

Epigenetic modulations by Endocrine Disruptors

Modulations épigénétiques par les perturbateurs endocriniens

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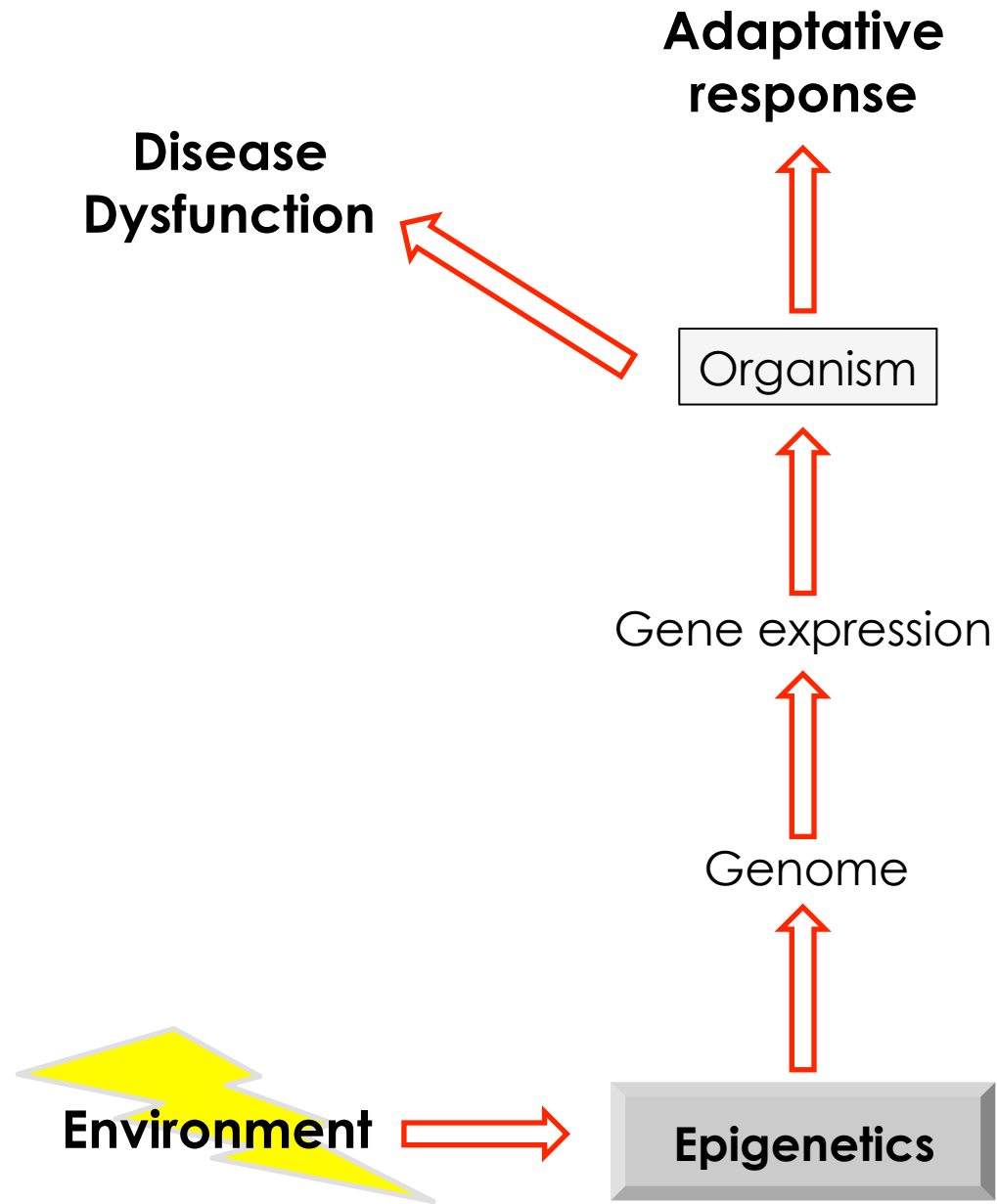
Epigenetics

Epigenetic modifications: reversible changes that, **without** affecting the DNA sequence, can affect gene expression.

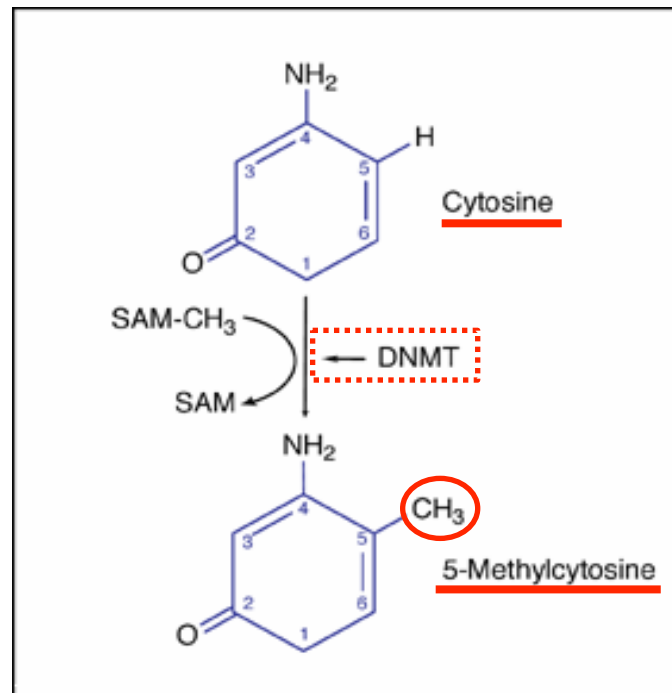
These changes **can be inherited** in such a way that the information that governs gene expression can be passed **from cell to cell**.

The best, although not the only, characterized epigenetic phenomenon is **DNA methylation** (other: histone acetylation, phosphorylation, ... all affecting chromatin structure, ...).

Epigenetic modifications themselves might explain how **environmental factors** may modulate gene expression

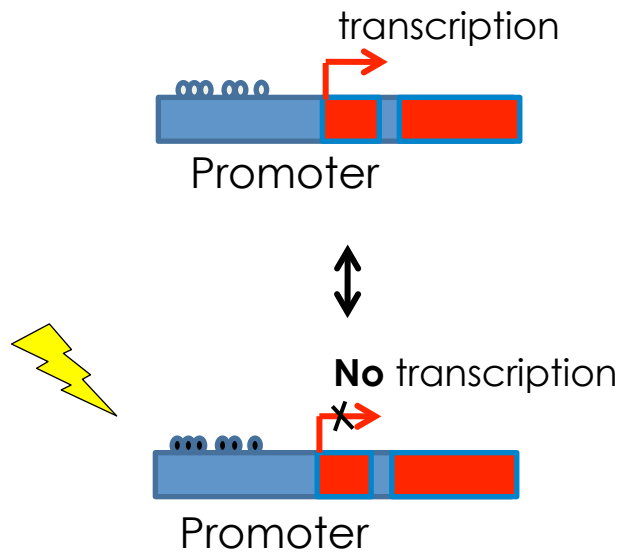


DNA Methylation : Cytosines (C) within CpGs (CG)

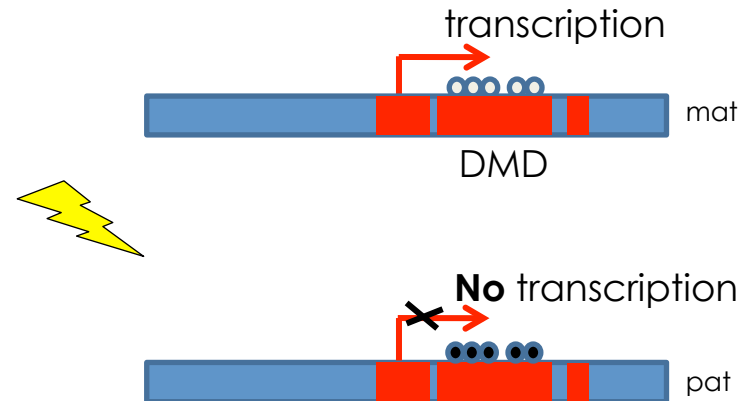


Methylation

Promoters of specific genes (housekeeping/tissue-specific)



