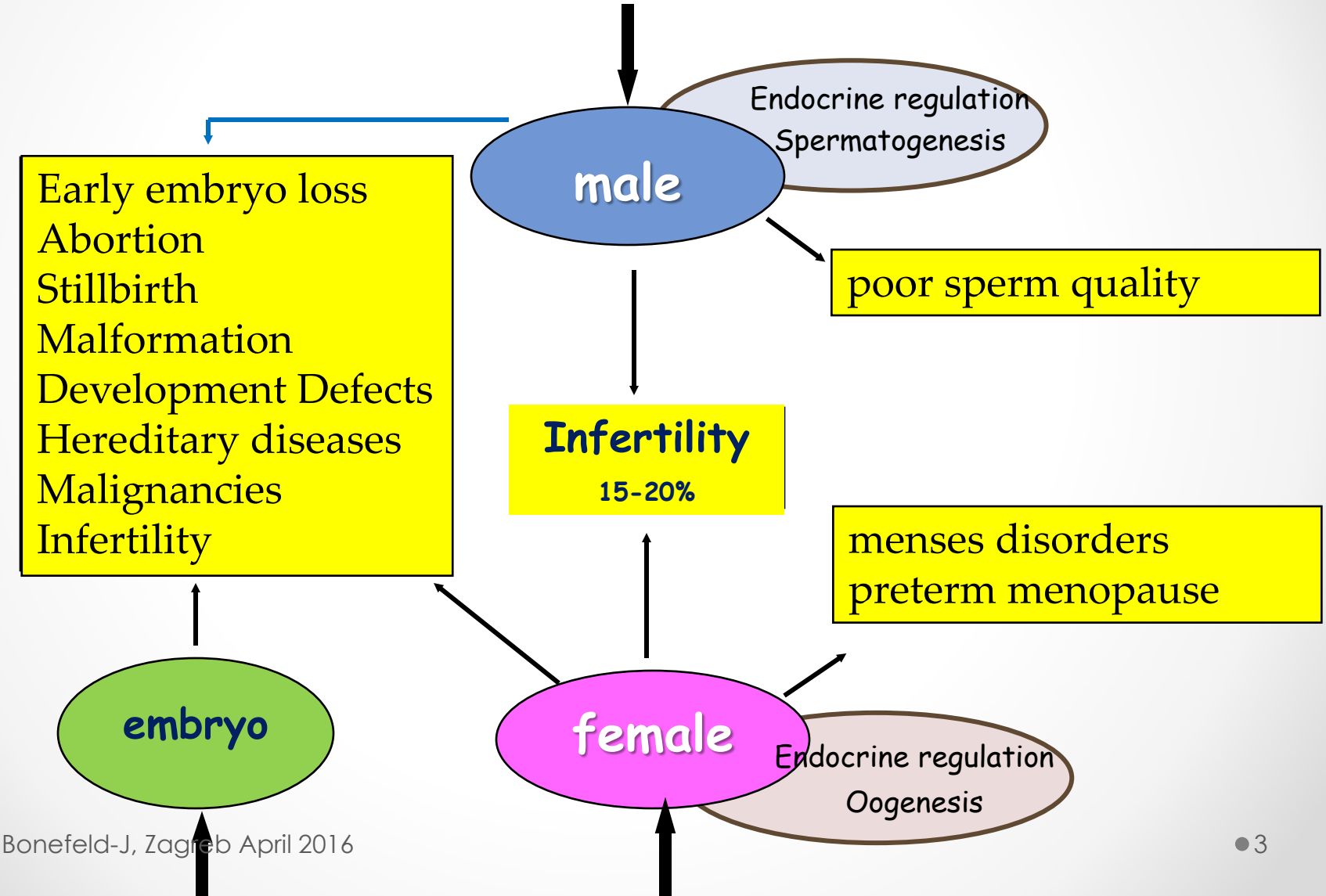
Reproductive and developmental toxicity

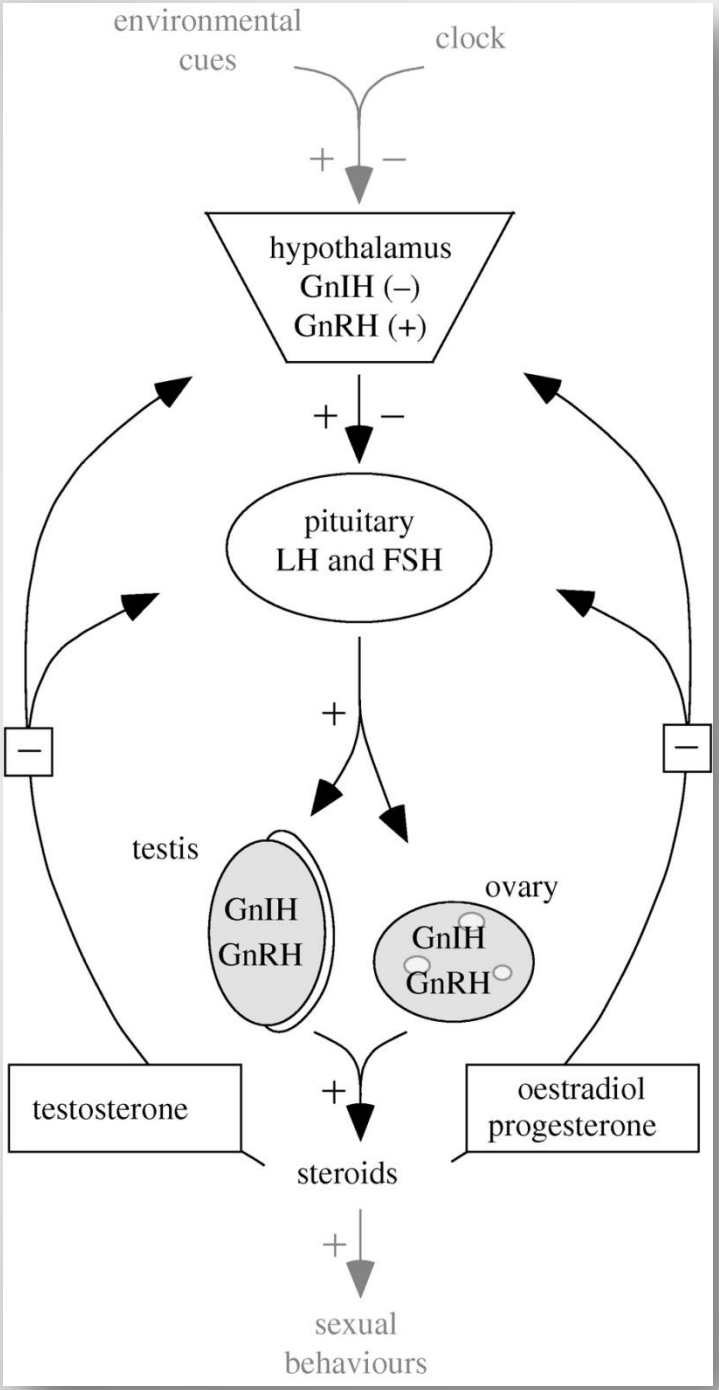
Eva Cecilie Bonefeld-Jørgensen
Professor, Director, PhD
Centre for Arctic Health &
The Unit of Cellular and Molecular Toxicology
Department of Public Health
Aarhus University

Outline of the talk

- Introduction to the male and female reproductive system
- Endocrine disrupters (EDs) and effects
  - the fetus and the neonate
  - men and women of childbearing age
- Lifestyle and occupational health
- Risk and Prevention

Possible external exposures and reproductive effects

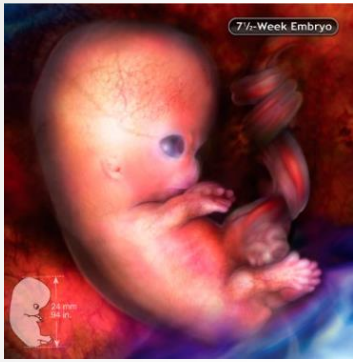




Sex hormones

- Sex hormones are divided into overall sex hormones and the male and female sex steroids.
- The overall sex hormones
  - Gonadotropin-releasing hormone (GnRH),
  - luteinizing hormone (LH),
  - follicle stimulating hormone (FSH)
  - are not steroids and not included in the sex steroid hormones
- Steroid hormones, the female and male sex steroids
  - androgens (testosterone) and estrogens (estradiol / progesterone)
  - are formed from cholesterol

Gonadotropin-inhibitory hormone (GnIH)



Embryo - Child

• • •

Causes of congenital malformations

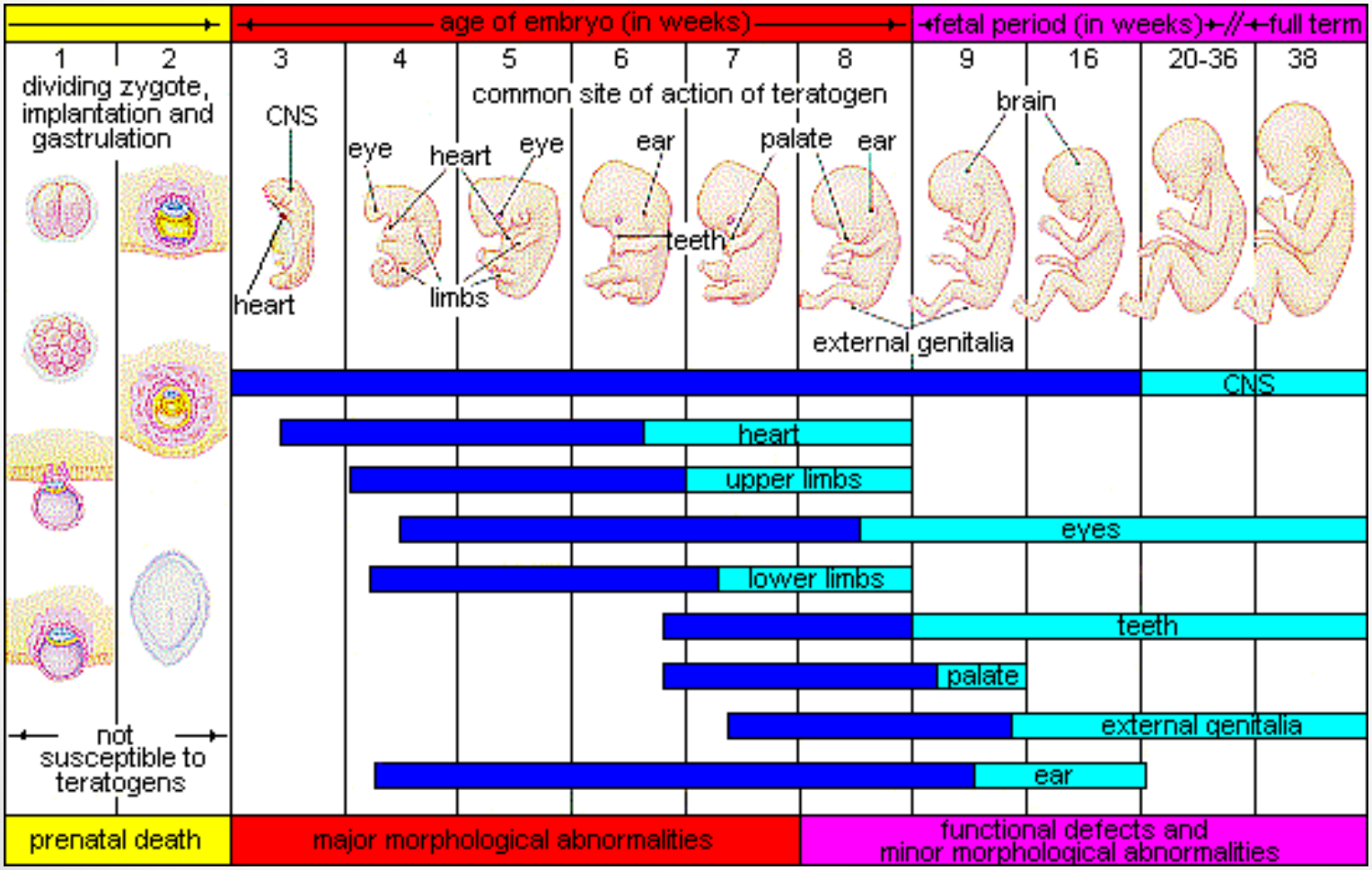
- Chromosomal abnormality 5%
- Genetic transmission 20%
- Infections 3%
- Metabolic diseases(e.g. diabetes) 1-2%
- Ionizing radiation 1%
- Pharmaceutical / chemical substances 4-6%

