[a,*]

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Received 16 January 2006; received in revised form 14 June 2006; accepted 20 June 2006

Exposure to PCBs & Dioxins

United Nations Environment Programme

UNEP

PCB Transformers and Capacitors

From Management to Reclassification and Disposal

PCBs were used since 1929 in various electrical applications.

While no PCBs are longer produced, they can be found in older electrical installations and marine sediments.

Dioxins are produced as a by-product during paper manufacturing, incineration and production of chlorinated aromatics.

These compounds are very persistent and continue to cycle in the environment.

Exposure to PCBs

Mean concentrations in woman adipose tissue

Mean PCBs ng/g	Mean OH-PCBs ng/g
566,318	0,0028

Spanish National Plan for PCB elimination
A census of 250.000 Tm

The Belgian PCB and Dioxin Incident of January–June 1999: Exposure Data and Potential Impact on Health

Nik van Larebeke,[1] Luc Hens,[2] Paul Schepens,[3] Adrian Covaci,[3] Jan Baeyens,[4] Kim Everaert,[4] Jan L. Bernheim,[5] Robert Viaminck,[6] and Geert De Poorter[6]

[1]Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, Ghent University, Belgium; [2]Human Ecology Department, Free University Brussels, Belgium; [3]Toxicological Center, Antwerp University, Antwerp, Belgium; [4]Department of Chemical Engineering, and [5]Department of Human Genetics, Catholic University, Leuven, Belgium; [6]Federal Ministry of Agriculture, Brussels, Belgium

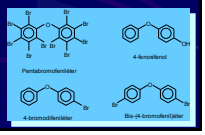
In January 1999, 100 tons of feed contaminated with approximately 50 kg of polychlorinated biphenyls (PCBs) and 1 g of dioxins were distributed to animal farms in Belgium, and to a lesser part of a PCB contamination, and average PCB/dioxin ratios were determined. During the first days of June, the Ministry of Public

Exposure to OBrs

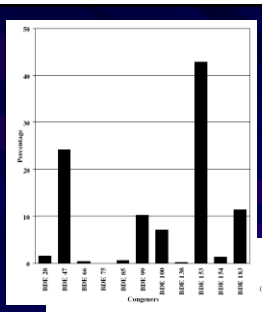
Brominated flame retardants (BFRs) used in polymers and textiles and applied in construction materials, furniture, and electronic equipment.

BFRs with the highest production volume are:

- polybrominated diphenyl ethers
- tetrabromobisphenol A (TBBP-A)
- hexabromocyclododecane (HBCD)



Inadvertent exposure to PBDEs and PBBs



CHEMOSPHERE

(2006) xxx–xxx

www.elsevier.com/locate/chemosphere

PBDEs and PBBs in the adipose tissue of women from Spain

M.F. Fernandez [a], P. Araque [a], H. Kiviranta [b], J.M. Molina-Molina [a], P. Rantakokko [b], O. Laine [b], T. Vartiainen [b,c], N. Olea [a,*]

[a] Laboratory of Medical Investigations, Clínico University Hospital, University of Granada, 18071 Granada, Spain
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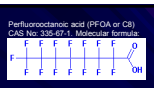
[c] Department of Environmental Sciences, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland

Exposure to OFs



Perfluorinated chemicals (PFCs), used since the 1950s, designed to repel grease and water in:

Stain- resistant coatings such as Scotchgard and Stainmaster for carpets, couches, and other upholstered furniture and automobile seat

Water-repellent like Gore-Tex

Exposure to phthalates

Phthalates have been widely used as plasticizers in many products since the 1930s.

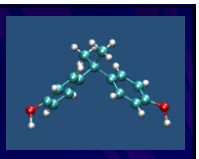
Found in plastic wrap, PVC, vinyl flooring, and ink used to print on plastic containers.

High levels of DEHP in some products used in vehicles like brake pads, serpentine belts and tires.

Phthalates are used in some cosmetics and some packaging.

Phthalates in perfumes and cosmetics

Exposure to bisphenols



More than 1.2 10^6 Tm/year of Bisphenol-A (BPA) are produced in the EU.

BPA is used in epoxy resins and polycarbonate plastic
It is an additive in acrylic and vinylic resins and in synthetic rubber, as well as in many other products: inks, tonners, brake fluids….

Beside BPA, Bisphenol-F, Bisphenol A-F, Bisphenol-S, Bisphenol-C, BADGE and Bis-GMA are bisphenols of concern

Exposure to bisphenols

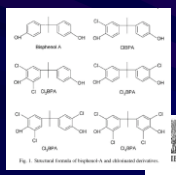


Table 2
Concentrations of BPA (ng/g) and chlorinated derivatives in adipose tissue samples

	n (%)	>LOD					
		Mean (±S.D.)	Median	5th	25th	75th	95th
BPA	11/20 (55)	5.83 (3.48)	4.79	2.07	3.54	7.12	11.88
Cl-BPA	3/20 (15)	3.05 (0.28)	3.14	2.82	2.94	3.21	3.26
Cl_2-BPA	16/20 (80)	9.21 (9.26)	7.77	2.61	5.62	8.83	21.49
Cl_3-BPA	2/20 (10)	0.74 (0.15)	0.74	0.66	0.69	0.80	0.84
Cl_4-BPA	0/20 (0)	<LOD	<LOD	–	–	–	–
∑BPA	16/20 (80)	9.00 (9.22)	8.12	0	1.93	9.60	28.25

n (%), number of subjects (percentage of detection); LOD, Limit of detection.

Available online at www.sciencedirect.com
ScienceDirect
Reproductive Toxicology xxx (2007) xxx–xxx
Reproductive Toxicology

Bisphenol-A and chlorinated derivatives in adipose tissue of women

M.F. Fernández[a,*], J.P. Arrebola[a], J. Taoufiki[b], A. Navalón[b], O. Ballesteros[b], R. Pulgar[a], J.L. Vílchez[b], N. Olea[a]
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[b] Research Group of Analytical Chemistry and Life Sciences, Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain

Food Additives and Contaminants, January 2007; 24(1): 95–102
Taylor & Francis

Oestrogenicity of paper and cardboard extracts used as food containers

M.-J. LOPEZ-ESPINOSA[1], A. GRANADA[1], P. ARAQUE[1], J.-M. MOLINA-MOLINA[1], M.-C. PUERTOLLANO[1], A. RIVAS[2], M. FERNÁNDEZ[1], I. CERRILLO[1], M.-F. OLEA-SERRANO[2], C. LÓPEZ[3] & N. OLEA[1]

[1]*Laboratory of Medical Investigations, San Cecilio University Hospital, University of Granada, E-18071 Granada, Spain,* [2]*Department of Nutrition and Food Sciences and* [3]*Department of Physical Chemistry, University of Granada, E-18071 Granada, Spain*

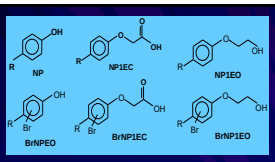
Table I. Estimated values of oestrogenicity (pM oestradiol equivalents Eeq g^{-1} of cardboard or paper) and the frequency of positive samples in the E-Screen assay of paper and cardboard extracts.