Differentially Methylated Domains of imprinted genes

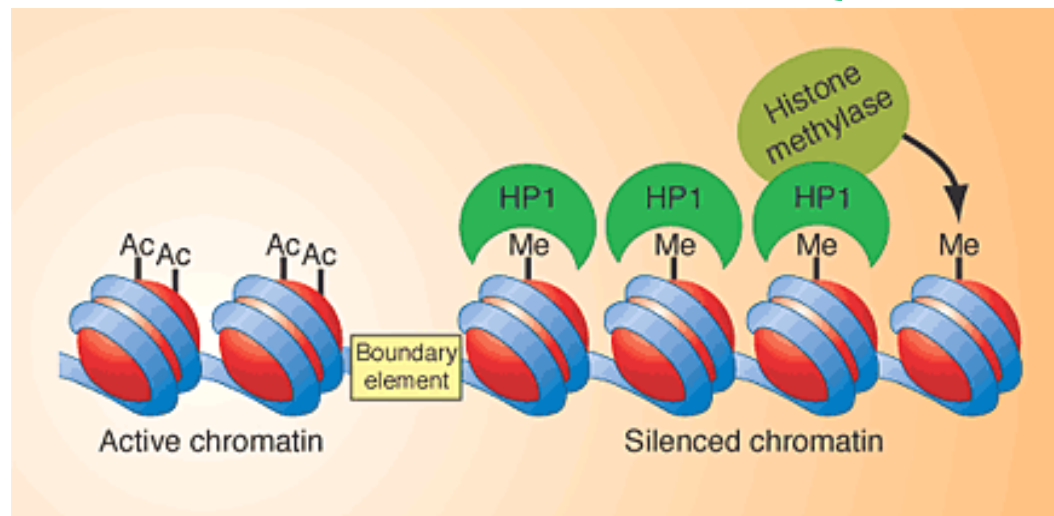


● methylation

Methylation I and II

Proteins **bind specifically to methylated DNA**: seem to account for the effects of DNA methylation on **chromatin structure and transcription**

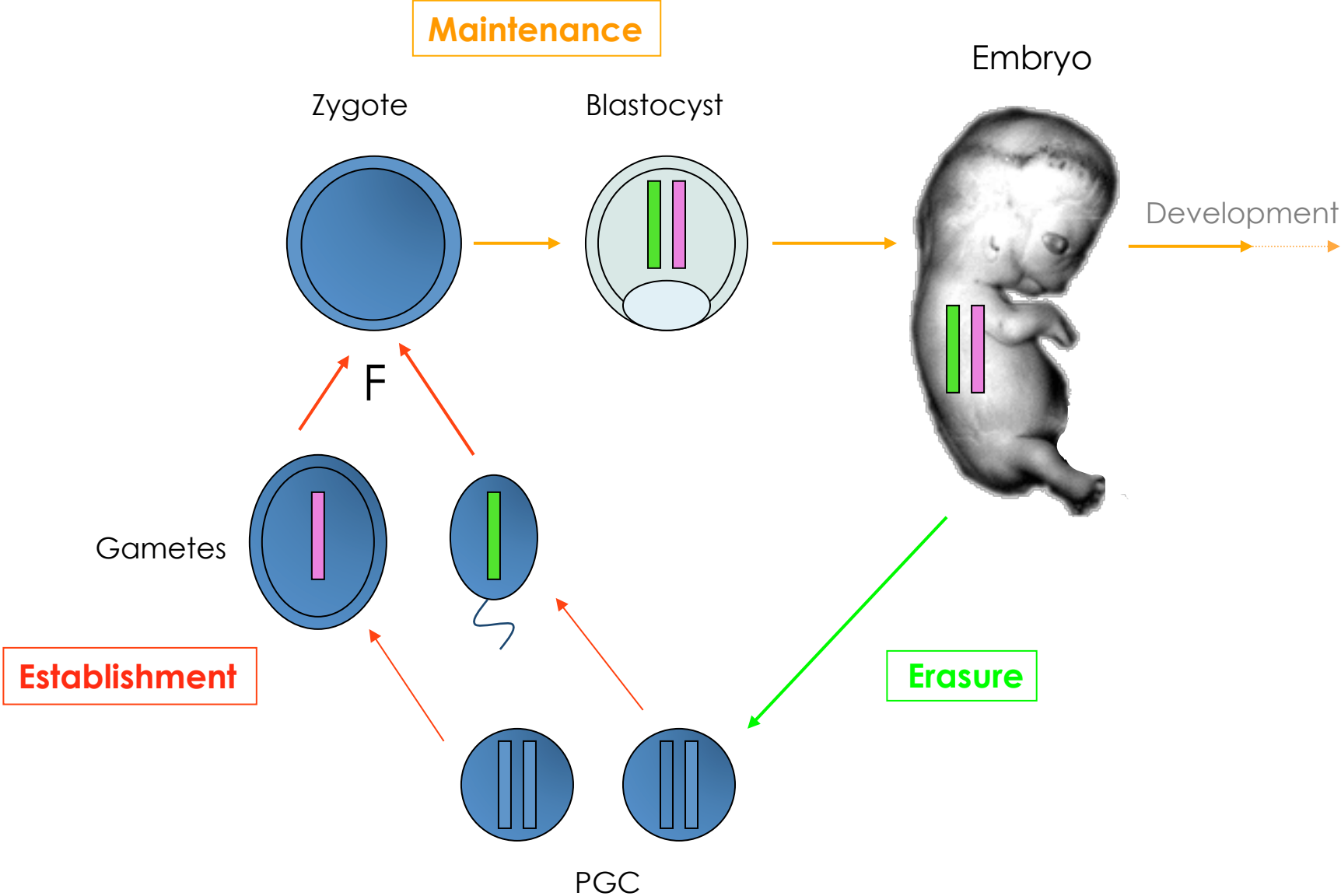
MBPs: methyl-CpG-Binding Proteins { HP1 : transcription-inhibiting protein



euchromatin

heterochromatin

Dynamic of genomic imprints : methylation **reprogramming**



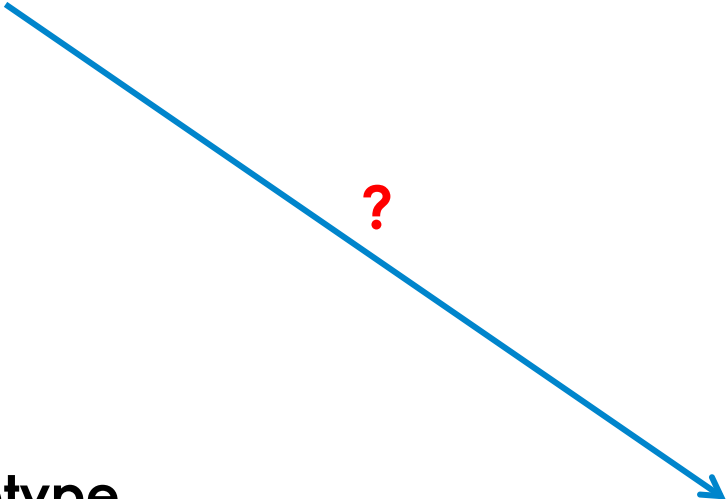
Endocrine Disruptors - mechanism of action

- ▶Mimic the effect of endogenous hormones (receptor activation)
- ▶Antagonize endogenous hormones (receptor blockage)
- ▶Affect the synthesis, transport, metabolism and excretion of hormones (i.e. alteration of the concentration of natural hormones)

What about :

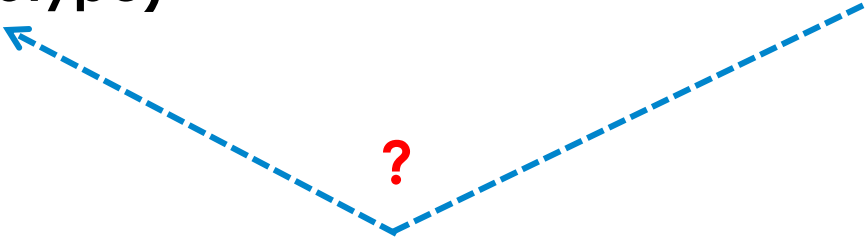
- ▶Epigenetic changes : **DNA methylation ?**