- **Unknown reasons** **65-70%**

  - Including suspected that a number of endocrine disruptors (EDCs) play a significant role

Exposure during fetal development is particularly critical

Exposure during the 38 gestation weeks is critical



Fetal development toxicity via maternal exposures

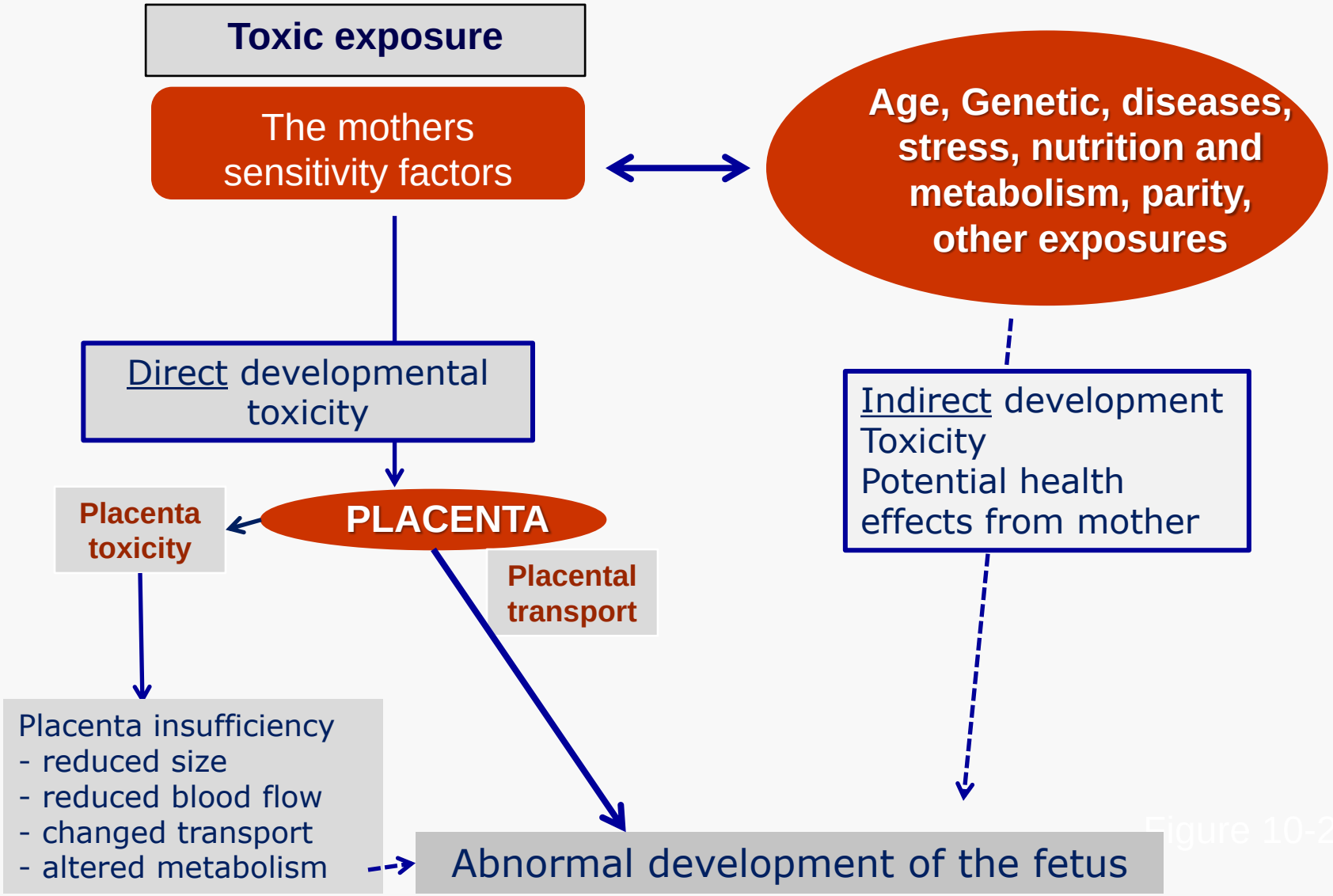
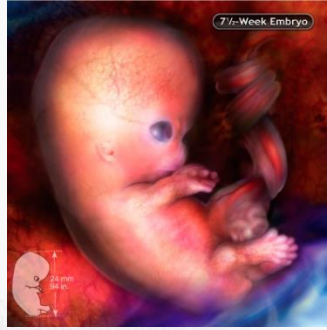
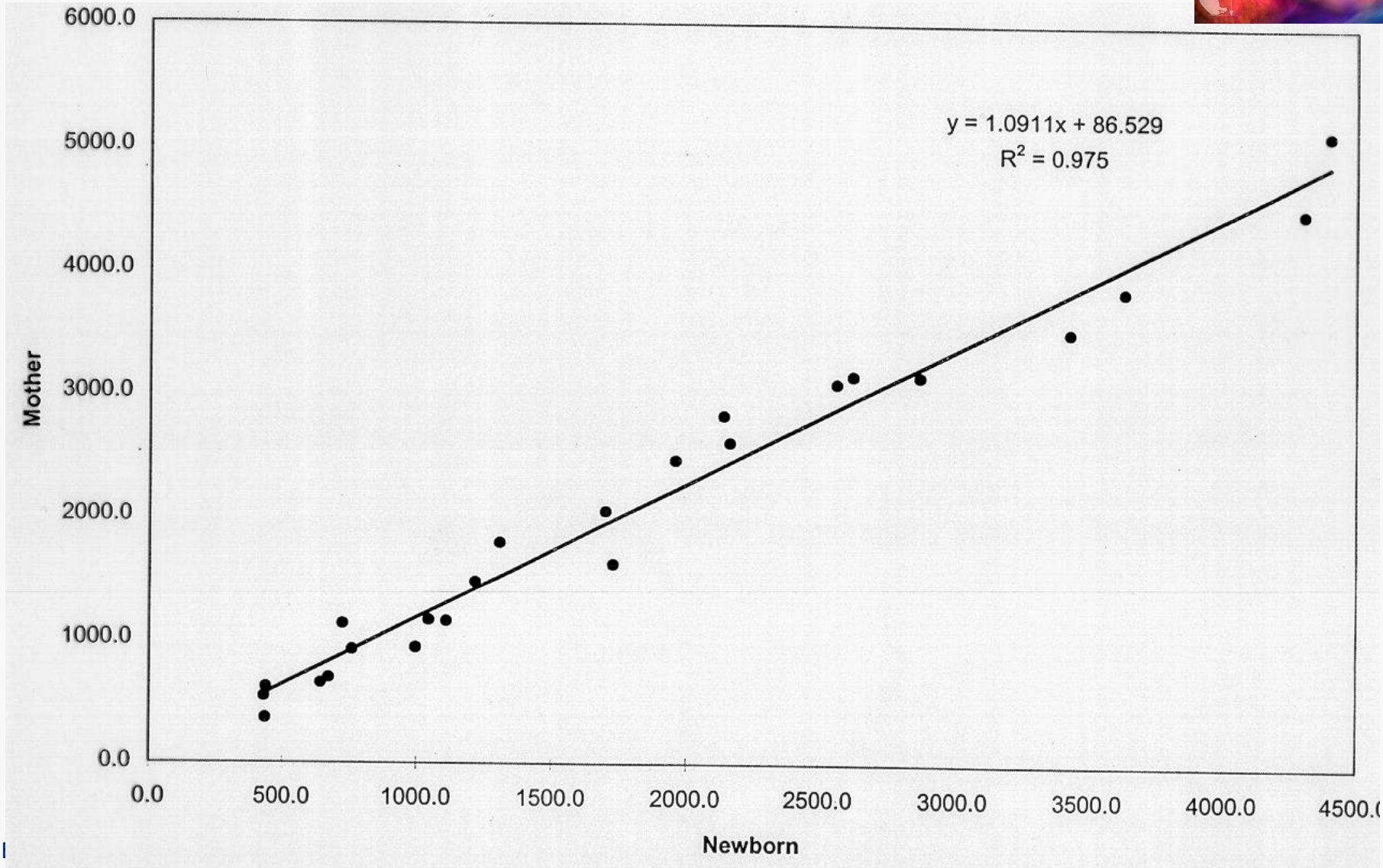


Figure 10-2

Total PCB (polychlor biphenyl's) in mother and cord plasma (ug/kg lipid)