	Total samples ($n=40$)	Cardboard ($n=32$)	Paper ($n=8$)
Arithmetic mean	60.52	64.24	45.61
Geometric mean	11.97	13.33	7.78
Median	18.69	21.51	16.80
Standard deviation	12.55	13.02	12.09
Range	0.01–355.50	0.01–355.50	0.08–280.90
Frequency (%)	90.00	90.63	87.50

Table II. Levels and frequency of BPA, DBP and DEHP (ng g^{-1} of cardboard or paper) in samples.

Residues	Total samples ($n=40$)	Cardboard ($n=32$)	Paper ($n=8$)
BPA			
Arithmetic mean	97.34	115.32	25.43
Geometric mean	2.38	2.74	1.35
Median	0.52	0.52	0.49
Standard deviation	16.33	17.68	12.72
Range	0.05–1817.00	0.05–1817.00	0.08–188.00
Frequency (%)	45.00	46.88	37.50
DBP			
Arithmetic mean	713.17	706.32	740.49
Geometric mean	37.39	20.97	377.63
Median	121.84	75.62	548.55
Standard deviation	35.61	41.65	3.95
Range	0.10–10774.00	0.10–10774.00	29.10–3049.00
Frequency (%)	67.50	59.38	100
DEHP			
Arithmetic mean	3901.56	706.32	740.49
Geometric mean	341.74	302.11	559.54
Median	893.48	814.44	2751.84
Standard deviation	23.27	22.53	31.36
Range	0.52–61013.00	0.52–61013.00	1.53–10198.00
Frequency (%)	77.50	78.13	75.00

Exposure to alkylphenols



Detergents contain surfactants, such us nonylphenol, used to improve cleaning.

Alkylphenols are also used as carriers for some pesticides to make the pesticide stick to the plant.

They are used as plasticizers and UV stabilizers in plastics.

Exposure to UV-filters

Benzophenone-3 (BP-3)
Octyl-methoxycinnamate (OMC)
3-(4-methylbenzylidene) camphor (4-MBC)

….once they are absorbed by the skin they modify hormone levels

Janjua et al., Journal of Investigative Dermatology (2004) **123**, 57–61
Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans

Some putative outcomes in humans

- Urogenital malformations (male)
- Impaired spermatogenesis (male)
- Breast cancer (female)
- Sexual maturation (female)
- Endometriosis (female)
- Sex ratio
- Immune effects
- Prostate and thyroid carcinogenesis

Working Hypothesis

Carlsen *et al.*, (2008), Human Reprod **23(1)**: 201-10

Swan *et al.*, Int J Androl 1997, 2000.

The exposure to environmental pollutants (EDs) may cause diseases in humans:

Several studies indicate that semen quality has been declining during the past half century in industrialized countries, with remarkably geographical variations

Epidemiology

•Epidemiology studies show a strong evidence of association between persistent organic pollutants **(PCBs, DDE, TCDD, PFOA,..)** and abnormal sperm quality, especially on **motility**

….while other effects (sperm count and morphology, or testicular function) are less convinced

Clinical Observation

Swan *et al.*, (2005), EHP **113**:1056-61

- Exposure to ED, during fetal life, may contribute to the increased in male reproductive health problems

- Decrease in anogenital distance among male infants with prenatal phthalates exposure

Clinical Observation

Shen *et al.*, (2008), Human Reprod **23(1)**: 201-10

Krysiak-Baltyn *et al.*, Int J Androl 2009.

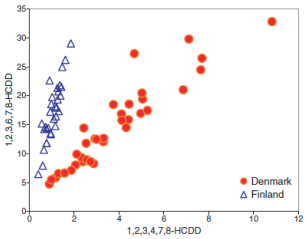


Figure. The distribution in the concentration of 1,2,3,4,7,8-HCDD in maternal milk is completely different (Denmark or Finland), maybe related with different incidence of male reproductive disorders

Exposure scenario in ED

- Multiple exposure
- "Low doses"
- During large periods of time
- Delay of effects against time of exposure
- Health problems cause by multiple factors

Exposure scenario in ED

- **The association remains elusive**

- The hypothesis cannot be tested on the basis of individual compounds

- A comprehensive study of all possible EDs --metabolites, isomers and congeners would be an ideal approach

Exposure scenario in ED

Synergetic, additive, and/or antagonistic interactions between chemicals and hormones should be considered

Different methods have been proposed by Soto and coworkers, Kortenkamp and coworkers, and others to overcome the unpredictability of xenoestrogen interactions.

Challenge for epidemiology

Take account of specific exposure scenario relevant to adverse health effects

Low level exposure to large numbers of chemicals

Total Effective Xenoestrogen Burden (TEXB)

Biomarker of exposure to mixtures of xenoestrogens

- Goes beyond the quantification of environmental estrogens
- Measures the biological activity resulting from xenoestrogens
- Biomarkers in a continuum of disease development

Higher TEXB-alpha: a risk factor for genital tract malformations

TEXB,

Fernandez *et al.* (2007), EHP **115 (S1)**, 8-14

Objective: To determine whether the combined effect of environmental estrogens measured as the total effective xenoestrogen burden is a risk factor for cryptorchidism and hypospadias

Prospective cohort study with 668 boys recruited at the time of delivery between October 2000 to June 2002.

- A nested case-control study was selected.

- 50 cases and 114 controls.

Higher TEXB-alpha: a risk factor for genital tract malformations

TEXB,

Fernandez *et al.* (2007), EHP **115 (S1)**, 8-14

-OR adjusted for mother's age and weight of newborn in a conditional regression analysis

OR = 2.82 (95% CI = 1.10-7.24)

For values above detection limit (DL= 0.5 pM Ee/g placenta) compared with values below DL

Mean values were not statistically significant different: 3.92 ± 9.12 and 2.08 ± 7.19 pM Ee/g plac, for cases and controls

Research questions

- What are the chemical classes and their potencies?
- What are the dose-response characteristics in the low-dose region?
- Are testing guidelines adequate?
- What extrapolation tools are needed?

Research questions

- How does exposure take place?
- What are the major sources and fates?
- What are the cumulative effects of exposure?
- What effects are occurring in populations?
- How can unreasonable risks be managed?

Thank you

Rigshospitalet

Department of Growth and Reproduction

Secular trends in Timing Puberty and Role for Environmental Exposures

Anders Juul

Rigshospitalet

Genetic influence on menarcheal age

Pubertal timing depends on:

- Genetic factors (no single ”puberty” gene)
- Environmental/lifestyle factors
  - Fat mass?
  - Insulin resistance?
  - Dietary factors?
  - Physical fitness?
  - Phychological factors?