Endocrine Disruptors



**Disease/Phenotype
(Epiphenotype)**

Methylation changes



Target genes

Target tissues

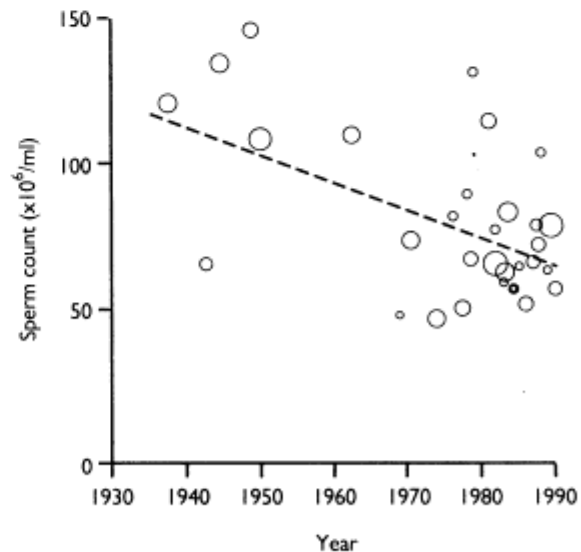
Male infertility

In a few decades in Western countries: almost **50% reduction**

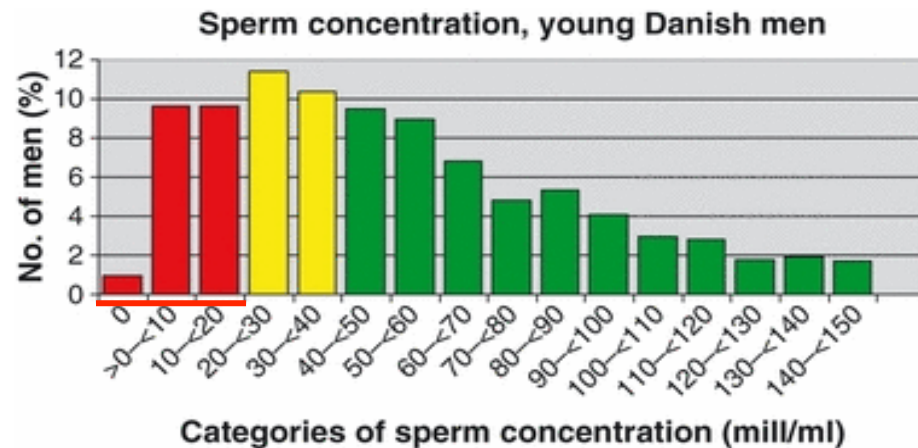
— from $113 \times 10^6/\text{mL}$ to $65 \times 10^6/\text{mL}$ —

in **mean sperm concentration**

as well as decrease in sperm quality



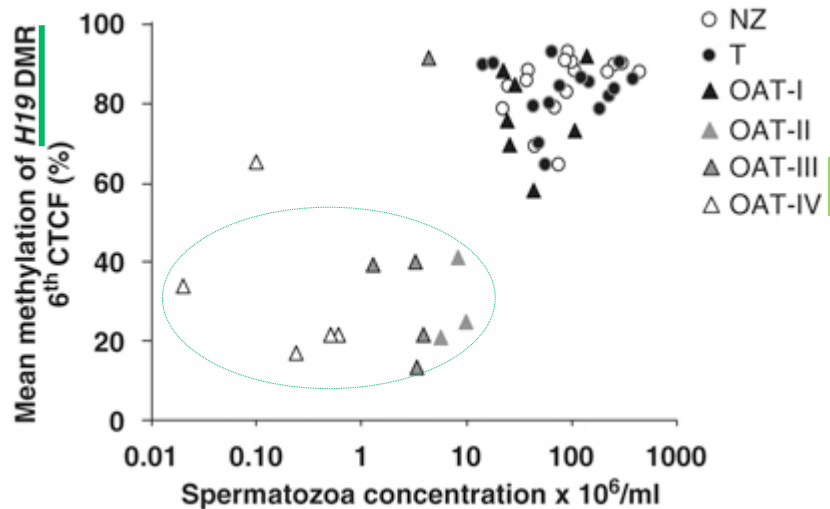
Carlsen et al., *BMJ*, 1992



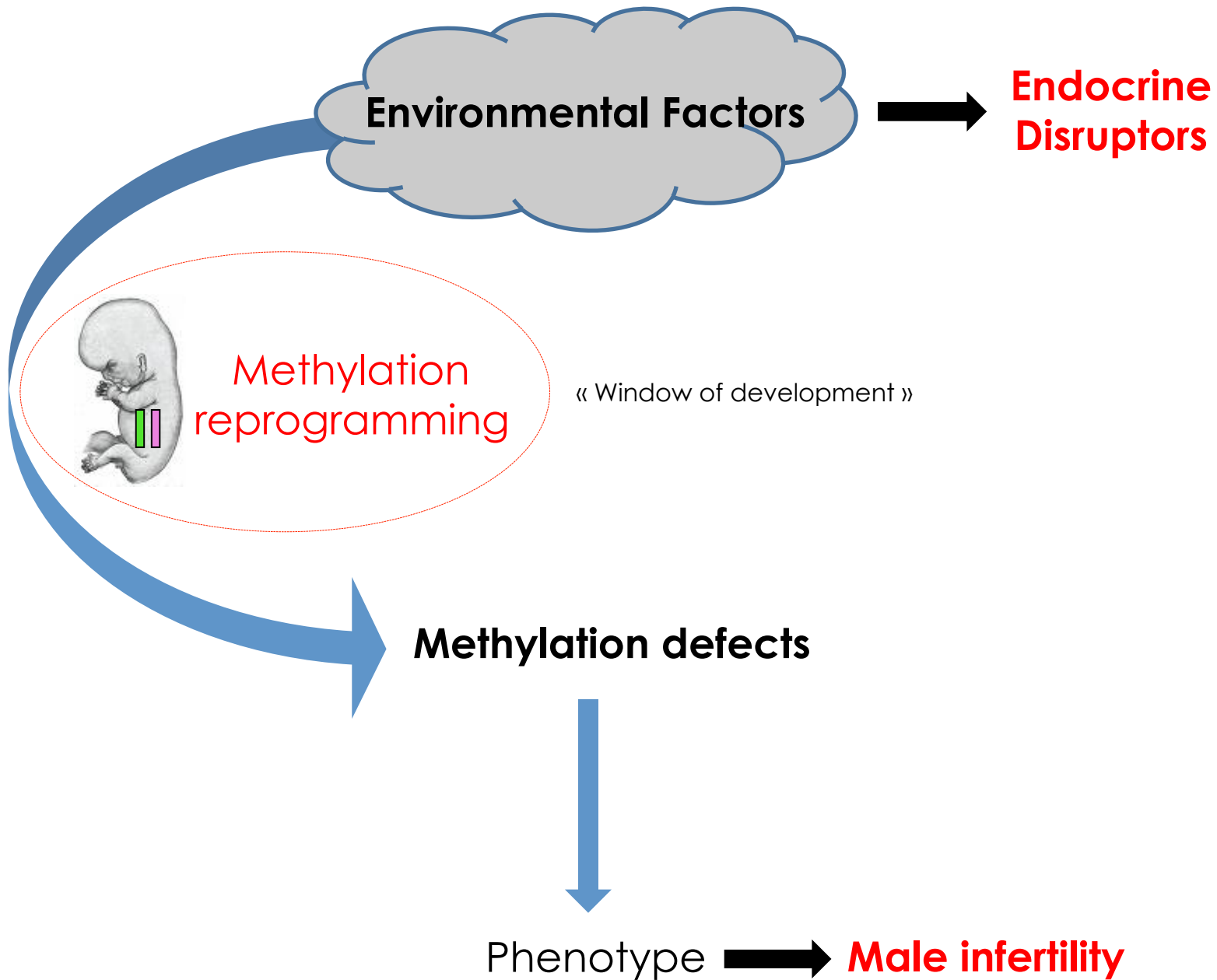
Andersson et al., *Int J Androl*, 2008

In human

- Idiopathic **male infertility** was associated with **aberrant methylation** of 2 imprinted genes : Peg1 and H19 – cohort of 148 infertile vs 33 fertile males
- **Aberrant methylation** of Peg1 imprinted gene was correlated to
↓ **sperm number + quality**



- Loss of methylation at H19-oligospermia



Methodology applied

In vivo system

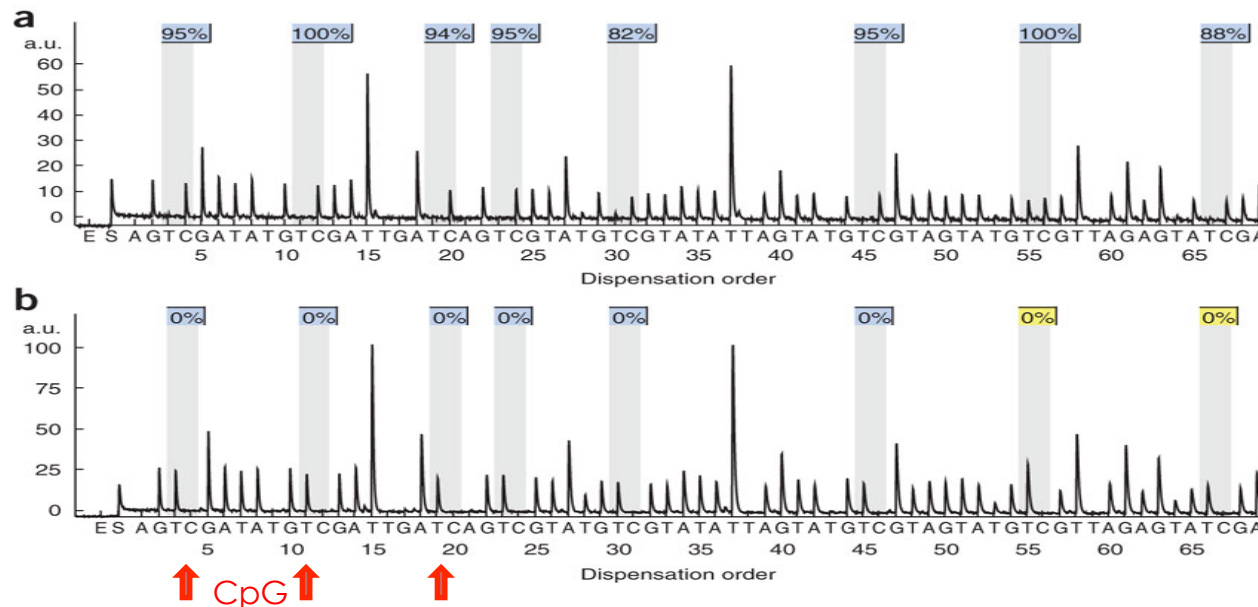
- FVB/N or C57BL/6J mice strain
- In utero (methylation reprogramming) or direct exposure to EDs
- Tissue- and gene-specific methylation analysis (pyrosequencing technology)
- Phenotype assessment (spermatogenesis)
- Transgenerational inheritance assessment