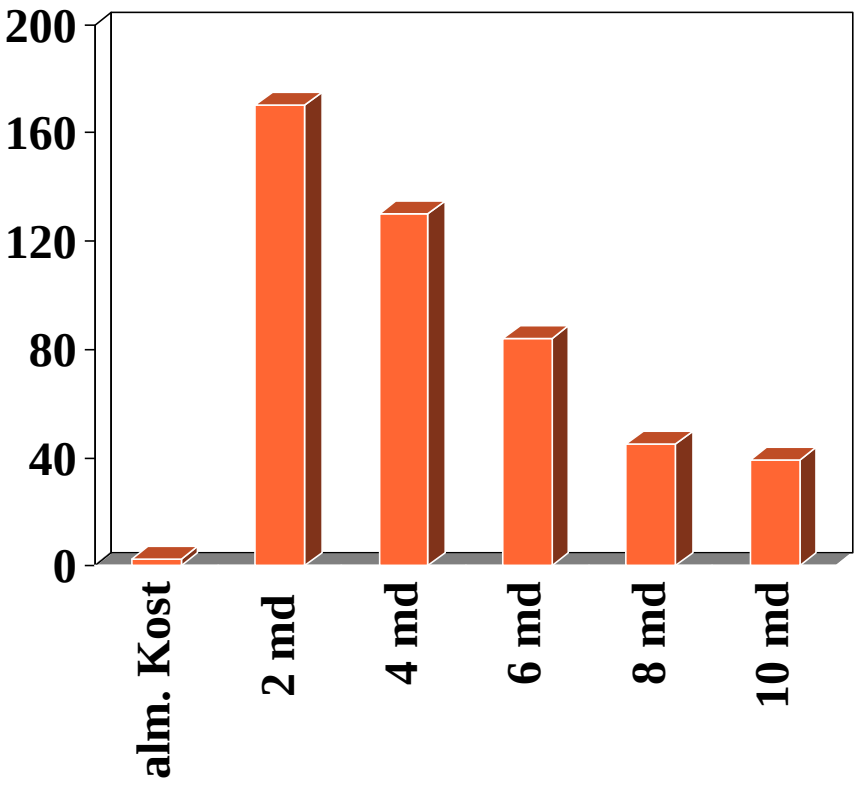




Effects on different embryo stages

- *Embryogenesis (5.-17. days)* *all or nothing*
  - implantation of the blastocyst in endometrium (5-7 days)
    - » Chemical compounds (Cd, Pb, PCB), HFS, *ionizing radiation*
- *Organogenesis (17.-56. days)* *(no placenta)*
    - » embryoletal, teratogen
      - Organic. solvents, thalidomide (structural malformation), diethylstilbestrol (DES) (endocrine disrupt.), methylmercury (mHg)(CNS), EDCs
- *Fetogenese 9th week to birth (week 40)*
  - organ growth / organ maturation
  - effect on endocrine-, immune-, urogenital-, central nerve-system (CNS)
      - mHg, Pb (CNS effects v. 0.5-1.0 mM), EDCs
- *Postnatal exposure via breast feeding*
  - Low molecular weight and fat-soluble substances

Infant intake of fat soluble dioxins and PCBs via breast milk pgTEQ/kg bw/dag



Harrison et al. 1998, Chemosphere

adult

Eksponering postnatal

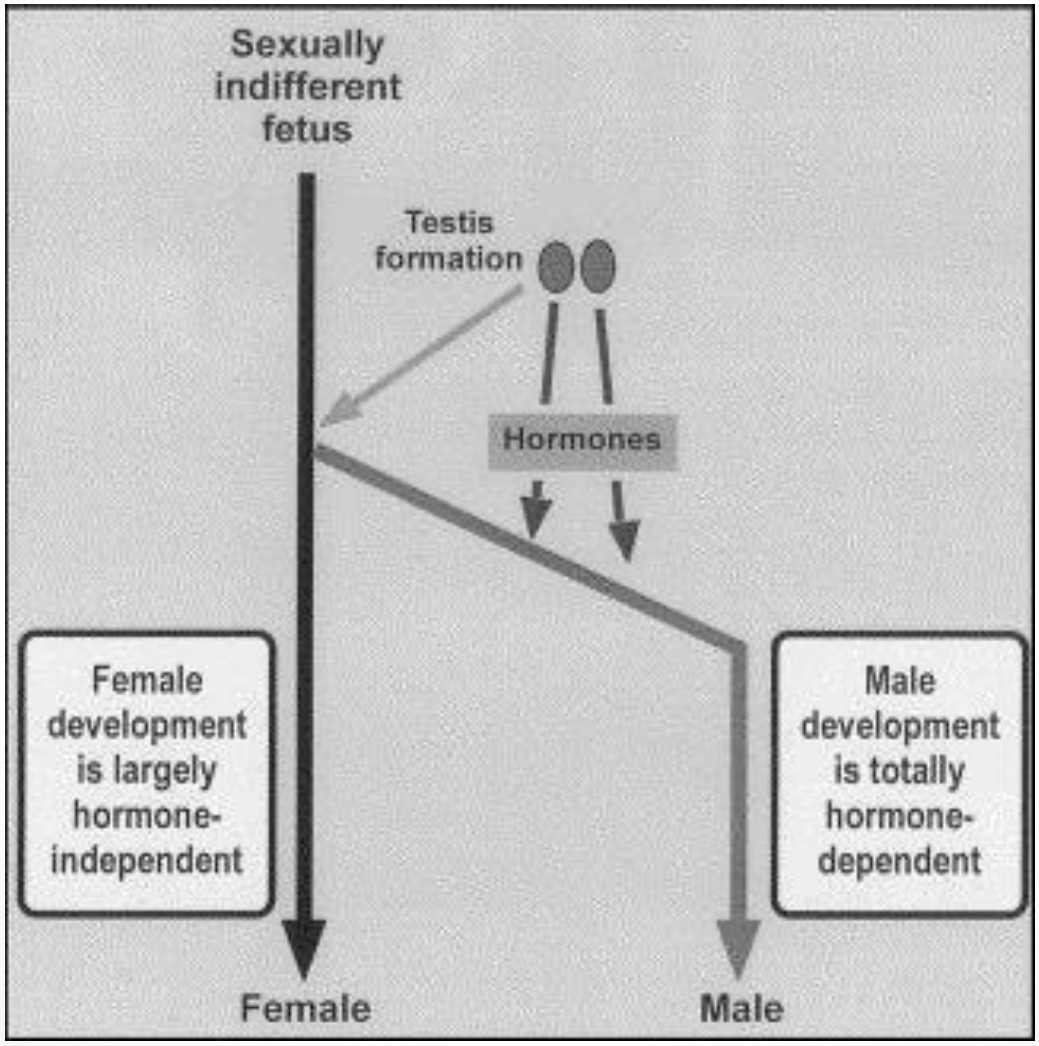
A sperm cell fusing with an ovum

Endocrine disrupters and reproduction

Male fertility and reproductive disorders

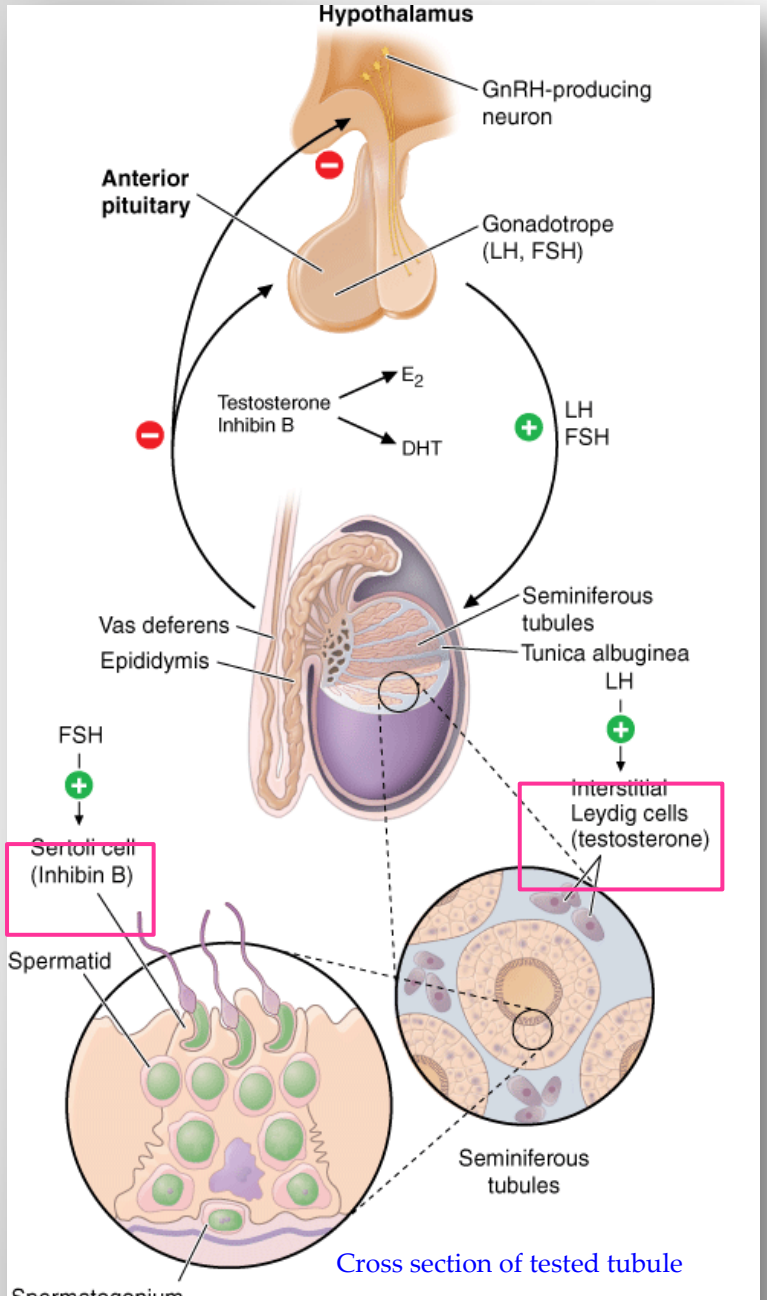
Schematic presentation of sexual differentiation in humans

The development of male fetus involves "diversion" from the (standard) girl embryonic development because of the formation of the testes and the production of hormones from the tested



Kilde: Sharpe, RM (2001) Toxicology Letters 120, 221-232.