Rigshospitalet

Background

Definition of Precocious Puberty

Development of secondary sexual characteristics < 8 years

Etiology

Central (CPP) { Idiopatic (ICPP) / Organic (OCPP) }

Peripheral (PPP)

Premature thelarche

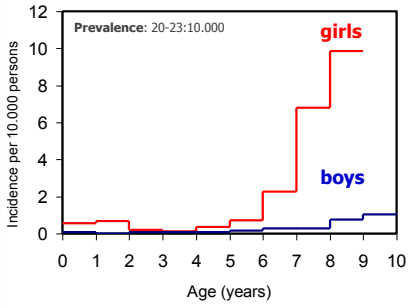
Premature adrenarche

Hypothyroidism

MR

Rigshospitalet

Incidence and prevalence of precocious puberty in Denmark



Teilmann et al. **Pediatrics 2005**

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Increasing incidence of Precocious Puberty in Denmark

Studies	Year	DK population	Incidence
Thamdrup	1961	4.5 mio	3-4 per year
Teilmann	2002	5.3 mio	50-70 per year*

Teilmann, et al. Pediatrics 2005

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Methods and materials

Registry from Department of Growth and Reproduction in the period 1993-2009 on the following patient categories (ICD10 codes):

- Precocious Puberty (DE228A)
- Premature thelarche (DE308A)
- Premature adrenarche (DE270B)
- Early pubertal variant (DE301)

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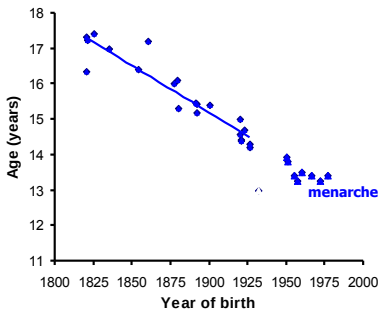
Epidemiological studies of menarche



Olesen et al. Ann Hum Biol 2000; 27:377-86

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Timing of pubertal growth spurt

- 149.992 girls born between 1930 and 1969 included
- Annual health examinations including height in Copenhagen Municipality were computerized.
- 135.223 girls fullfilled criteria for determination of OGS and PHV.

Aksglæde et al. **PLoS ONE 2008**; 3(7):e2728.

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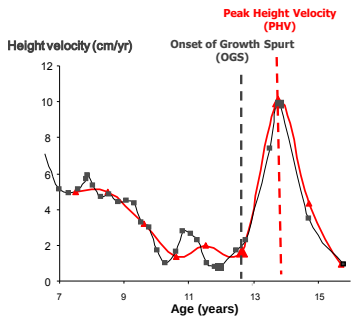
Mathematical modelling of pubertal growth



Aksglæde et al. **PLoS ONE 2008**; 3(7):e2728.

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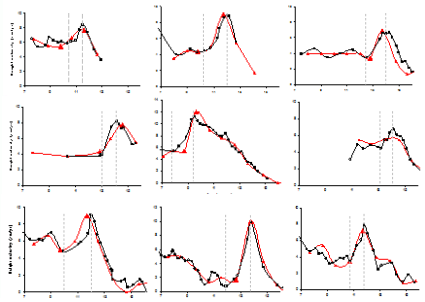
Timing of pubertal growth spurt



Aksglæde et al. **PLoS ONE 2008**; 3(7):e2728.

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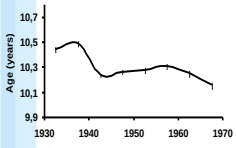
Secular trend in timing of pubertal growth spurt

Age at OGS



Age at PHV

N=135.223 girls

Aksglæde et al. **PLoS ONE 2008**; 3(7):e2728.

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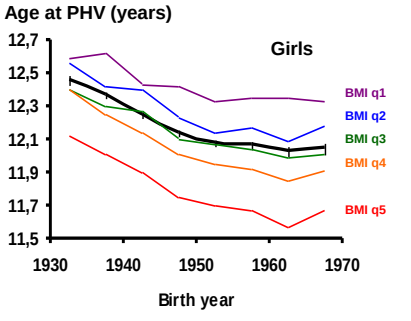
Obesity and secular trend in puberty timing



Aksglæde et al. **PLoS ONE 2010, in press**

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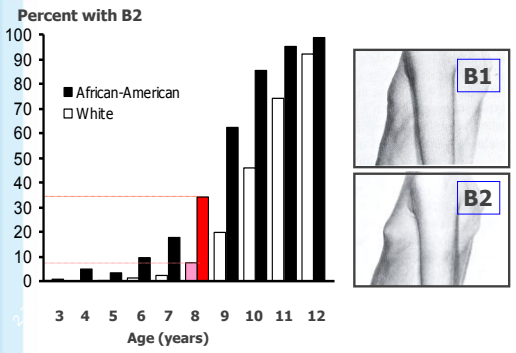
Breast Development in US Girls



Herman-Giddens et al. Pediatrics 1997; 99: 505-12

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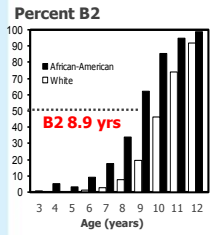
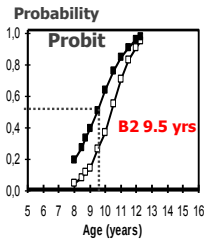
Breast Development in US Girls

PROS (1991-92)



Herman-Giddens et al. **Pediatrics 1997**; 99: 505-12

NHANES III (1988-94)



Sun et al. **Pediatrics 2002**; 110: 911-9 (B2)
Chumlea et al. **Pediatrics 2002**; 110: 752-7 (M)

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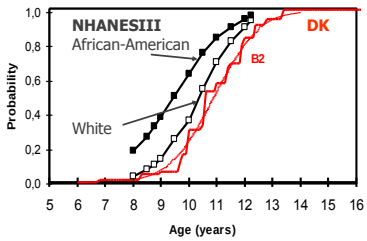
Breast development in Girls in Denmark 1992



Juul A et al. **Int J Androl 2006**; 29: 247-255
Sun et al. **Pediatrics 2002**; 110: 911-19

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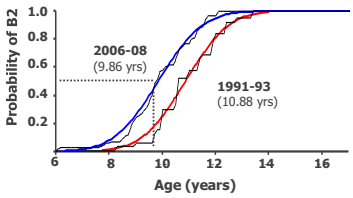
The COPENHAGEN Puberty Study - GIRLS



P<0.0001

Juul A et al. Int J Androl 2006; 29: 247-255, Aksglaede L et al. Pediatrics, 2009, May;123(5):e932-9

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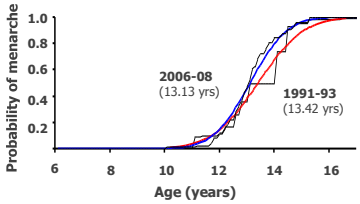
The COPENHAGEN Puberty Study - GIRLS



P=0.023

Juul A et al. Int J Androl 2006; 29: 247-255, Aksglaede L et al. Pediatrics, 2009, May;123(5):e932-9