Pyrosequencing – DNA analysis

sequencing-by-synthesis method : **quantitatively** monitors the real-time incorporation of nucleotides through the enzymatic conversion of released pyrophosphate into a proportional light signal



degree (%) of methylation at each CpG position in a sequence determined from the ratio of T and C at the corresponding position



Compounds investigated - I

Pesticides - Endocrine Disruptor properties

Vinclozolin (antiandrogenic activity), fungicide

Effects (reported in rodent studies, different doses):

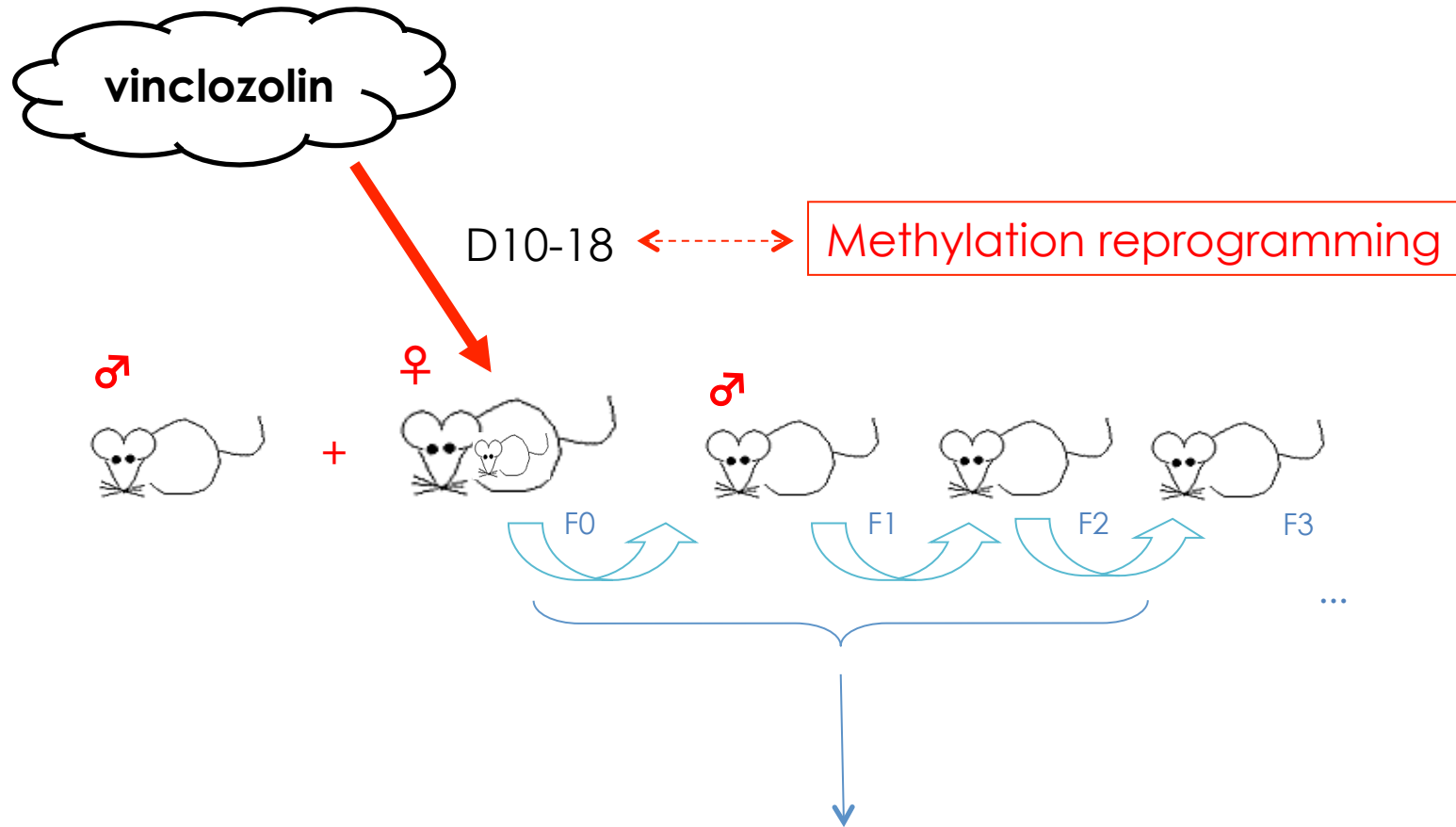
► **genital abnormalities in males/females**