Male fertility



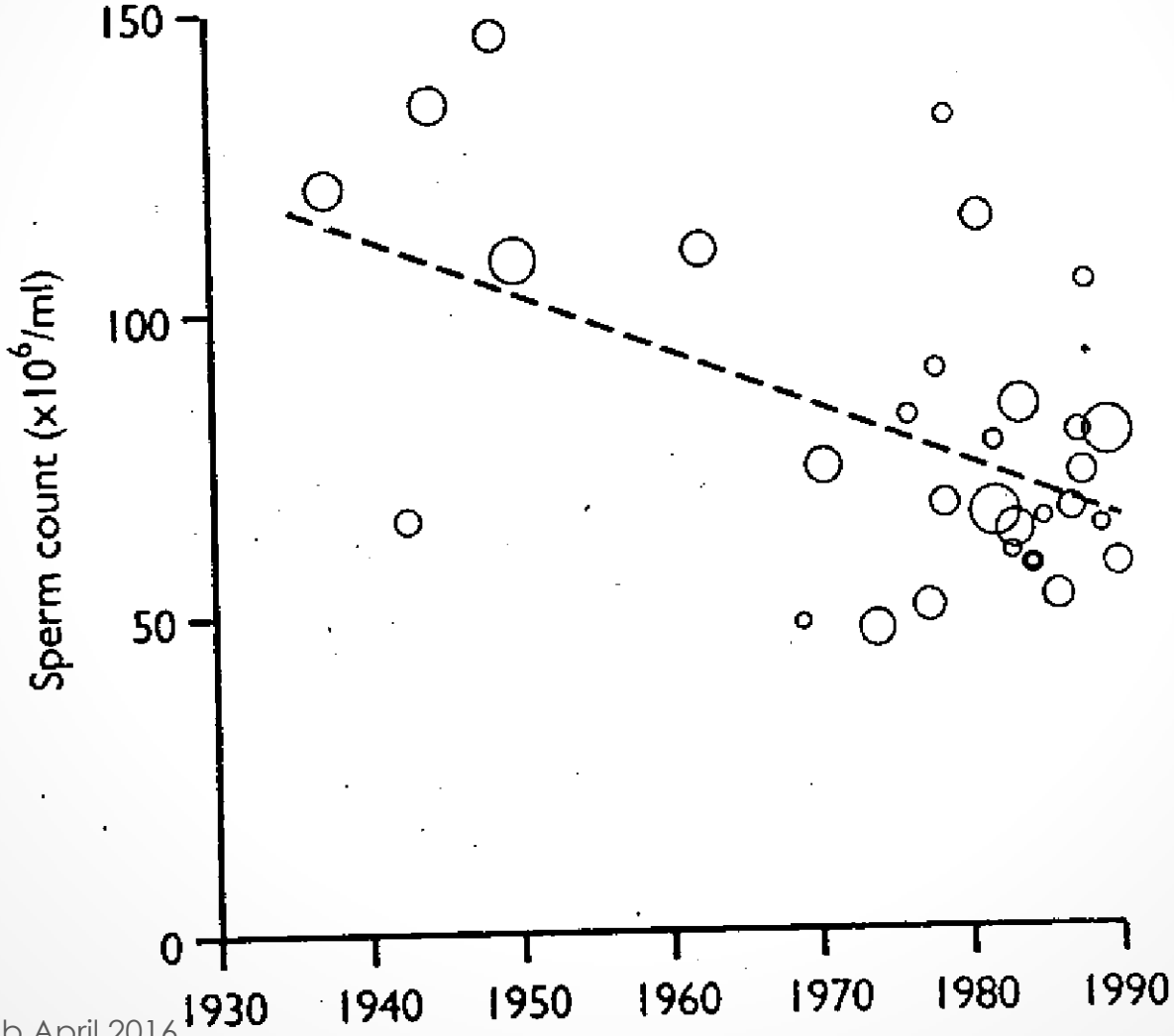
- Leydig cells produce testosterone of from about 16-20. Weeks of gestation. Releasing androgens when stimulated LH.
- Sertoli cells (regenerate not after puberty) release
  - anti-Müllerian hormone (AMH) early in the fetal life.
  - Inhibin and activin; excreted after puberty, and work together to regulate FSH secretion.
  - Androgen binding protein (increases testosterone in tubules).
  - Sertoli cell aromatase converts testosterone to 17 beta estradiol.
  - FSH positively affects the Sertoli cells and increases the response of Leydig cells to LH by increasing the number of the LH receptors expressed on Leydig cells

Male fertility: mechanisms of action

- **Spermatogenesis (74 dg); is regulated by pituitary hormones FSH and LH**
  - Spermatogonia (mitotic-meiotic cell divisions) = spermatids (23 chrom.)
- **Toxic effects on cells:**
  - Early stages: spermatogonia / spermatocytes
    - azoospermia (destruction) - decreased sperm numbers, altered morphology / mobility (pesticide: dibromochloropropane, DBCP); (effect time 60 dg)
  - Late stages: epididymis / testis
    - transient reduced mobility (welding: chlorine methane, heating / radiant heat);
  - Endocrine disruption by environmental and occupational health exposures
    - hormone regulation (EDCs, anti-androgens, organic solvents, lead)
    - Dependent on exposure time: permanent - temporary reduced sperm quality
    - By selective toxicity on Leydig cells affected testosterone production and thus low sperm count
- **Prenatal exposure of male embryo**
  - EDCs with androgen and/or estrogen like functions
    - permanent reduced **Sertoli cells** (regenerate not after puberty) result in reduced sperm number

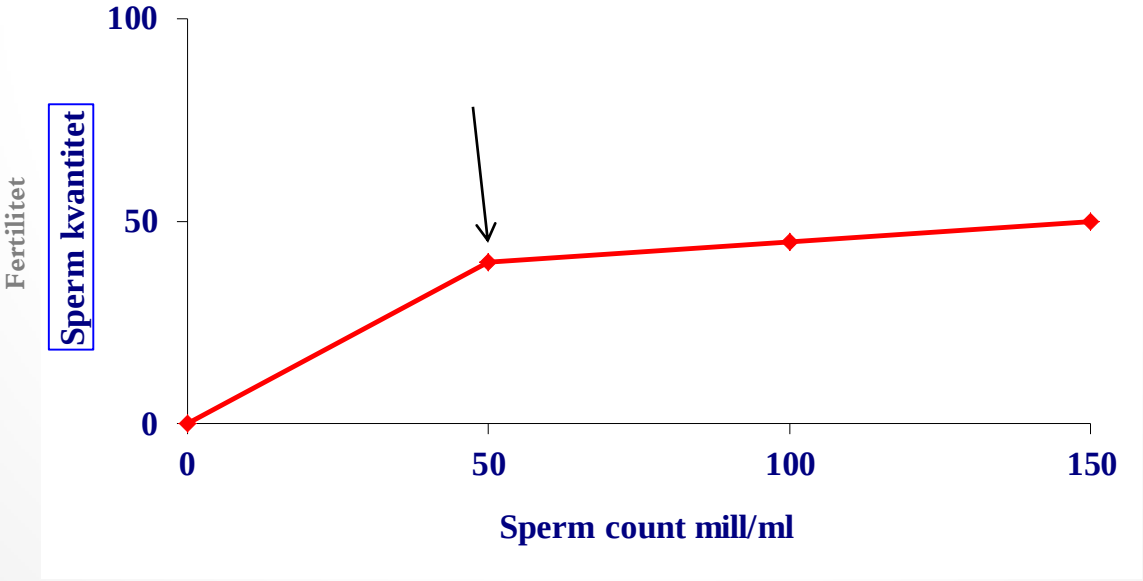
Temporal decline in sperm number

Reproduced from Carlsen et al., 1992, Br. Med. J., ,



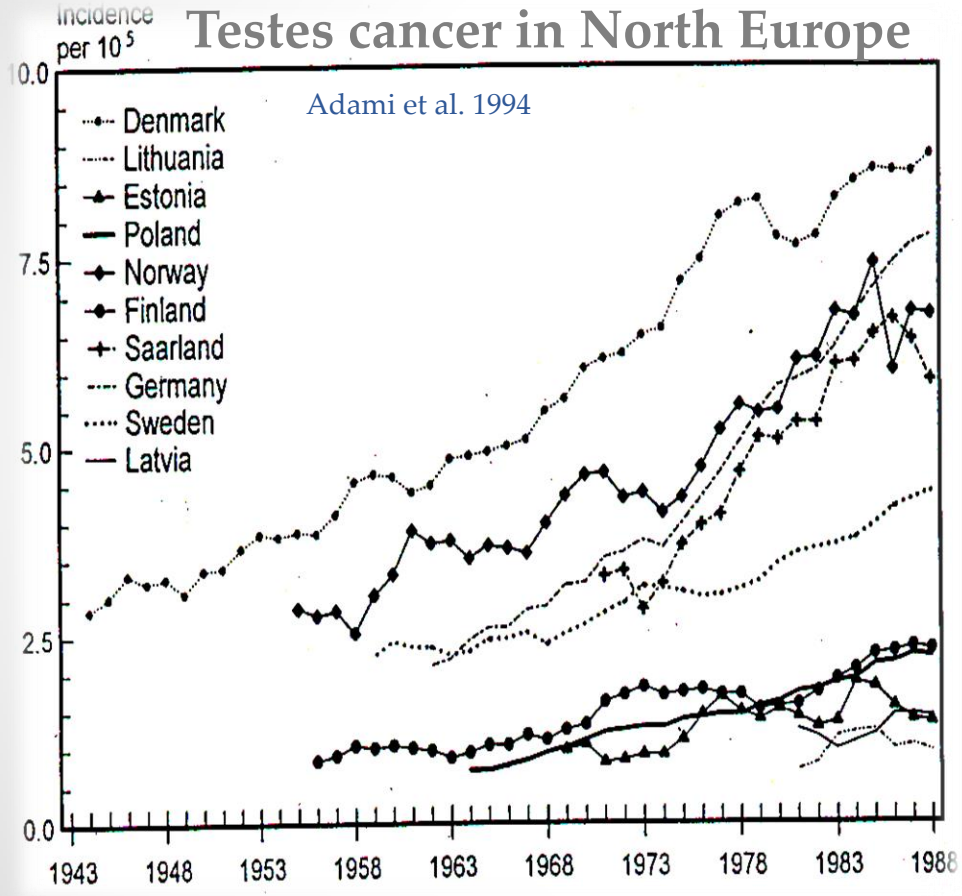
Sperm Quantity / Quality / fertility

- WHO normal level: ~ 60 - 80 mill/ml
- DK 40% : ~ 40 mill/ml
- DK 20% : ~ 20 mill/ml
  - n = 708; young Danish males, age ~ 20, study for the suitability for military service