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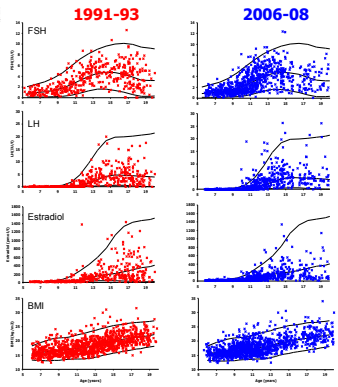

Aksglæde et al. **Pediatrics 2009**

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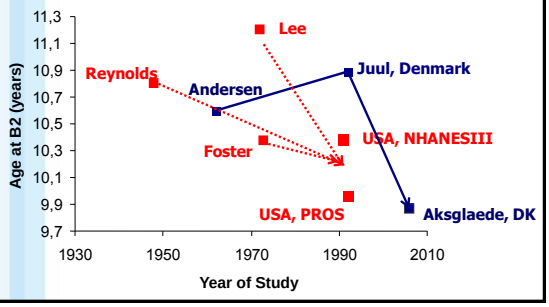
The COPENHAGEN Puberty Study - GIRLS



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The COPENHAGEN Puberty Study - GIRLS

Lise Aksglæde et al. Pediatrics
April 27, 2009

Tara Parker-Pope, New York Times
May 4, 2009

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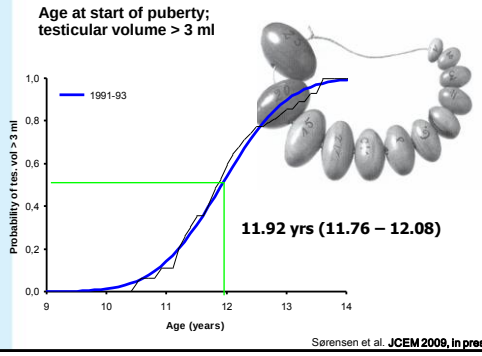
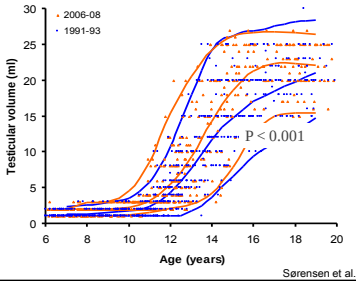
The COPENHAGEN Puberty Study - BOYS



Sørensen et al. **JCEM 2009, in press**

Rigshospitalet

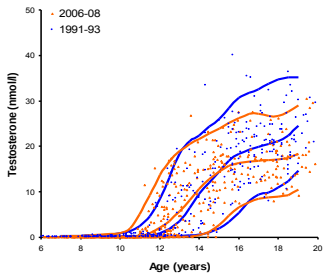
The COPENHAGEN Puberty Study - BOYS



Sørensen et al. **JCEM 2009, In press**

Rigshospitalet

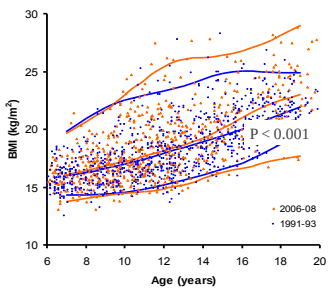
The COPENHAGEN Puberty Study - BOYS



Sørensen et al. **JCEM 2009, In press**

Rigshospitalet

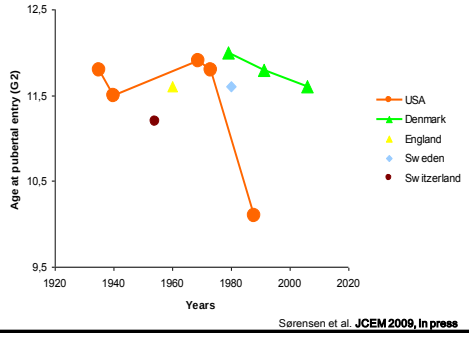
The COPENHAGEN Puberty Study - BOYS



Sørensen et al. **JCEM 2009, In press**

Rigshospitalet

The COPENHAGEN Puberty Study - BOYS



Sørensen et al. **JCEM 2009, In press**

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CONCLUSION

1. **GIRLS**

   Marked earlier breast development (1 year) in Danish girls from 1991 to 2006

   - effect of BMI

2. **BOYS**

   Slightly earlier testicular growth (0.35 years) in Danish boys from 1991 to 2006.

   + effect of BMI

3. **Secular changes must be attributed to environmental factors** (i.e. non-genetic)

Lifestyle Factors as Indicators of Male Reproductive Function

Sally Perreault Darney, Ph.D.

Basic course on environment and human male reproduction

ESHRE, June 27, 2010, Rome

Are Lifestyle factors associated with semen quality?

- Factors (amount of evidence)
  - Smoking (+++)
  - Alcohol (++)
  - Caffeine (+)
  - Obesity (+)
  - Drugs" (who me?)
  - Mobile phones (+)
  - Stress (+)
- Endpoints (amount of evidence)
  - Sperm count/conc (+++)
  - Sperm motility (++)
  - Quality of motion (+)
  - Sperm morphology (++)
  - Sperm DNA damage (+)
  - Sperm chromosome abnormalities (+)

Smoking & Semen Quality

Is smoking an independent risk factor for poor semen quality or fertility?

- If so, then doctors can advise patients accordingly and with confidence ("Quit smoking and your sperm count will improve.")
- Evidence is extensive for lower sperm numbers and plentiful for morphology and motility, but inconsistent in findings, with some studies showing associations and some not (Collodel et al. 2010; Vine et al., 1994)

Impact may depend on both amount of exposure (# cigarettes/day) and duration (pack years)

Lifestyle factors may co-occur. Additive or synergistic?

- Smoking and drinking? ("Pub" lifestyle, Rubes et al., 1998)
- Abuse of alcohol and drugs? Unhealthy lifestyles, poor nutrition.
- Smoking and vitamin C (protective?)

Context: Environmental Exposures

Does smoking change the relationship between an exposure and an outcome?

- If so, then it needs to be considered a confounder in environmental or occupational studies
  - E.g. Air pollution + cigarette smoke share physical/chemical properties
- Challenge: Impact likely small in both cases and hard to distinguish

Biological Plausibility

- Cigarette smoke contains:
  - Poly-cyclic aromatic hydrocarbons that can be metabolized to carcinogenic intermediates
  - Cadmium
  - Particles resulting from combustion (not unlike air pollution)
  - Nicotine
- Creates hypoxia (CO)
- Could have acute or chronic effects on testis/sperm

Are men with inherently poor semen quality more susceptible to effects of smoking?

- Collodel et al., 2010, evaluated effects of smoking in men with idiopathic infertility but without other risk factors (screened for genetics, alcohol & drug abuse, occupational exposures or health factors).
- And were either smokers or never smoked.
- Smokers classified: Cigs/day: 1-10 (mild), 11-20 (moderate) or >20 (heavy).
- Ultrastructure, and routine semen measures
- Infertile men differed from a control group, i.e., men with normal semen quality (WHO), but within the infertile group, smokers did not differ from non-smokers except for sperm concentration and a Fertility Index which were reduced in the heavy smokers only.