feminization of males, hypospadias, masculinization of females

► **testis histological defects + sperm count ↓** and motility/shape

our dose: 50 mg/kg/d

Experimental setup: 1 condition ▶ in utero exposure

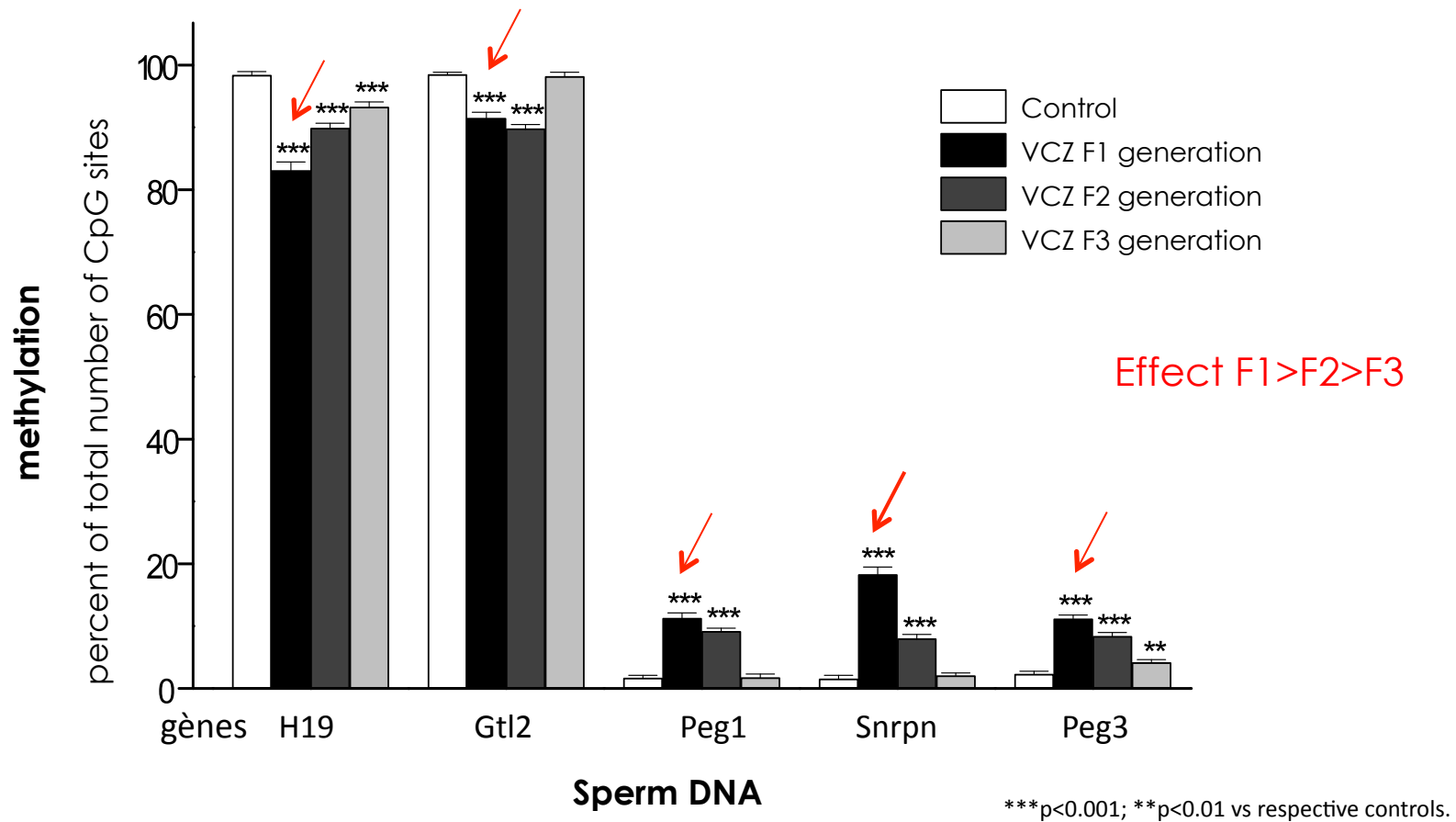


Target genes – imprinted genes

DNA methylation – different tissues

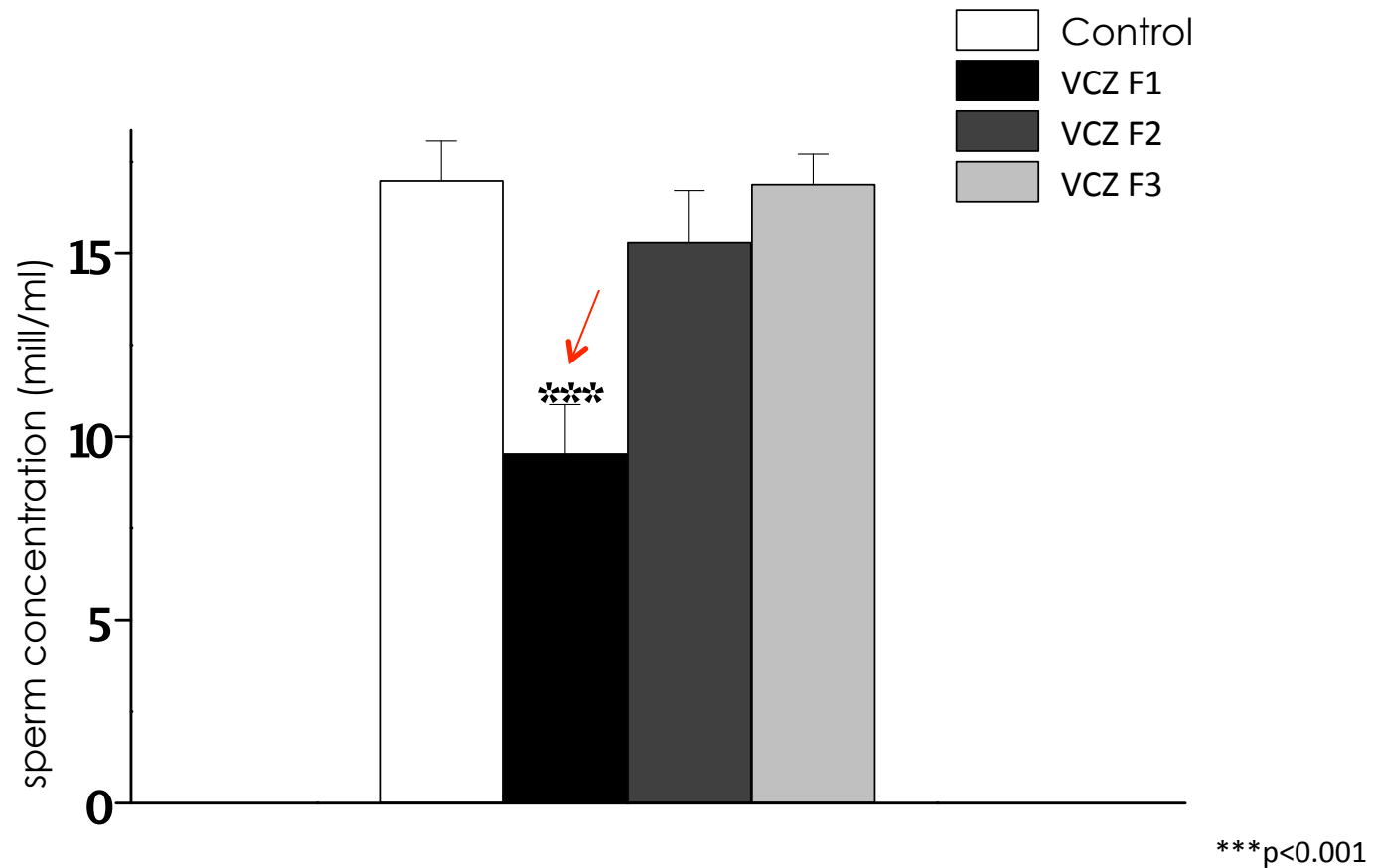
Phenotype (fertility) ?

Vinclozolin exposure - Sperm results



- ▶ Epigenetic modifications (imprinting)
- ▶ Tissue-specific effect (gametes >>)
- ▶ Transgenerational effect: F1 and F2 (5 genes), → F3 (2 genes)

Vinclozolin exposure - phenotype results



◇ Spermatogenic impairment (F1>F2-F3)

◇ No other observed phenotype (weight, litter size, sex ratio)

Compounds investigated - II

Pesticide - Endocrine Disruptor properties

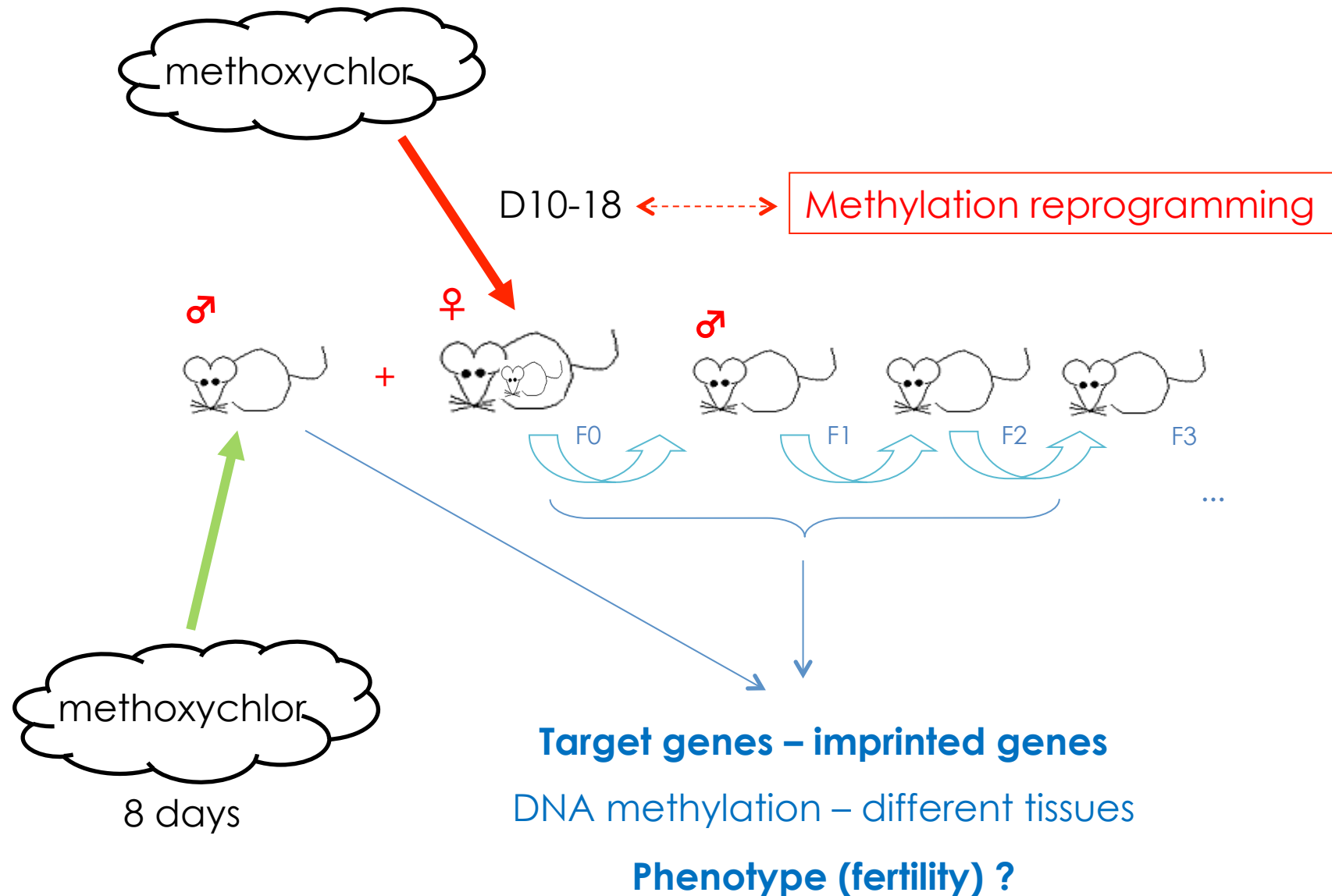
Methoxychlor (metabolites: estrogenic, antiestrogenic and antiandrogenic activities), insecticide