Andersen et al. , 2000. Hum. Reprod. 15

Testis cancer

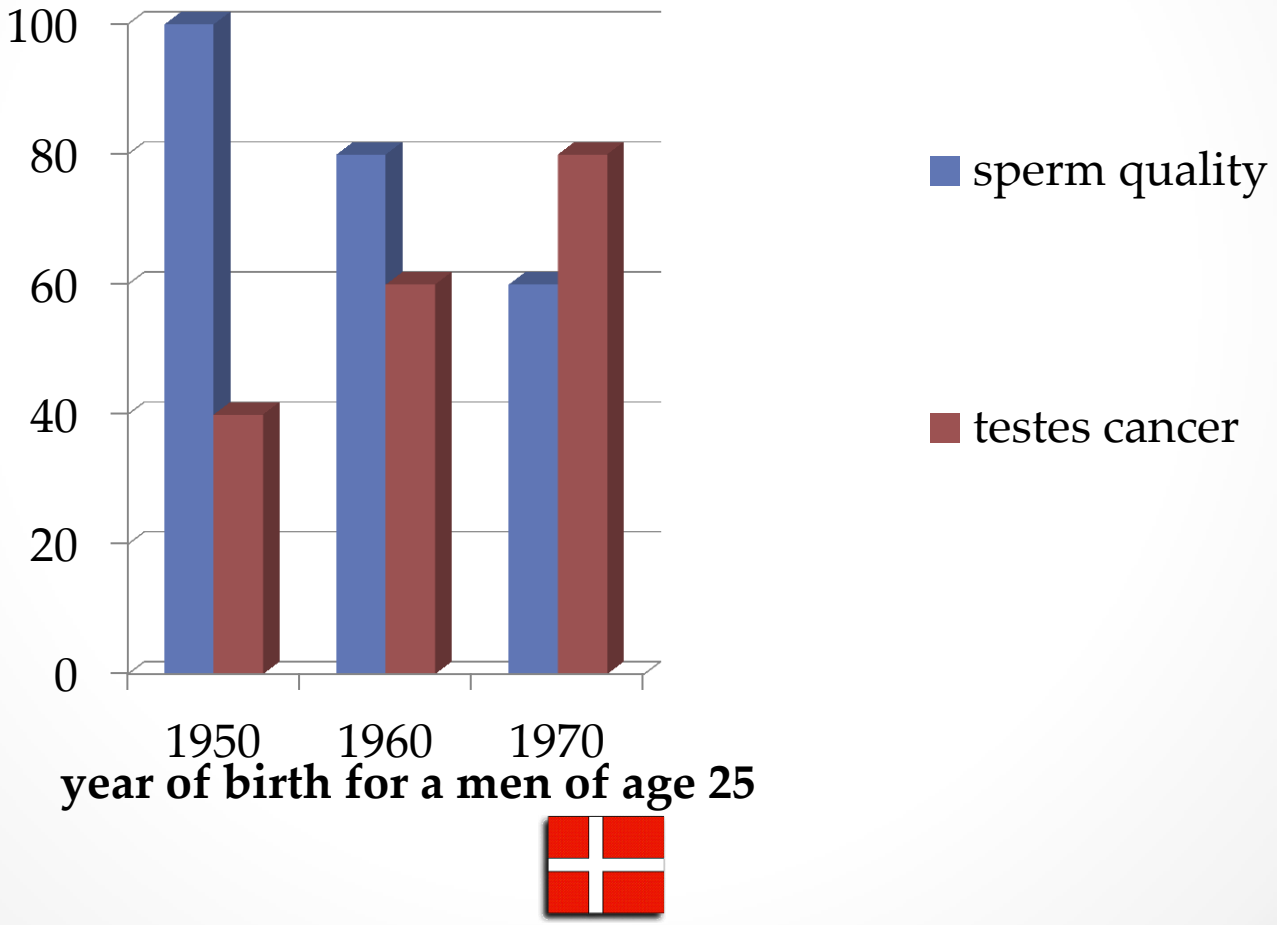


Fakta, March 2011			
Testes Cancer (TC)	**Incidence pr 100.000 males**	**High social economy / Low social economy**	**White/ black**
Scandinavia,	**6,7**		
USA	**3.7**		**0.2% / 0.05%**
Japan	**0.8**		
South Africa	**seldom**		
Probability of development of TC		**2/1**	

The disease is approximately 4-5 X so frequent in Caucasians than African American.
Scandinavia, Germany and New Zealand have the highest incidence of cancer tested; Asia and Africa the lowest

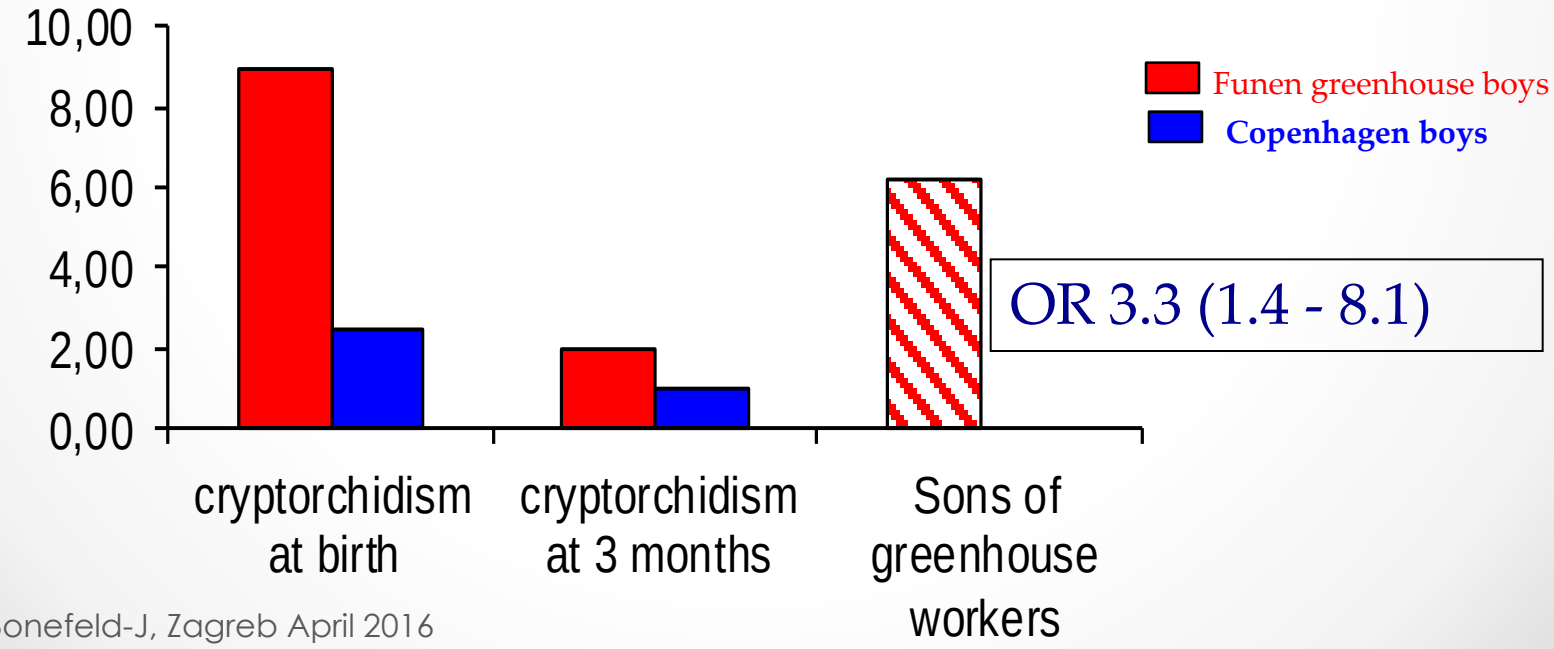
The disease most frequent between the ages of 15-35 years.
Risk factors; heredity, exposure to endocrine disruptors (fetal), congenital defects of the reproductive organs (tested injuries; tested atrophy)? Low physical activity

Relative association of sperm quality, testicular cancer



Pesticide exposure: Sons of staffs in greenhouses have increased risk of cryptorchidism

1996-2000: 288 pregnant women were recruited from Funen greenhouse workers with 203 children (113 boys) and compared with 982 in Copenhagen boys
Study at 3 months