Are fertile men less susceptible to smoking and other lifestyle exposures?

- **The "Healthy Men Study" (HMS)**
  - Partners of pregnant women in a pregnancy outcome study
  - Exposure of interest: Disinfection byproducts (DBPs) in drinking water
  - Men lived in community with low DBPs, or high chlorinated DBPs or high brominated DBPs.
  - Exposure carefully characterized
  - Semen: Count/conc., morphology, and DNA damage (SCSA-%DFI) and immaturity (SCSA-%HDS)
  - No differences were found based on DBP exposures (Luben et al, 2007), adjusting for other factors.

Analysis of Lifestyle Exposure factors in HMS

- **Smoking**: current, former or never; 0, 1-10, or >10 cigarettes/day, and # years smoked (0, 1-5, 6-10 and >10). Pack years: #/day /20 x #years.
- **Alcohol**: calculated based on average # drinks [beers (12 oz), wine (4 oz) and hard liquor (1oz)] and categorized by # drinks/week: 0-7, 8-15 and >15.
- **Caffeine**: Based on Coffee (and other caffeinated drinks), mg caffeine/day was calculated and categorized: none, >0 to 150 (low), >150-300 (moderate) and >300 (high = 3 cups coffee).

Statistical Analysis in HMS

- Lifestyle exposure factors were examined (controlling for study site, age, income, education, abstinence interval, history of chronic or serious illness, body mass index (BMI), with other study exposures (smoking, alcohol, caffeine) as potential confounders.
- Multiple linear regression was used to estimate associations of each lifestyle exposure factor and each outcome. Full model (with all covariates) was evaluated for each covariate and only those that changed the parameter estimate of the exposure variable by at least 10% were retained. Age, sexual abstinence, income and study site were retained as obligate, along with any factor that met the criteria for confounding
- Semen outcomes were also dichotomized when possible for logistic regression: percent normal forms at <15%; and, SCSA %DFI at >30% according to the literature.

HMS: Characteristics

- 229 men from 3 study sites
  - Young: 25-34 years of age (70%)
  - White, non-hispanic (84%)
  - Most college educated with income >$40K
  - Overweight or obese by BMI (73%)
  - Smokers: 16% (vs. 23.4% nationally)
    - Former smokers: 18%
    - Only 2 men smoked >1 pack/day
  - Non-drinkers (29%) or <1 drink/day (55%) with only 6% heavy drinkers (>14 drinks/week)
  - Caffeine consumers (83%) with 26% ingesting >300mg/day

HMS semen quality: Better than most published cohorts

- Mean concentration: 110 mil/ml
- Mean count: 348 mil
- Mean % normal forms: 14
- Mean % DFI: 19
- Mean % HDS: 8

HMS examined independent effects of lifestyle exposures

- **Current smoking** was not associated with semen decrements, even SCSA %DFI. Previous smoking (>30 days ago) was associated with improved semen quality (unexplained).
- **Drinking** was not associated with semen decrements; on the contrary light to moderate drinking appeared to be protective for some outcomes.
- **Caffeine intake** was weakly associated with fewer normal forms

BMI and semen quality in the HMS

- **Excess body fat** is thought to alter the endocrine balance which could impact sperm production and function but very few studies have examined the impact of BMI on semen quality.
- A few studies found BMI associated with decrements in semen measures (e.g. Kort et al., 2006; Hammoud et al., 2008) while others did not (e.g. Aggerholm et al., 2007)
- Among HMS volunteers, nearly half were overweight (BMI 25-29.9) and 25% were obese (BMI >30)

Obesity was not a risk factor in HMS

- After controlling for confounders and other factors, BMI was not associated with semen decrements; rather obese men had higher sperm concentrations.
- HMS participants, in general, appear to have healthy lifestyles with fewer smokers than the general population and few heavy drinkers in this cohort.
- Perhaps this combination of healthy living contributes to their above average semen quality even though, as a group, they are overweight.
- The literature on obesity is limited and more studies, including those that evaluate both semen quality and hormone profiles, will be needed to explain discrepant findings across existing studies.

Are some men more susceptible than others? What about susceptibility genes?

- A negative association between air pollution and DNA damage (SCSA DFI) was more significant when men were stratified by GSTm1 genotype.
- Men with GSTm1 null genotype were more susceptible than those with the normal gene (and enzyme) to air pollution-induced DNA damage. (Rubes et al., 2007).
- Other semen outcomes were not significantly associated with air pollution or genotype. Results were comparable with or without smoking in the model.

Conclusions

- A number of lifestyle exposure and metabolic factors may impact male reproduction, either singly or together.
- It is difficult to detect significant impacts of any one factor against a backdrop of exposures from other factors and from exogenous environmental exposures to man made chemicals.
- New exposure factors such as cell phones also have the potential to disrupt male reproduction.
- Very little is currently known about the mechanisms behind observed associations, how lifestyle factors may interact, and whether some men are inherently more vulnerable than others.
- The HMS findings show that, on average, men in this fertile cohort have above average semen quality and below average consumption of cigarettes and alcohol.

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- Vine MF, Margolin BH, Morrison HI, Hulka BS. Cigarette smoking and sperm density: a meta-analysis. Fertil. Steril. 1994;61:35-43.

HYPOSPADIAS AND CRYPTORCHIDISM AND ENVIRONMENT

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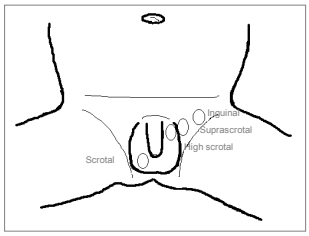
ESHRE 2010

Disclosures

- I have received lecture honoraria from Eli Lilly, Merck-Serono, Novo Nordisk Pharma, and Pfizer during the past year
- The studies have been supported by grants from
  - EU, Envir. Reprod. Health, Expored, Eden, DEER
  - Academy of Finland
  - Pediatric Research Foundation
  - Sigrid Juselius Foundation
  - Turku University Hospital

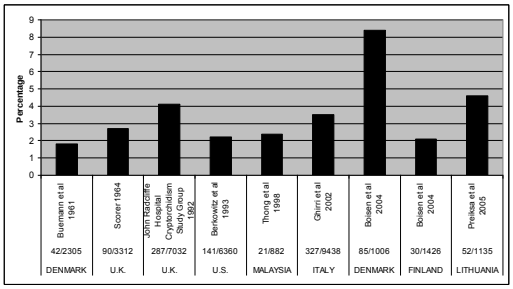
Cryptorchidism: classification



Incidence of cryptorchidism

- Congenital cryptorchidism
  - Increasing trends in some countries
  - Diagnostic controversies
- Acquired cryptorchidism (ascending testis)
- Orchiopexy rate