Effects (reported in rodent studies, different doses):

- ▶ **genital abnormalities in males (testis, prostate, seminal vesicles)**
- ▶ decreases spermatogenesis
- ▶ decrease in sexual arousal
- ▶ **reduction in pregnancy outcome**
- ▶ reduction in ovulatory rates

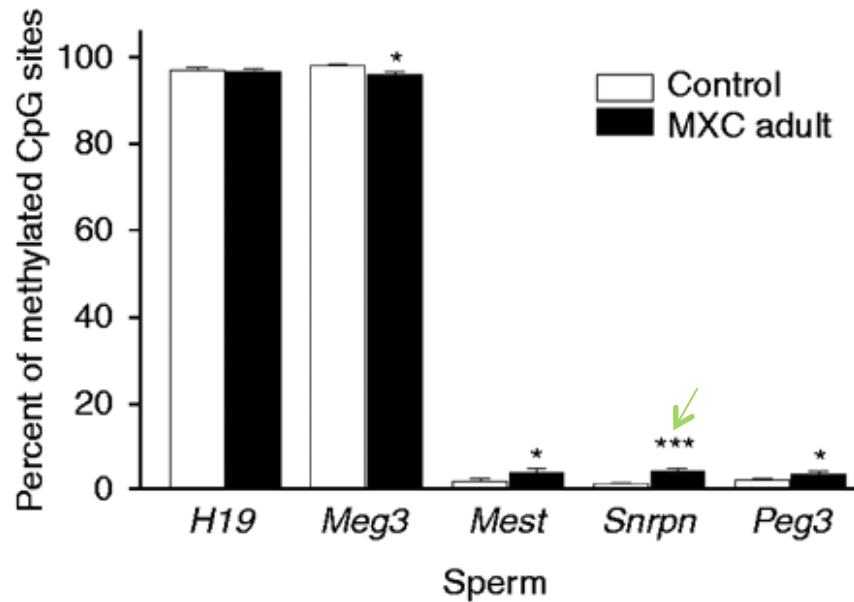
our dose: 10 mg/kg/d

Experimental setup: 2 conditions ▶ in utero exposure / direct exposure

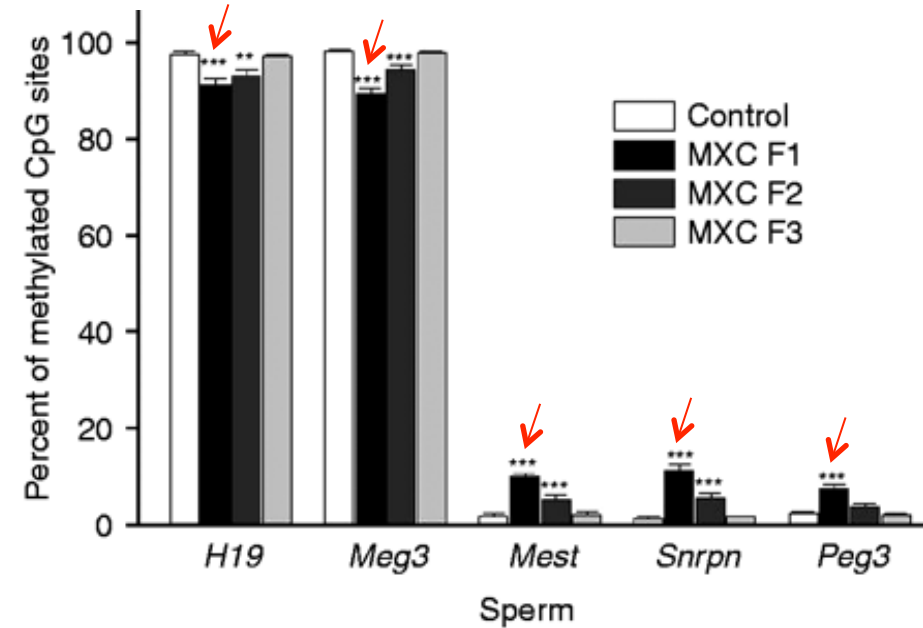


Methoxychlor exposure - Sperm DNA results

Direct expos. (8D)



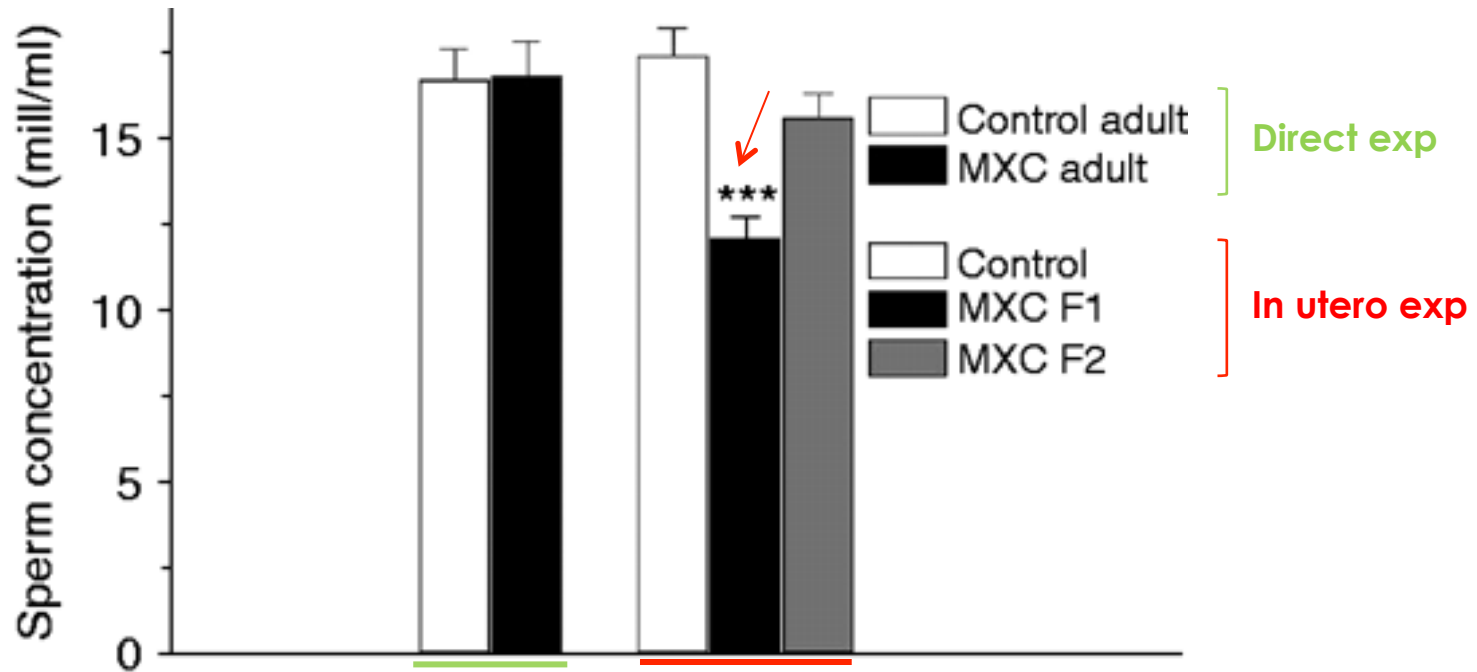
In utero expos. (D10-18)



***p<0.001; **p<0.01 vs respective controls.

- ▶ Effect is **discrete** for **direct** exposure, **important** for **in utero** exposure
- ▶ Transgenerational effect for in utero (3 genes → F2)