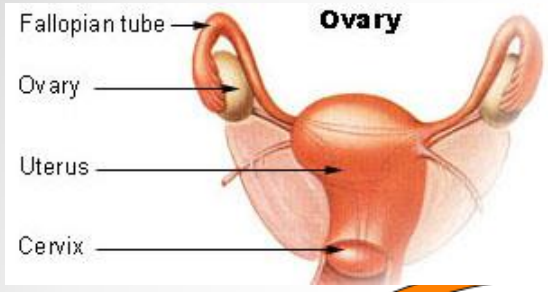


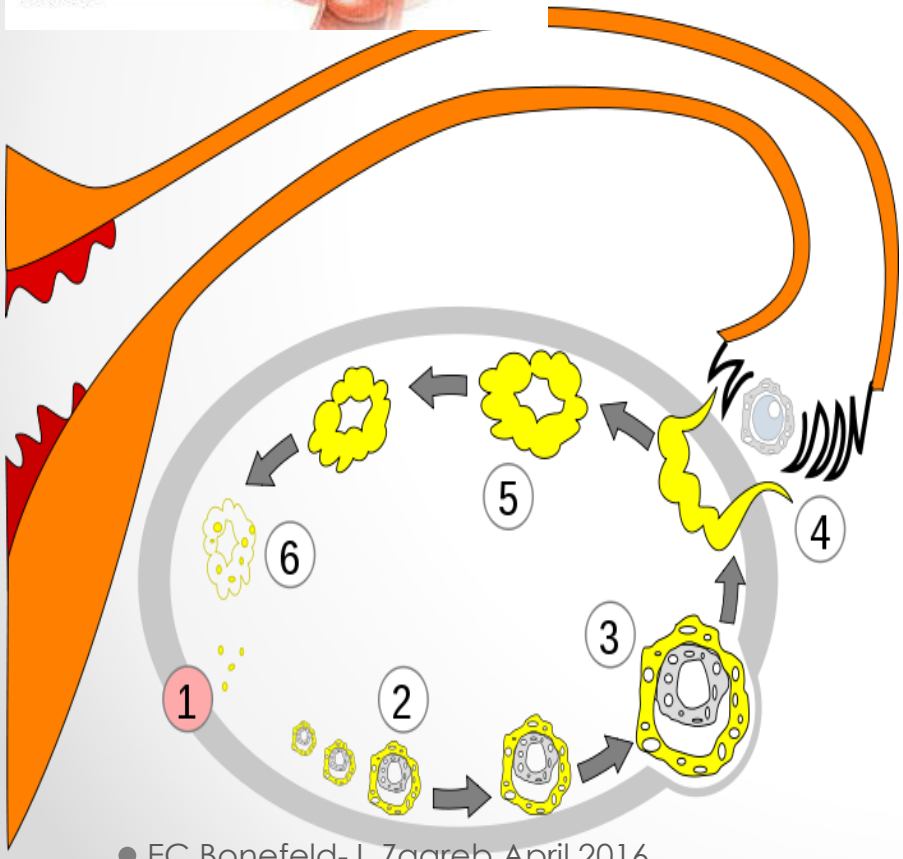
A sperm cell fusing with an ovum

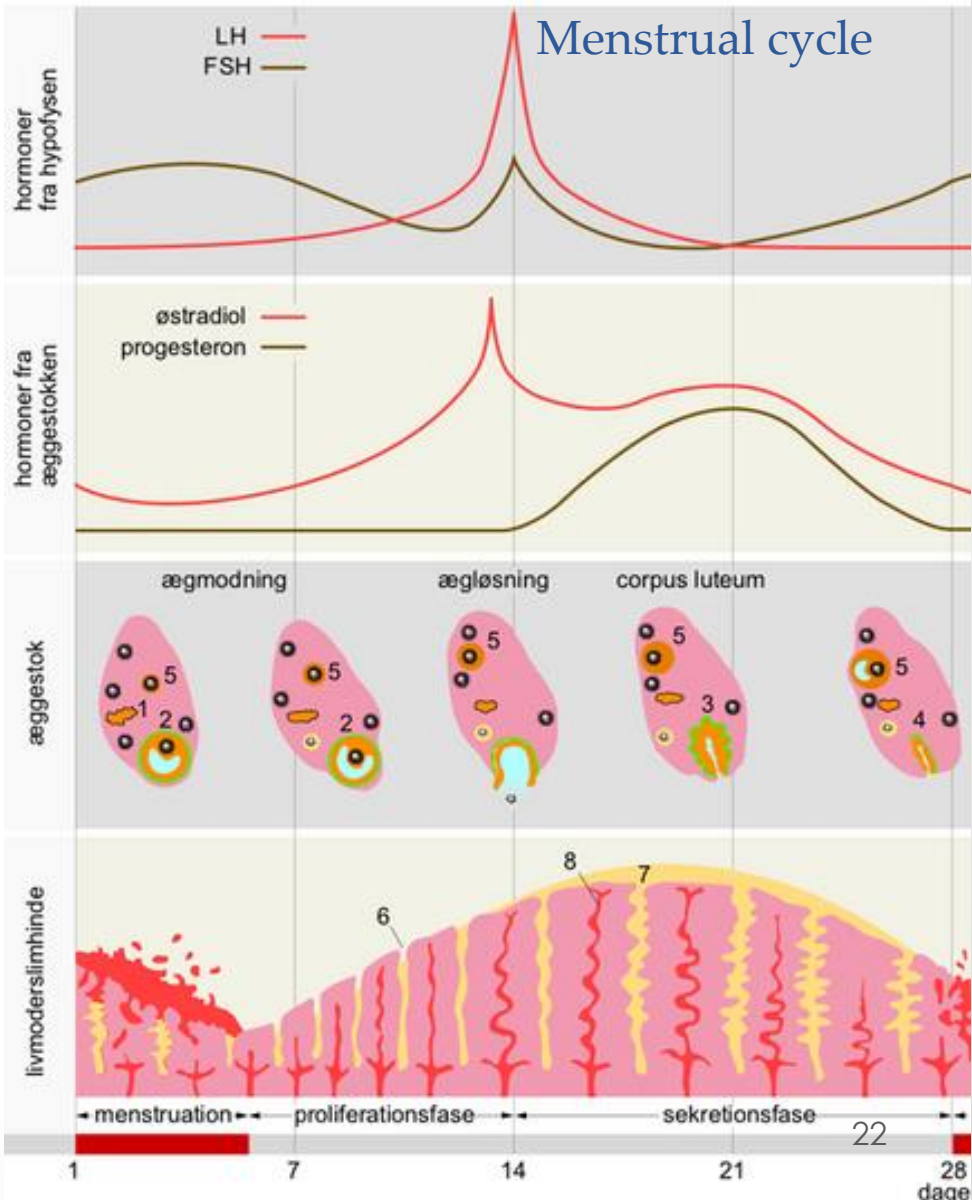
Endocrine disrupters and reproduction

Female fertility and reproductive disorders

Female fertility: Ovarium morphology and function





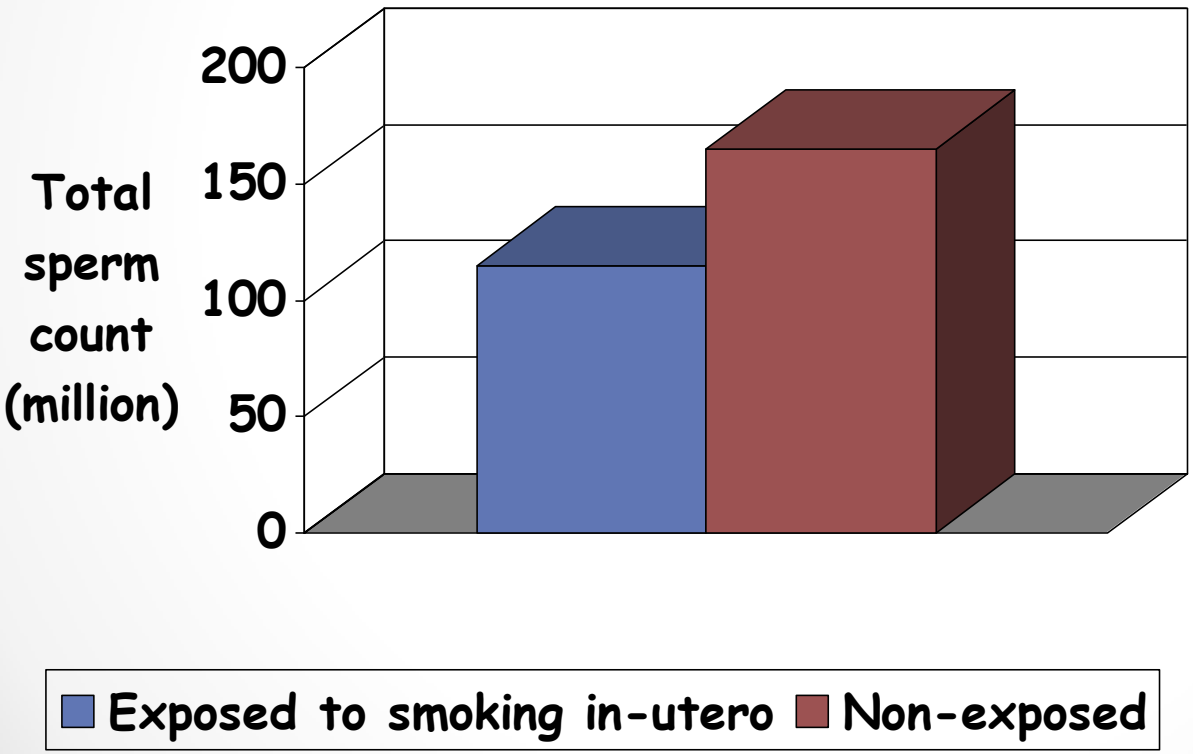


Female fertility: Mechanisms - oogenesis

- Oocytes are not generated after birth
  6 million. in utero - 2 million. by birth - 400,000 puberty
- Endocrine disruption
  - Hormone regulation (hypothalamus, pituitary)
    - anovulation and menstrual disorders
      - (Pb, org. Opel., organochlorine compounds, e.g. PCBs and pesticides (DDT / DDE)
- Oocyte destruction
  - polyaromatic hydrocarbons (PAH)(cigarette smoke)
  - Caffeine (excessive intake of coffee)?

Exposure to smoking by the mother during pregnancy affects the sperm count for men

Smoking and Reproduction



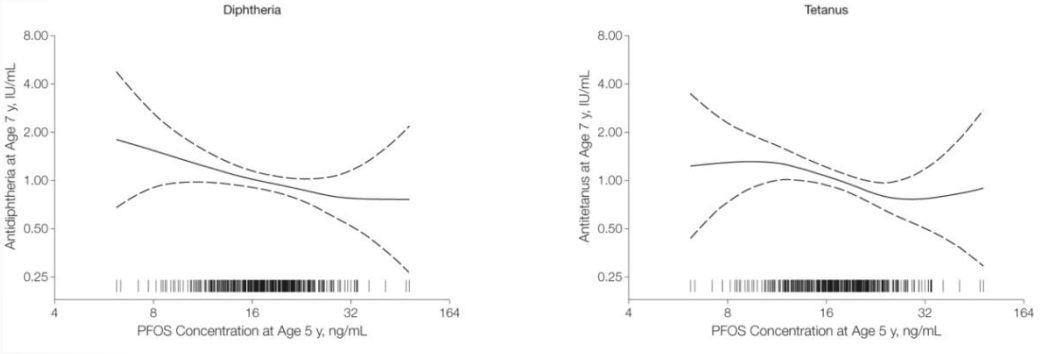
Jensen et al. 2004, Am. J. Epidemiol 159:49

Repported effects.
Benoff et al 2009:
Inverse relation between **Cadmium (Cd)** concentration and sperm numbers and mobility

Izawa et al 2007, 2008:
Diesel Exhaust **P**oly**A**romatic **H**ydrocarbons **(PAH)** reduce sperm quality

EDCs : The woman and her fetus / child health

- Studies have shown that
  - **Low-dose PCB[1] og PFCs[2]** exposure affect birth weight ([1]Govarts E, et al. 2011; [2]Fei et al. 2007)
  - **PCB and PFC** inhibit vaccination response to diphtheria and tetanus in children(Heilmann et al. 2006; Grandjean et al 2012