Incidence of cryptorchidism



Virtanen HE and Toppari J, Hum Reprod Update, 2007

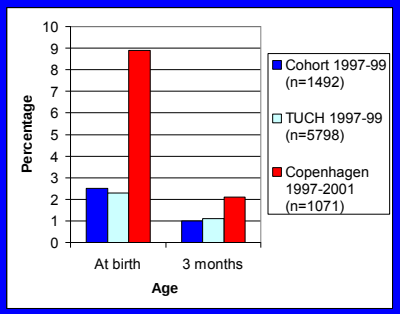
Material (Cohort study)

- All newborn boys of volunteer mothers (born 1997 -1999 in Turku, n=1455, 1997-2001 in Copenhagen, n=1046)
  - Questionnaire during pregnancy
  - Clinical examinations
    - All boys: At birth and at 3 months of age
    - Cases and controls: Also at 18 months of age
  - Biological samples
    - Blood sample around 10 - 15 gestational weeks
    - Placenta
    - Breast milk (4-8 weeks after birth)
    - Blood sample at 3 months of age

Case-control -study

- All boys with genital malformations and healthy control boys of volunteer mothers (born in TUCH 1.1.1997 - 28.2.2002)
  - Questionnaire after birth
  - Clinical examinations
    - At birth, at 3 months of age and at 18 months of age
  - Biological samples
    - Blood sample around 10 - 15 gestational weeks
    - Placenta
    - Breast milk (4-8 weeks after birth)
    - Blood sample at 3 months of age

Prevalence of cryptorchidism

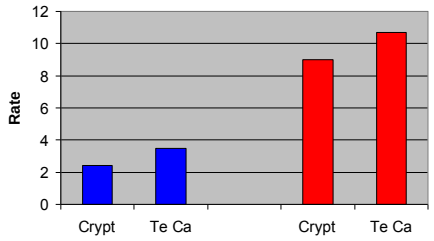


Boisen et al., Lancet 2004

Risks related to cryptorchidism

- Testicular cancer
  - 4-5 fold risk; 5-7 % of testis cancer patients
  - Age at treatment may or may not modify risk
- Infertility
  - 35-50 % of bilateral, 10-20 % of unilateral
  - Lower age at treatment decreases the risk

Incidence of cryptorchidism (%) and testicular cancer (n/100 000) in Finland and Denmark



Cryptorchidism data from Boisen et al., Lancet 2004;
Cancer data from WHO/IARC 1998

Prevalence and risk of cryptorchidism at expected date of delivery

	No cryptorchid / no examined		Prevalence %		Odds Ratio (95%)
	Denmark	Finland	Denmark	Finland	Denmark/Finland*
BW< 2500g	9/40	5/29	22·5	17·2	1·5 (0·4-5·5) †
BW≥ 2500g	85/1006	30/1426	8·4	2·1	4·8 (3·0-7·5) †
GA< 37 wk	13/60	4/61	21·7	6·6	3·8 (1·1-12·7) ‡
GA≥ 37 wk	81/986	31/1394	8·2	2·2	4·3 (2·8-6·7) ‡
WGA<-2SD	6/39	2/26	15·4	7·7	2·2 (0·4-12·5) §
WGA≥-2SD	88/1007	33/1429	8·7	2·3	4·5 (2·9-7·0) §

BW = birth weight, GA = gestational age, and WGA = weight for gestational age

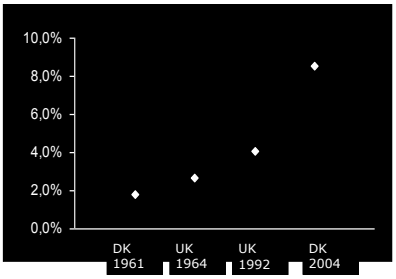
SGA and risk of cryptorchidism

- Finland: total hospital cohort
  - Relative risk of cryptorchidism in boys being SGA:

    3.0 (95% CI 1.7-5.5), p=0.002

    (at the expected date of delivery)

Trends in cryptorchidism



DK 1961: Buemann B et al. Acta Chir Scan 1961

UK 1964: Scorer CG. Arch Dis Child 1964

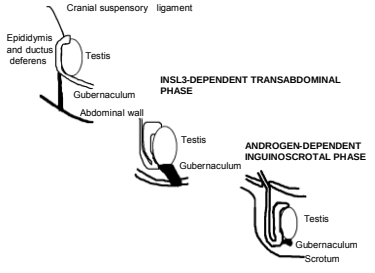
UK 1992: John Radcliffe Hospital Cryptorchidism Study Group. Arch Dis Child 1992

DK 2004: Boisen et al. Lancet 2004

Incidence of acquired cryptorchidism

- 1.1 – 2.2 % between 6-13 years; Hack et al. Arch Dis Child 2007; Sijstermans et al. Int J Androl 2008
- 0.3 - 0.6 % between ½ - 3 years; Wohlfahrt Veje et al. Int J Androl 2009
- Cumulative 7 % at 2 years; Acerini et al. Arch Dis Child 2009

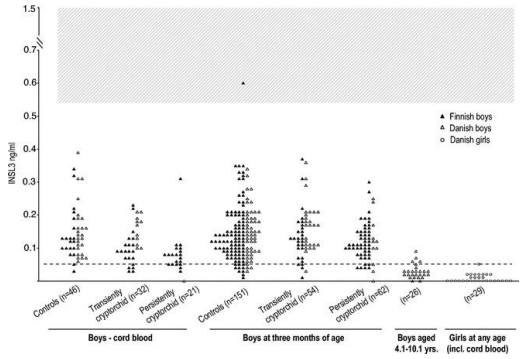
Phases of testicular descent



Toppari et al. Mol Cell Endo 2006

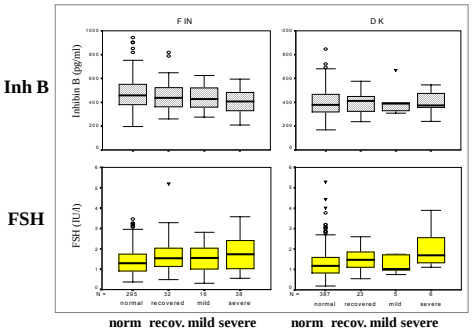
Regulation of Insl3

- expression in fetal and adult Leydig cells
- indirect regulation by LH (Leydig cell effect)
- down-regulated by diethylstilbestrol (Emmen et al. Endocrinology 141: 846, 2000)
- mechanism of estrogen-induced cryptorchidism (Nef et al. Dev Biol 24: 354, 2000)