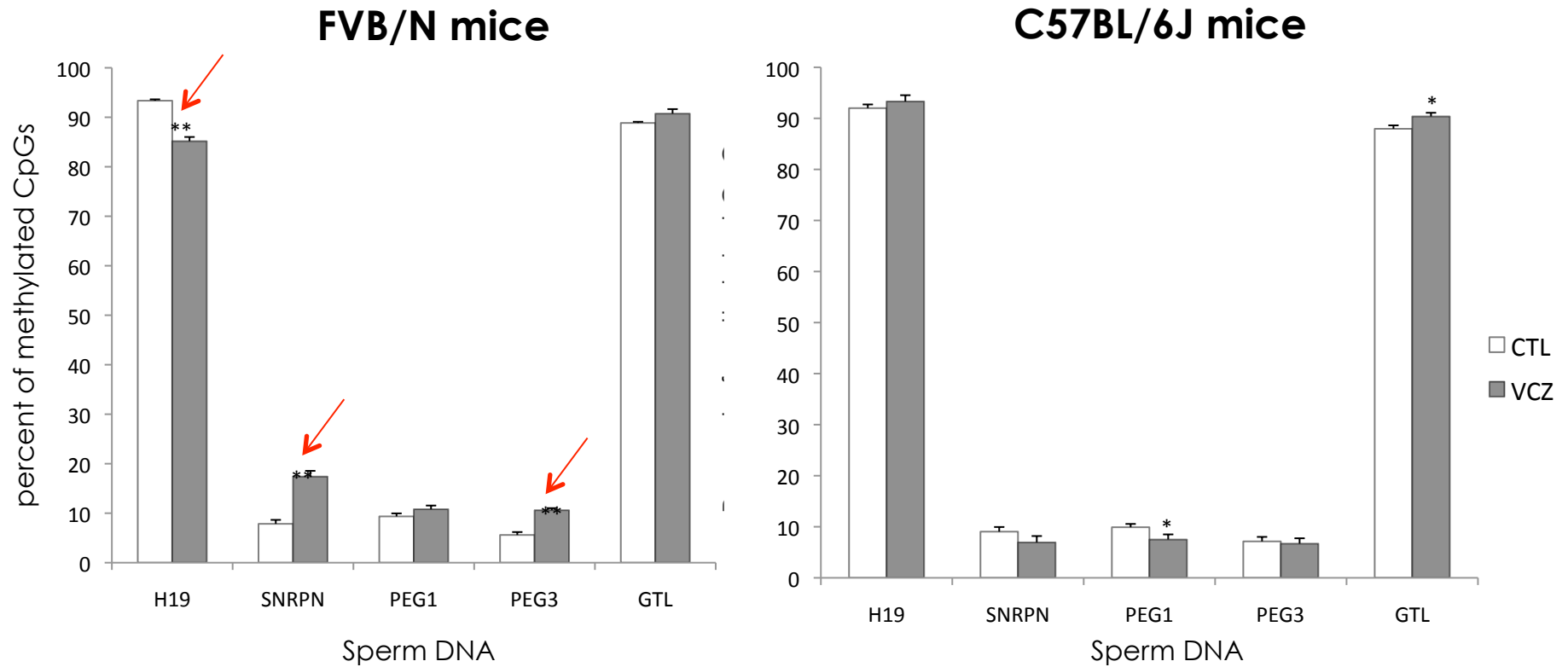
Methoxychlor exposure - phenotype results



- ▶ **Direct exposure : 0 effect on fertility, ++ for in utero**
 - ▶ 0 other phenotype (weight, litter size,...)

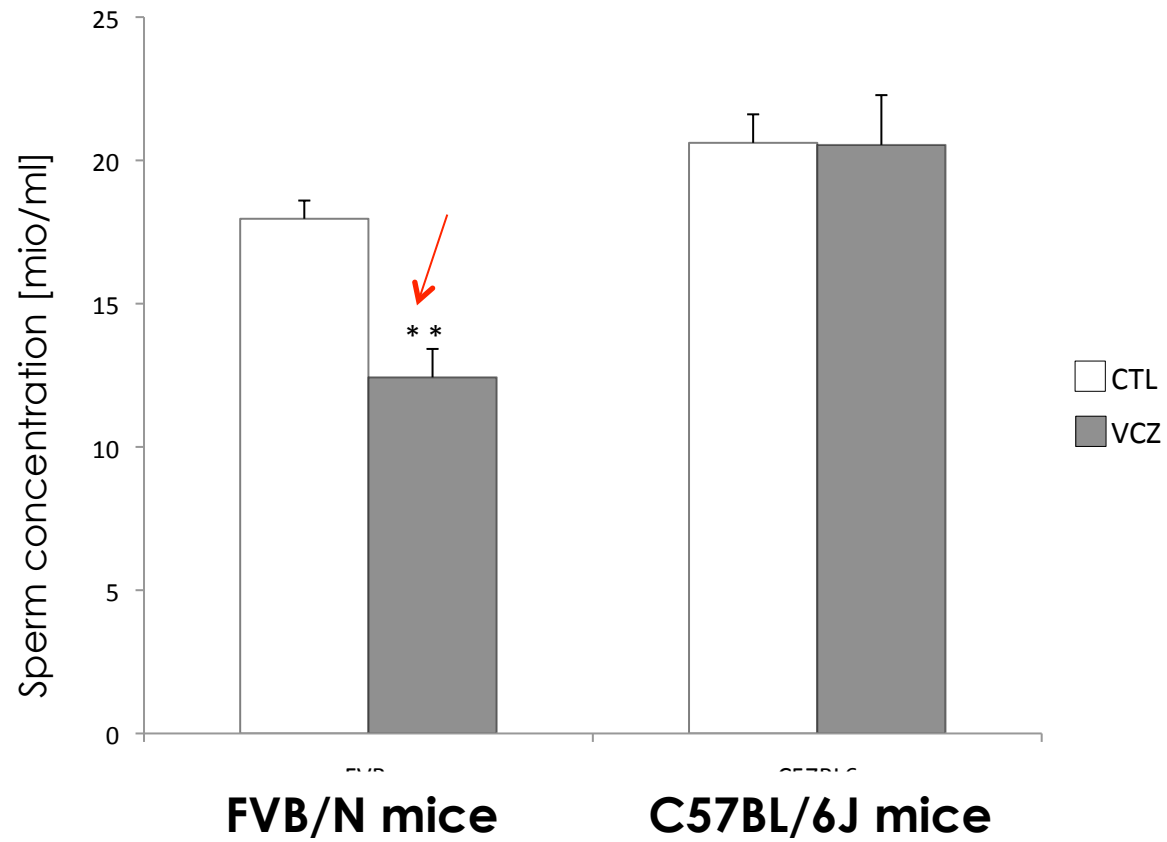
Different genetic backgrounds - same exposure

Vinclozolin exposure, 20 mg/kg/day, Days 10-18 of pregnancy



► Strain-specific epigenetic effect

Different **genetic backgrounds** - phenotype



► Phenotype correlated to methylation changes

strain specificity

Compounds investigated - III

Dioxin (TCDD : 2,3,7,8-tetrachlorodibenzo-p-dioxin)

Endocrine Disruptor properties

by-product of numerous industrial processes,
resistant to degradation, accumulates in the environment

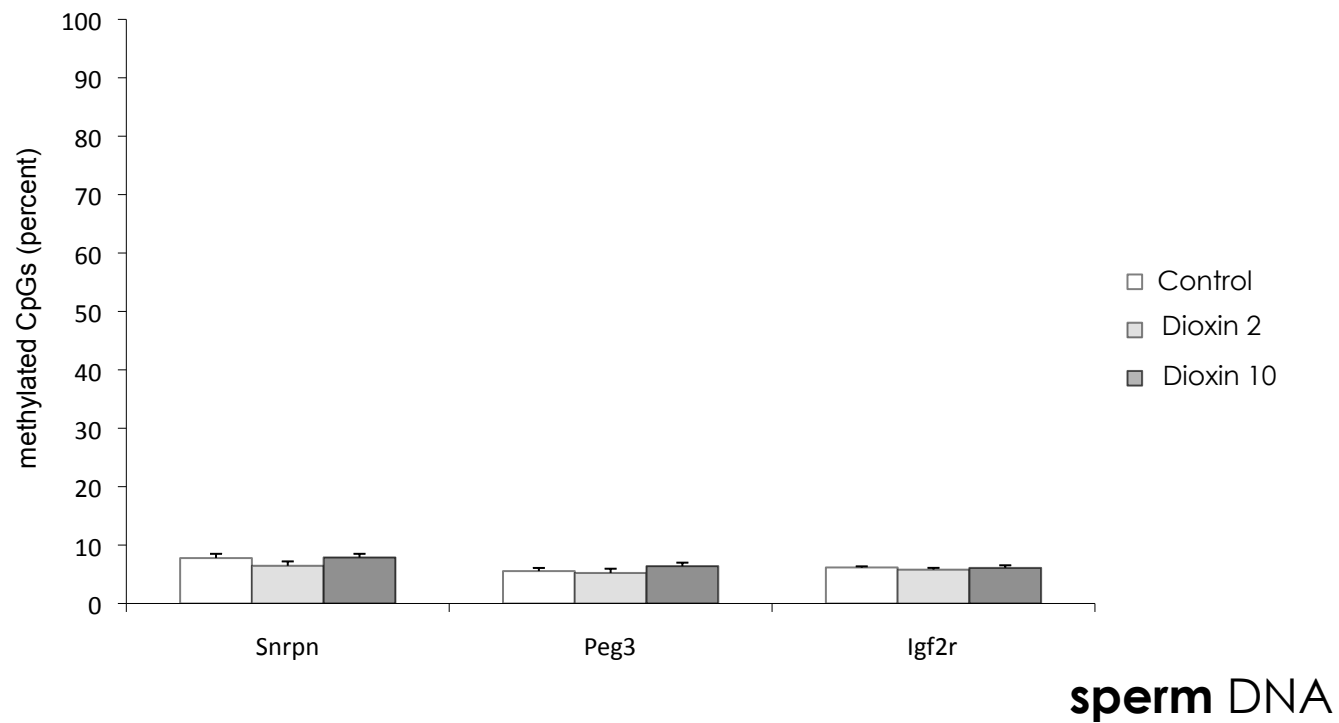
Rodent and human data

▶ decreased fertility, reduced sperm counts, endometriosis,
changes in sex ratio in offspring, effects on breast ..