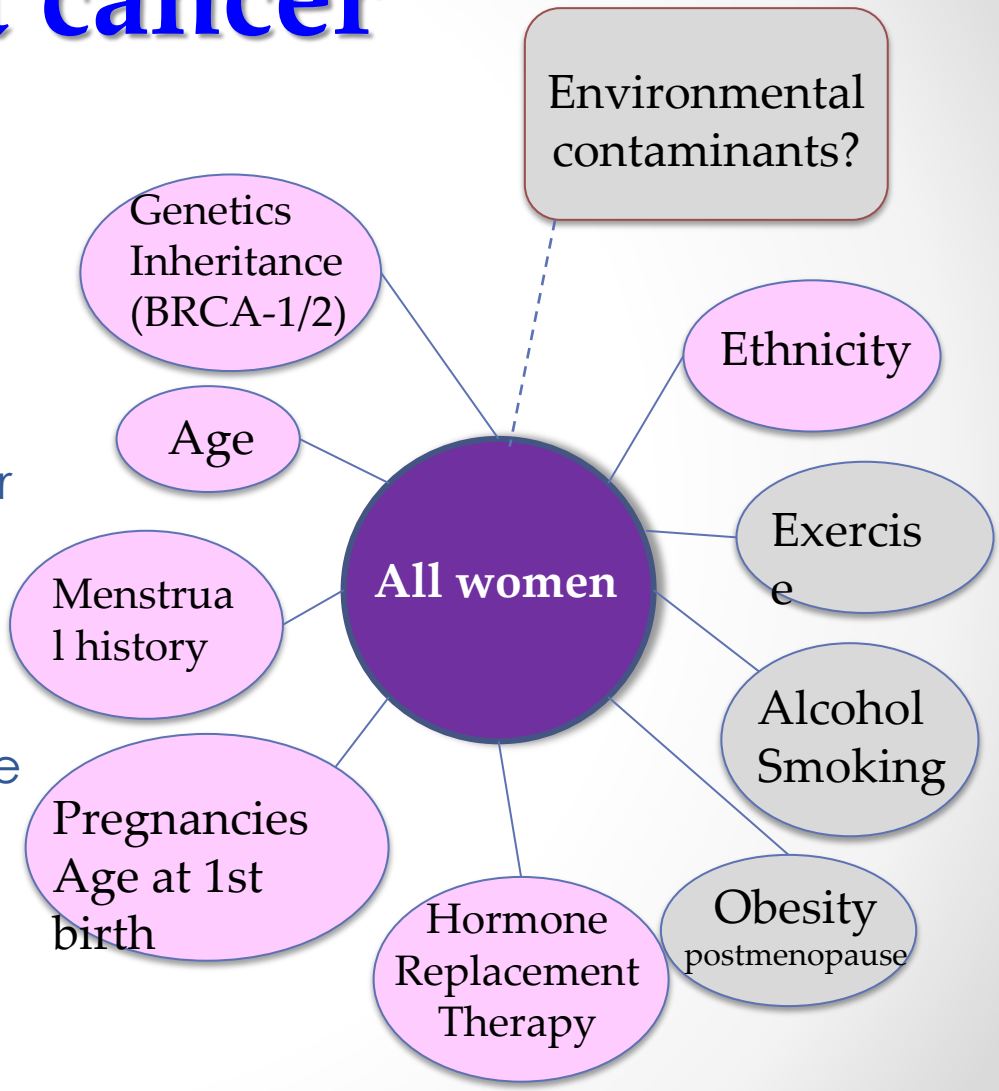
The additive models were adjusted for age, sex, and vaccine booster type. Dashed lines indicate 95% confidence intervals; vertical bars on the horizontal scale indicate individual observations.

  - **Perfluorinated compounds (**PFCs) are suspected of increasing the risk of the offspring developing autism / ADHD(Stein & Savitz, 2011; Hoffman et al 2010)
  - **PFCs and women's health**
  - affects women's ability to get pregnant(Fei et al., 2009; Lyngsø et al 2014); Vestergaard et al 2012;
  - PFC can be a risk factor for developing breast cancer (Bonefeld-.et al 2011, and Bonefeld-.et al 2014)

Fredslund SO, Bonefeld-Jorgensen EC: IJCH Review 2012

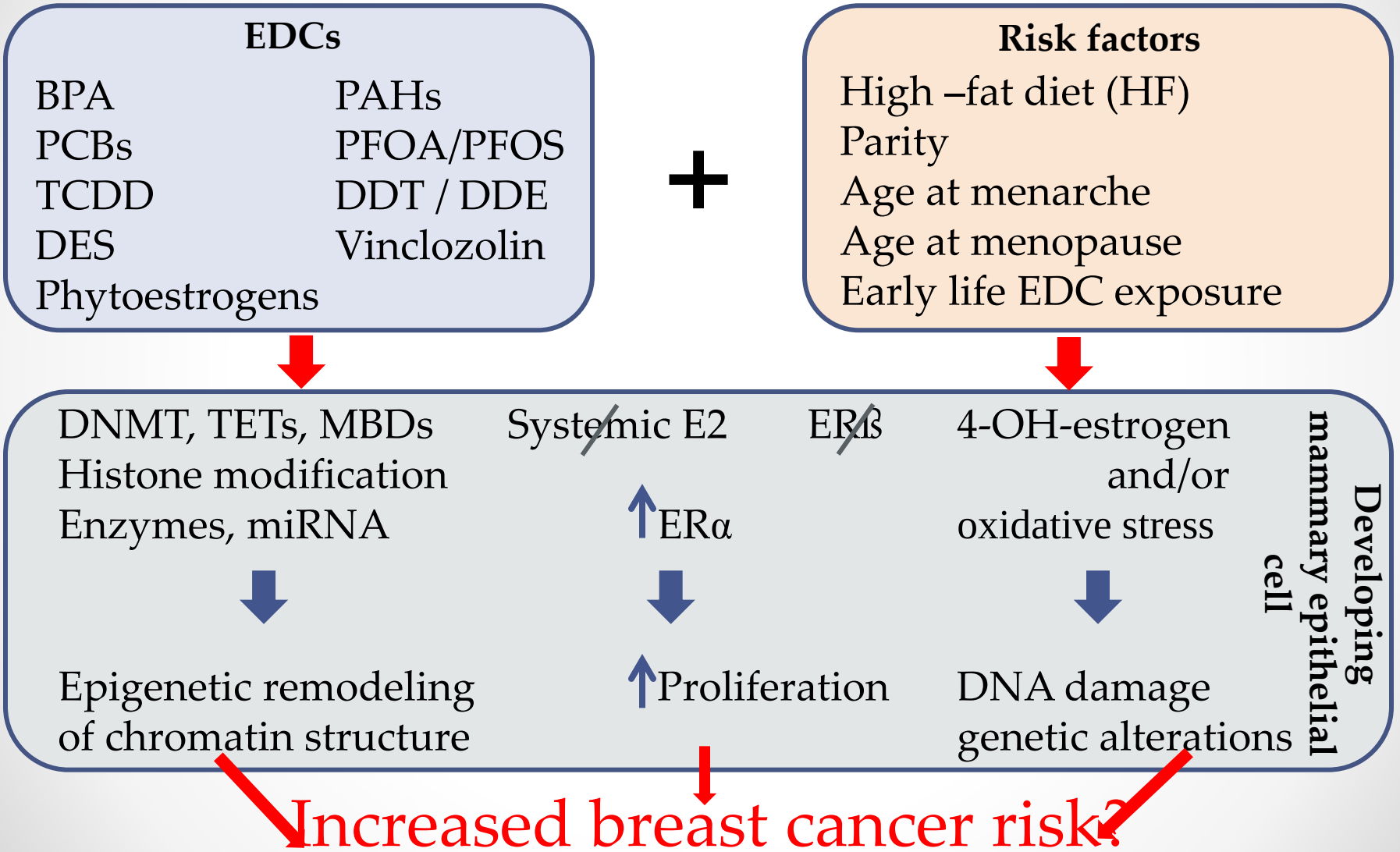
Breast cancer

- Most common cancer in women
  - 1 out of 9 women in DK
- Incidence rate (age standardized)
  - DK: 142,1/100,000 women/year (2012)
  - US: 122.8 / 100,000 women / year (2007-2011)
  - GRL: 41.3/ 100,000 women/year (2007-2011)
- In the Arctic: Increasing incidence since 1970s
  - Still lower (app. 30-50%) compared to western countries (Denmark)
- Multifactorial disease



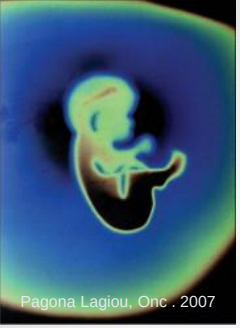
More than 70% of breast cancer cases cannot be explained by known risk factors

Exposure to EDCs and risk factors mediate epigenetic changes and increasing breast cancer risk