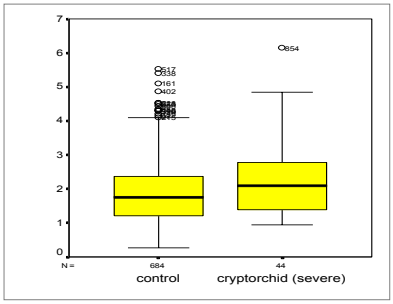
Bay et al., JCEM 2007

Inhibin and FSH

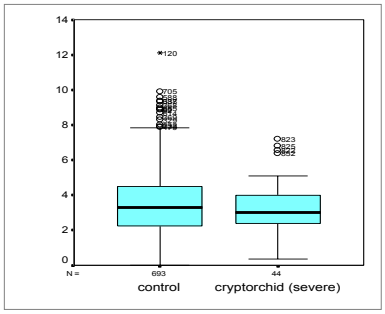


Suomi et al. JCEM 2006

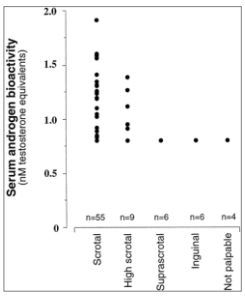
LH



Testosterone



Androgen bioactivity



Raivio et al., JCEM 2003

Hormone levels in cryptorchidism

- At 3 months, boys with severe cryptorchidism have
  - elevated LH and FSH levels
  - low inhibin B levels
  - unmeasurable testosterone bioactivity
  - normal testosterone levels
  - decreased INSL-3 levels
- These findings point to primary testicular failure associated to cryptorchidism

Risk factors

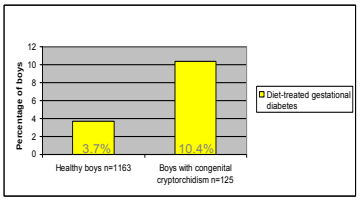
- Small for gestational age
- Prematurity
- Impaired glucose tolerance during gestation
- Alcohol with no lower limit
- Exposure to estrogenic and anti-androgenic compounds

Abnormal maternal glucose metabolism and cryptorchidism

- 1163 singleton newborn Finnish boys with normal testicular descent
- 125 newborn singleton Finnish boys with cryptorchidism

- Information about abnormality of maternal glucose metabolism during pregnancy was obtained from hospital records after delivery

Prevalence of diet-treated gestational diabetes



Diet-treated GDM: cryptorchid boys vs. controls

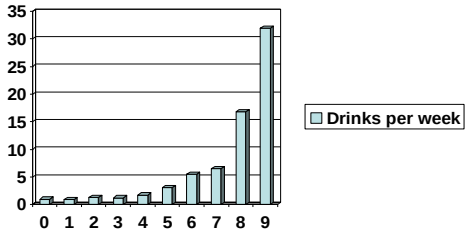
OR=3.98*

(95% CI 1.97 – 8.05)

*adjusted for maternal age >39 years at delivery, maternal smoking during pregnancy, prematurity, weight for gestational age

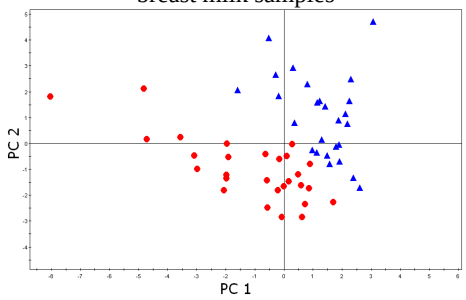
Virtanen et al. JCEM 2006

Alcohol and risk of cryptorchidism

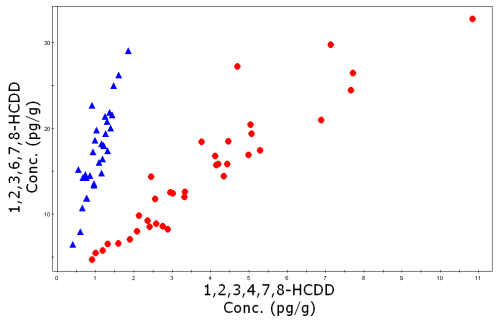


Damgaard et al., Environ. Health Perspect. 2007

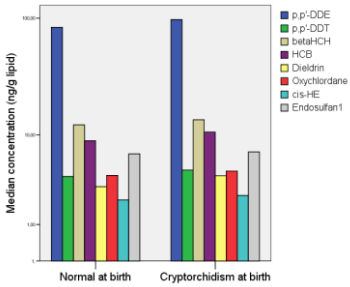
Chemical signatures in Danish and Finnish breast milk samples



Typical dioxin profiles in Danish and Finnish breast milk samples



Pesticides in Human Breast Milk



Damgaard IN et al, EHP 2006

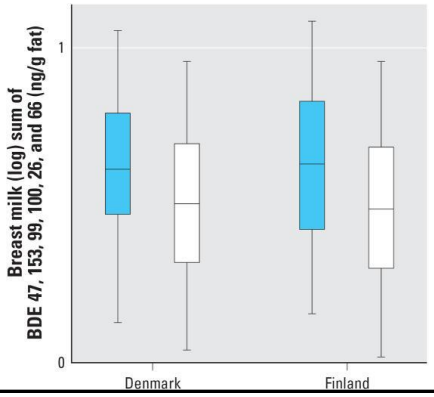
Results

Pesticides (ng/g lipid)	Cryptorchidism (n=62)[1]		Normal (n=68)[1]
p,p´-DDE	**97.3** *(51.6-152.4)*		**83.8** *(58.0-144.7)*
p,p´-DDT	**4.6** *(3.3-6.6)*		**4.0** *(3.1-6.1)*
β-HCH	**13.6** *(10.2-19.3)*		**12.3** *(9.3-18.0)*
HCB	**10.6** *(7.9-13.5)*	>	**8.8** *(7.6-12.6)*
α-endosulfan	**6.9** *(4.1-10.8)*		**6.7** *(3.9-8.4)*
dieldrin	**4.1** *(2.4-5.6)*		**3.1** *(2.2-5.3)*
oxychlordane	**4.5** *(3.3-6.6)*		**4.1** *(3.2-5.2)*
cis-HE	**2.5** *(1.9-3.2)*		**2.2** *(1.6-3.0)*

[1]Medians (*25-75 percentiles*)

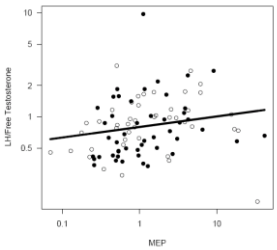
p = 0.03

Polybrominated flame retardants



Main et al., EHP 2007

LH / free testosterone ratio and mEP µg/L



Change in serum hormone levels with 10-fold increase of phthalate

- mEP, mBP: ↑ **+ 15,+8 %** SHBG
- mBP: ↓ **- 15%** free testosterone
- mMP, mEP, mBP: ↑ **+ 26, +19, +18 %** LH /free testosterone ratio
- miNP: ↑ **+ 97%** LH

Main et al, EHP 2006

Pesticides and cryptorchidism

- Cryptorchid boys in Denmark and Finland show a higher exposure to the sum of 8 most prevalent pesticides than the normal boys (Damgaard et al., Environ. Health Perspect. 2006)