Our dose: 2 or 10 ngr/kg/d

Dioxin (TCDD): relation dose-methylation defect ?

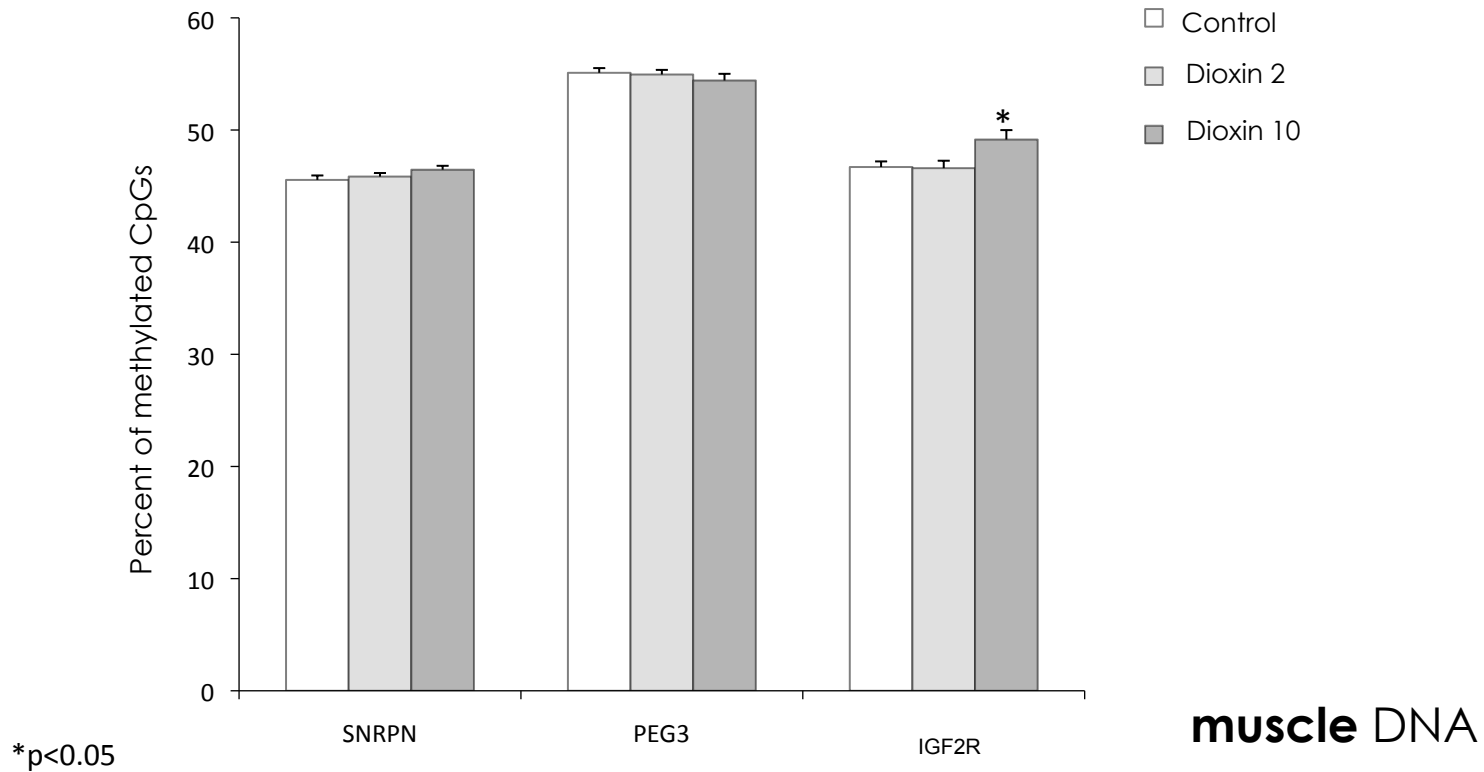
Dioxin 2 or 10 ngr/kg/d, per pregnancy



► 0 effect at 2 ngr/kg/d or 10 ngr/kg/d

Dioxin (TCDD): relation dose-methylation defect ?

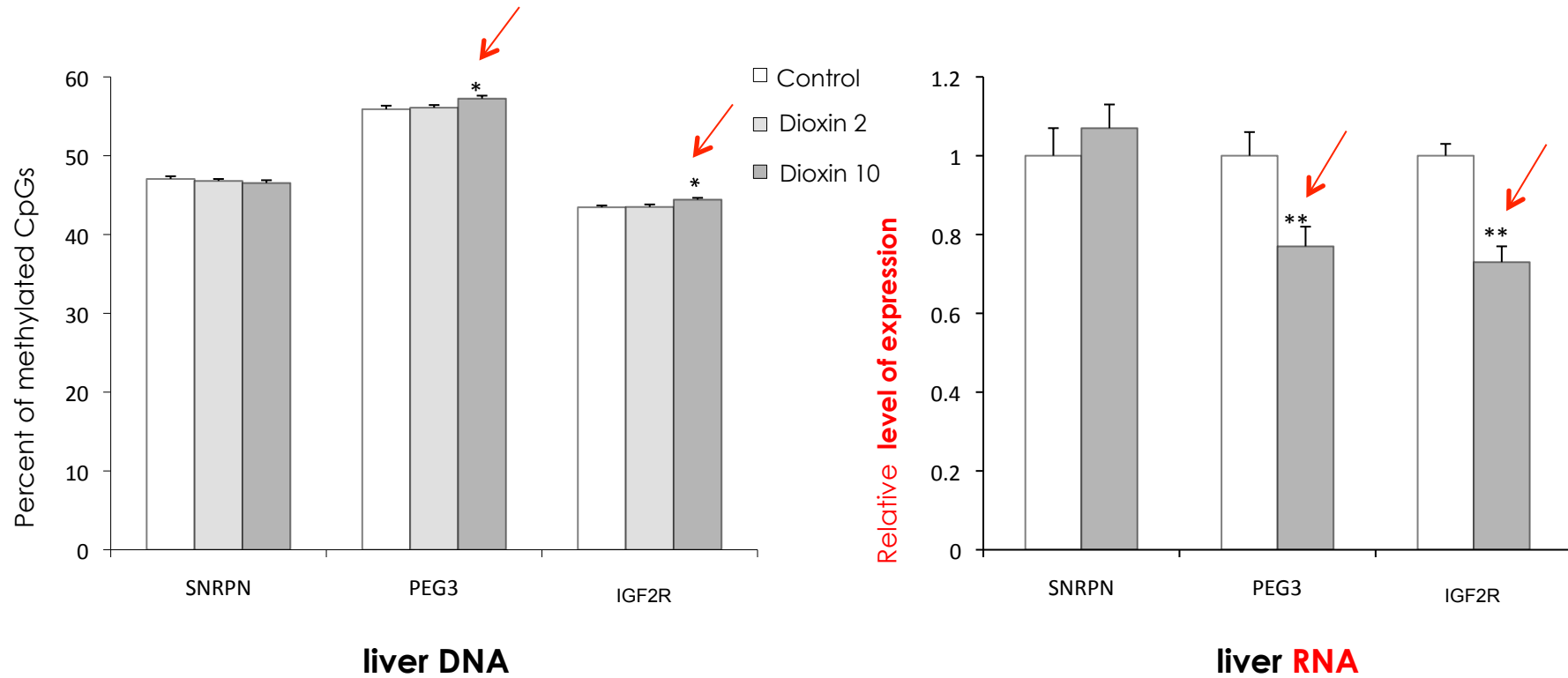
Dioxin 2 or 10 ngr/kg/d, per pregnancy



- ▶ 0 effect at 2 ngr/kg/d, discrete effect appearing at 10 ngr/kg/d
- different tissue-different effect

Dioxin (TCDD) exposure: methylation-gene expression ?

2 or 10 ngr/kg/d, per pregnancy



► Correlation ↑ methylation - ↓ gene expression