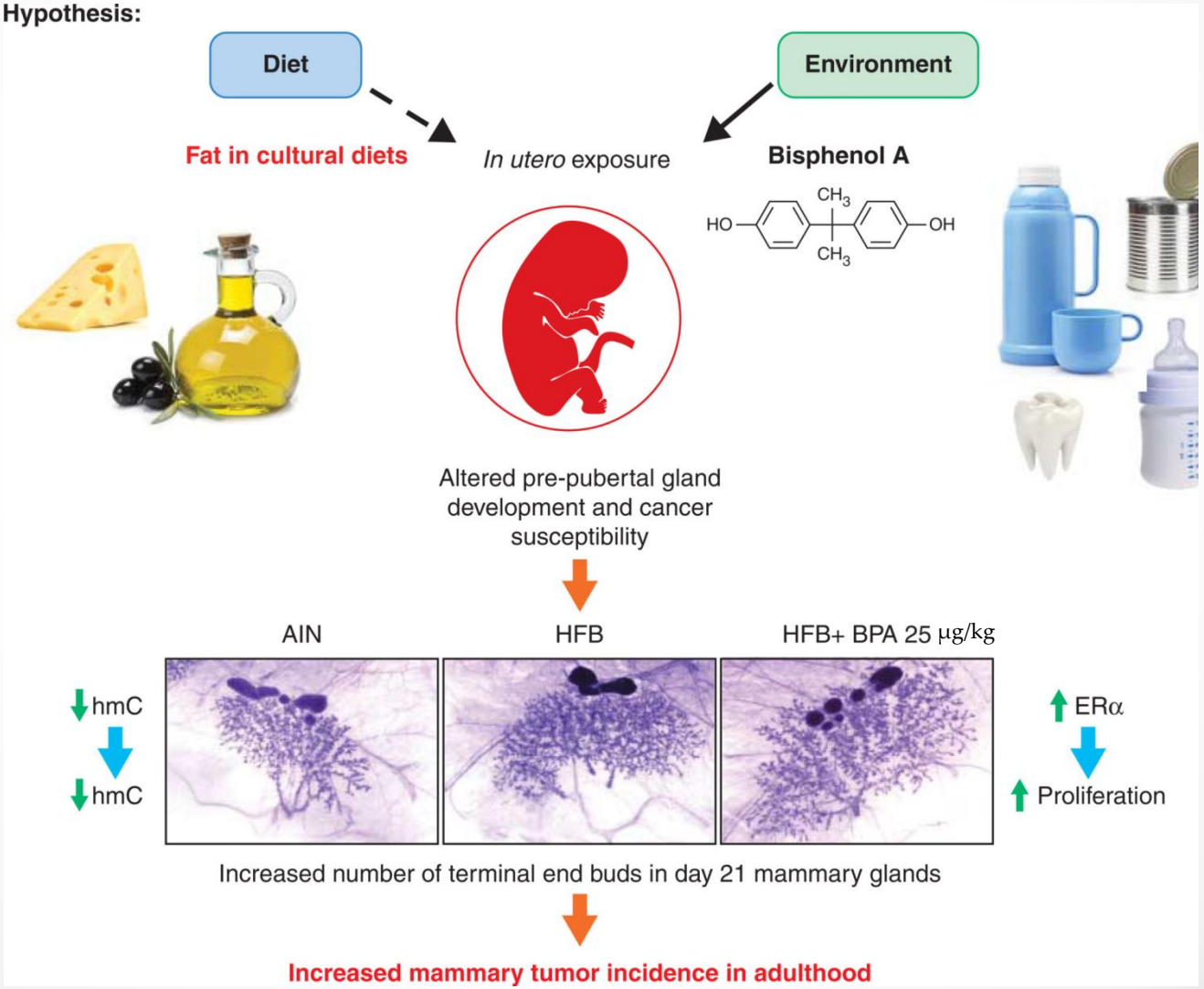


EC Bonefeld-J, Zagreb April 2016

Modified from Kevin C Knower et al 2014: based on animal and human experimental models



Endocrine disruption of the epigenome: a breast cancer link



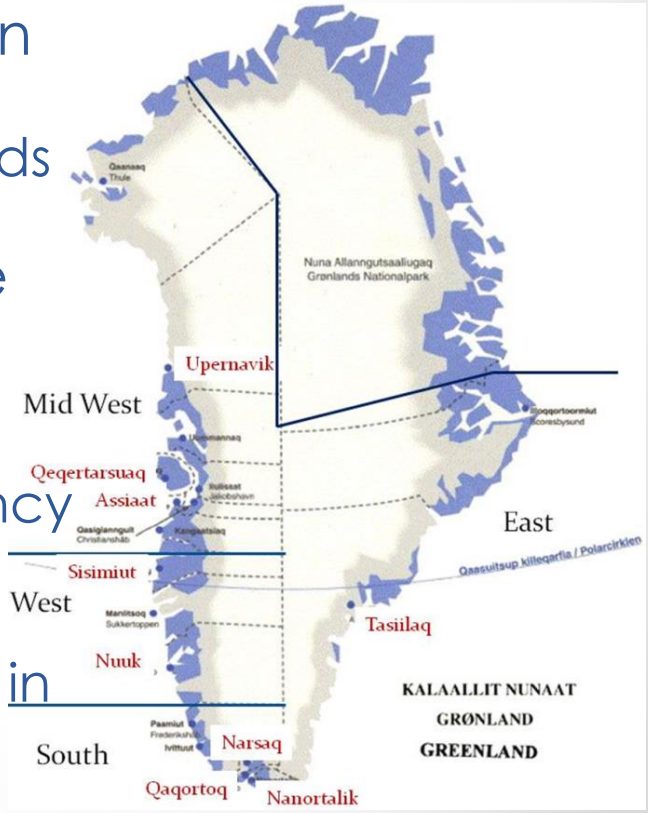
Knower et al. Endocr Relat Cancer. Review 21(2) 2014

AIN: control; HFB: High fat

Breast cancer case-control study in Greenlandic Inuit women

Aim:

- To elucidate factors involved in the increasing incidence of breast cancer in Greenlandic Inuit:
  - serum levels of perfluorinated compounds (PFAS) and lipophilic POPs
  - genetic polymorphisms in candidate genes
- Study population:
  - 31 breast cancer cases and 115 frequency matched controls
  - sampled in 2000–2003
  - Included 80% of all breast cancer cases in this period



Bonefeld-Jørgensen et al. 2011; Environ.

(Bonefeld-Jørgensen et al. 2011)

Increased risk of breast cancer in Inuit related to serum PFAS and lipPOP

- Breast cancer risk was associated with serum levels of PFOS, PFOSA and PFHxS
- Cases had higher lipophilicPOP induced xenoandrogenic activity in serum increasing the risk of breast cancer

Variable	n (case /control)	OR (95% CI)	P value	n (case/ control)	OR (95% CI)	P value
Raw	Raw	Raw	Raw	Adjusted	Adjusted	Adjusted
PFOS (ng/ml)	31/98	**1.01 (100; 1.02)**	**0.02**	9/69	**1.03 (1.00; 1.07)**	**0.05**
PFOSA (ng / ml)	31/98	1.83 (0.86-3.89)	0.12	9/69	**6.13 (1.12-33.64)**	**0.04**
PFHxS (ng/m)	31/98	**1.19(1.02-1.40)**	**0.03**	9/69	1.40 (0.95-2.05)	0.09
Xenoandrogenic transactivation	27/58	**8.52 (1.55-46.8)**	**0.01**	11/49	**44.1 (1.99-75.7)**	**0.02**

Adjustment: age, BMI, pregnancy, cotinine, breastfeeding, menopausal status

Gene-environment interaction

- Positive association between serum PFAS and breast cancer risk (Bonefeld-Jørgensen et al. 2011)
- The breast cancer risk was further increased in women with at least one of the following risk gene-allel (Ghisari et al 2015):

Polymorphism	Genotype	OR (95%CI)
CYP1A1 (Ile->val)	Val (Het/Variant)	2.6 (1.5-4.8)*
COMT (val->Met)	Met (Het/Variant)	2.7 (1.4-4.9)*
CYP17 (A1->A2)	A1 (wt)	4.9 (1.3-18.7)*

*Adjusted for age,smoking, mnopause

Breast cancer risk upon exposure to PFAS: a prospective case-control study in Danish women

- **Aim:** A prospective study to evaluate the correlation between serum levels of PFASs of in young Danish women and breast cancer risk later in life
- **Methods:** Blood and questionnaire data were taken from the "Danish National Birth Cohort" in the period 1996-2002
- About 10-15 years later 250 pregnant women (nulliparous) diagnosed with breast cancer, and 233 matched controls were selected for further analysis.
- Serum level for 16 PFAS was determined
- Data adjusted for known risk factors:
  - age at sampling, BMI before gravidity, use og contraceptive pills age at first menses, smoking, alcohol intake (Beer & Wine), maternal education and physical activity

Bonefeld-Jørgensen EC, Long M, Fredslund SO, Bossi R, Olsen J *2014. Cancer Causes Control*

Cases had significantly higher Relative Risk for breast cancer

PFAS (ng/ml)	Age < 40 yrs		Age > 40 yrs	
	case/control	Adj.RR (95% CI) [a]	case/control	Adj.RR (95% CI) [a]
PFHxS	132/120	0.64 (0.39-1.05)[b]	118/113	0.71 (0.47-0.94)[b]
<0.76	40/19	1.00 (ref)	24/19	1.00 (ref)
0.76-0.92	29/29	**0.39 (0.17-0.88)**	20/17	1.23 (0.44-3.42)
0.92-1.12	23/21	0.56 (0.23-1.35)	29/25	1.04 (0.40-2.68)
1.12-1.35	19/26	**0.30 (0.12-0.72)**	18/30	0.52 (0.20-1.35)
>1.35	21/25	**0.41(0.17-0.96)**	27/22	1.01 (0.40-2.54)
PFOSA	132/120	**1.07 (1.00-1.14)**[b]	118/113	1.01 (0.97-1.07)[b]
<0.93	29/33	1.00 (ref)	19/16	1.00 (ref)
0.93-1.70	32/27	1.53 (0.70-3.32)	21/17	1.30 (0.48-3.56)
1.70-2.83	22/24	1.04 (0.45-2.40)	22/29	0.96 (0.37-2.51)
2.83-5.75	22/24	1.10 (0.46-2.59)	24/26	1.37 (0.52-3.61)
>5.75	27/12	**2.45 (1.00-6.00)**	32/25	1.62 (0.61-4.29)

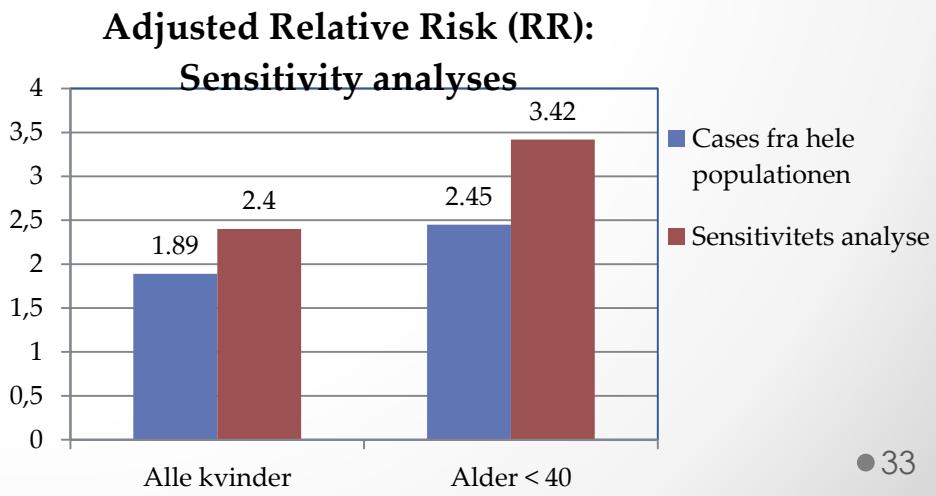
For women at age < 40 years PFOSA serum level showed a significantly RR for breast cancer development

Sensitivity analyses: 72 cases were after 1 year removed from the Danish Cancer Registry assessed not being breast cancers

5. Quintile

All women: **RR 2.40; CI, 1.20-4.83**

Women < 40 år: **RR 3.42; CI, 1.25-9.36**



Summary

- EDCs can potentially alter the epigenome and mammary development and increase breast cancer risk
- Animal studies suggest that in utero exposure to EDCs can increase the risk for breast cancer later in life
- Recent human data suggest that exposure to POPs/ PFAS can increase the risk for breast cancer
- How EDCs modify the epigenome of specific genes deserves further investigation