Phthalates and cryptorchidism

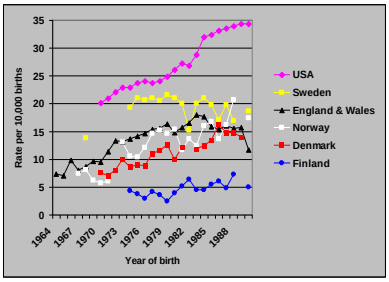
- fetal exposure of rats to high doses of dibutyl phthalate causes cryptorchidism (Mahood et al., Int. J. Androl. 2006)
- in human, high exposure to phthalates is associated with a decreased anogenital distance (Swan et al. Environ. Health Perspect. 2005) and a high LH/testosterone ratio (Main et al., Environ. Health Perspect. 2006)

Polybrominated diphenyl ethers

- Concentration of PBDEs is higher in breast milk of cryptorchid boys than in controls
- This difference is not present in placenta samples that have also much lower PBDE levels

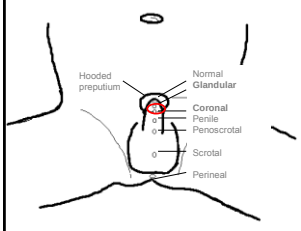
Main et al., Environ. Health Perspect. 2007

Rate of hypospadias



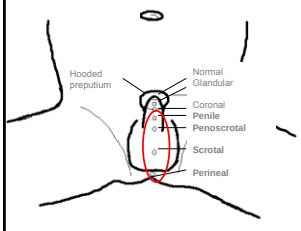
Toppari et al., 2001 (based on ICBDMS 1991; Paulozzi et al., 1997)

Hypospadias: Classification



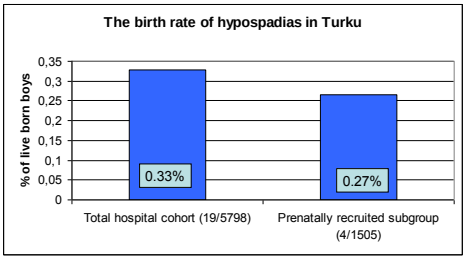
Distal hypospadias

Hypospadias: Classification

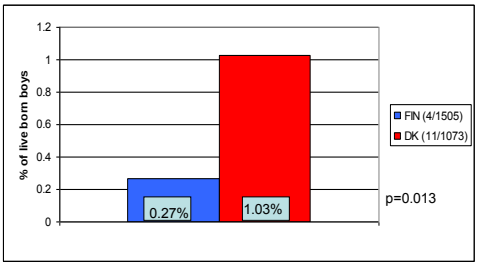


Proximal hypospadias

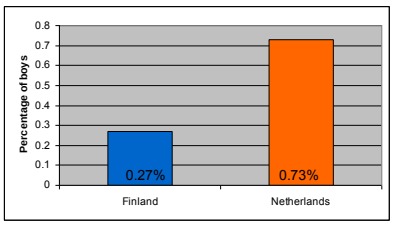
The birth rate of hypospadias in Turku University Central Hospital 1997-1999



The birth rate of hypospadias: Finland versus Denmark

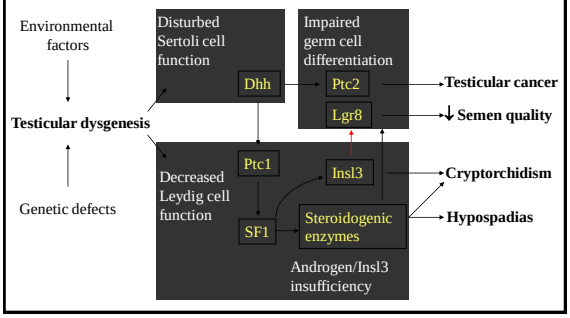


Prevalence of hypospadias in Finnish and Dutch cohorts

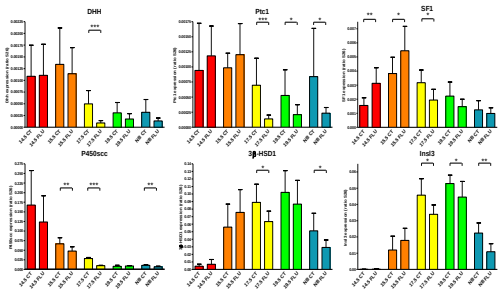


Pierik et al 2002

Testicular Dysgenesis Syndrome



Altered gene activity after anti-androgen exposure



Summary

- Prevalence of cryptorchidism and hypospadias shows large regional and temporal variation, suggesting environmental connection
- Both disorders can be found in some cases of TDS
- Risk of cryptorchidism is associated with the levels of several endocrine disrupters in breast milk

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- Damgaard IN et al. Risk factors for congenital cryptorchidism in a prospective birth cohort study. PLoS ONE 3(8): e3051, 2008

Mark your calendar for the upcoming ESHRE campus workshops!

- **Basic Genetics for ART Practitioners**
  organised by the SIG Reproductive Genetics
  16 April 2010 - Porto, Portugal

- **Array technologies to apprehend developmental competence and endometrial receptivity: limits and possibilities**
  organised by the Task Force Basic Science in Reproduction
  22 April 2010 - Brussels, Belgium

- **The management of infertility – training workshop for junior doctors, paramedicals and embryologists**
  organised by the SIG Reproductive Endocrinology, SIG Embryology and the Paramedical Group
  26-27 May 2010 - Kiev, Ukraine

- **Preimplantation genetic diagnosis: a celebration of 20 years**
  organised by the SIG Reproductive Genetics
  1 July 2010 - Rome, Italy

- **EIM 10 years' celebration meeting**
  organised by the European IVF Monitoring Consortium
  11 September 2010 - Munich, Germany

- **The determinants of a successful pregnancy**
  organised by the SIGS Reproductive Surgery, Early Pregnancy and Reproductive Endocrinology
  24-25 September 2010 - Dubrovnik, Croatia

- **Basic training workshop for paramedics working in reproductive health**
  organised by the Paramedical Group
  6-8 October 2010 - Valencia, Spain

- **Forgotten knowledge about gamete physiology and its impact on embryo quality**
  organised by the SIG Embryology
  9-10 October 2010 - Lisbon, Portugal

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- **Promoting excellence in clinical research: from idea to publication**
  5-6 November 2010 - Thessaloniki, Greece
- **"Update on pluripotent stem cells (hESC and iPS)" and hands on course on "Derivation and culture of pluripotent stem cells"**
  8-12 November 2010 - Valencia, Spain
- **Women's health aspects of PCOS (excluding infertility)**
  18 November 2010 - Amsterdam, The Netherlands
- **Endoscopy in reproductive medicine**
  24-26 November 2010 - Leuven, Belgium
- **Fertility and Cancer**
  25-26 November 2010 - Bologna, Italy
- **The maternal-embryonic interface**
  2-3 December 2010 - Valencia, Spain
- **GnHR agonist for triggering of final oocyte maturation – time for a paradigm shift**
  3 December 2010 - Madrid, Spain
- **Raising competence in psychosocial care**
  3-4 December 2010 - Amsterdam, The Netherlands

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