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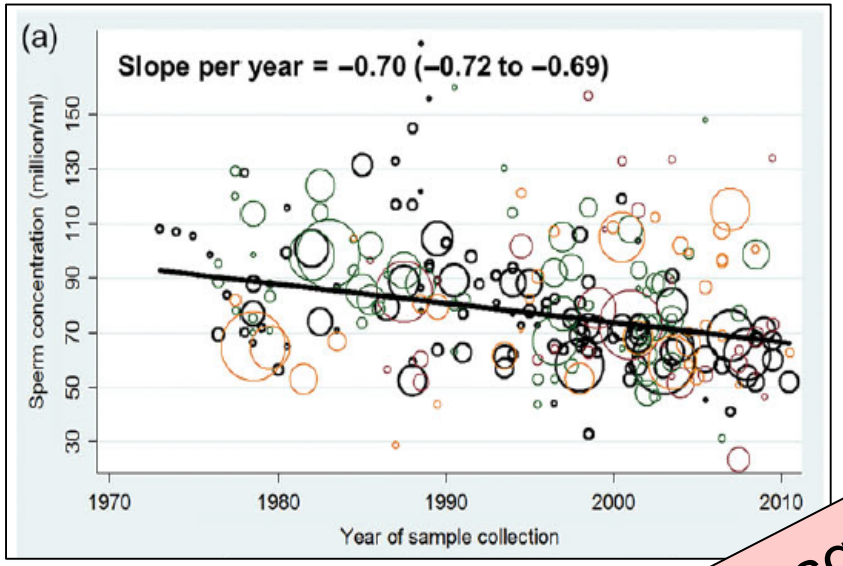
The household dog as a sentinel for chemical effects on male reproductive function

Richard Lea

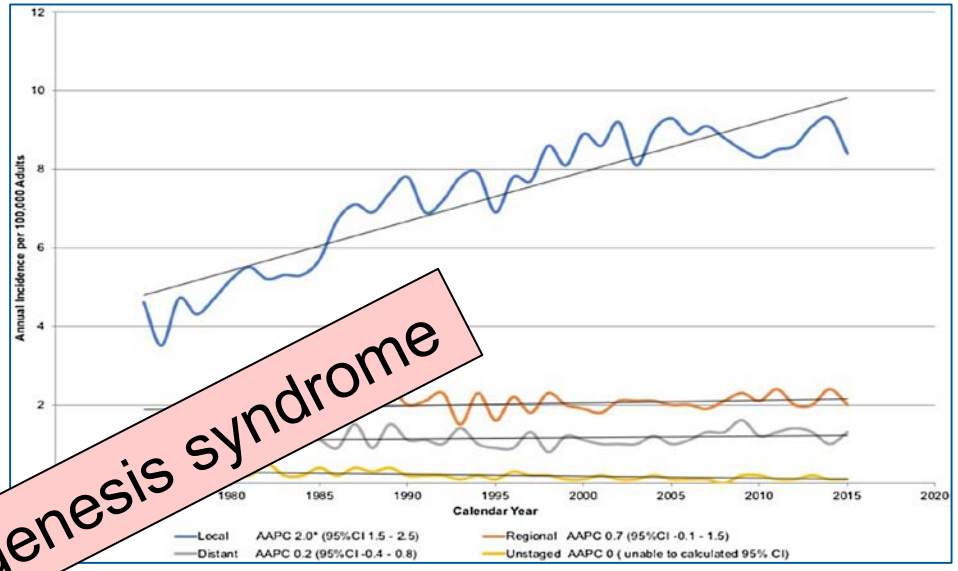
Temporal and regional influences on human male reproductive health: environmental?

Declining human sperm counts



Levine et al., 2017

Testicular Cancer USA: 1975-2015

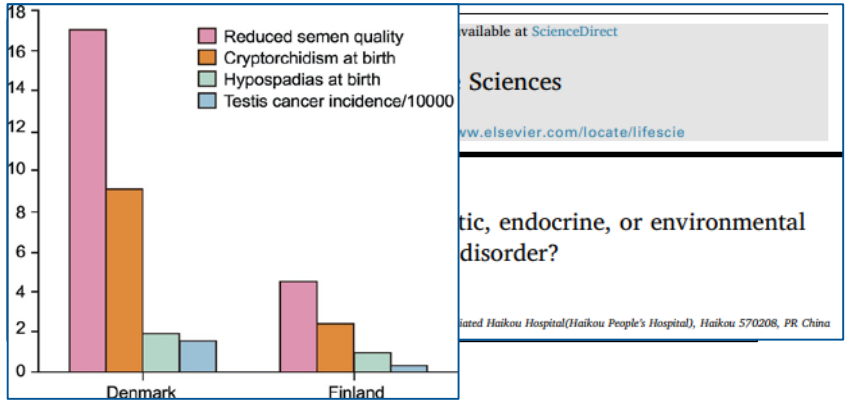


Reese et al., et al., 2021

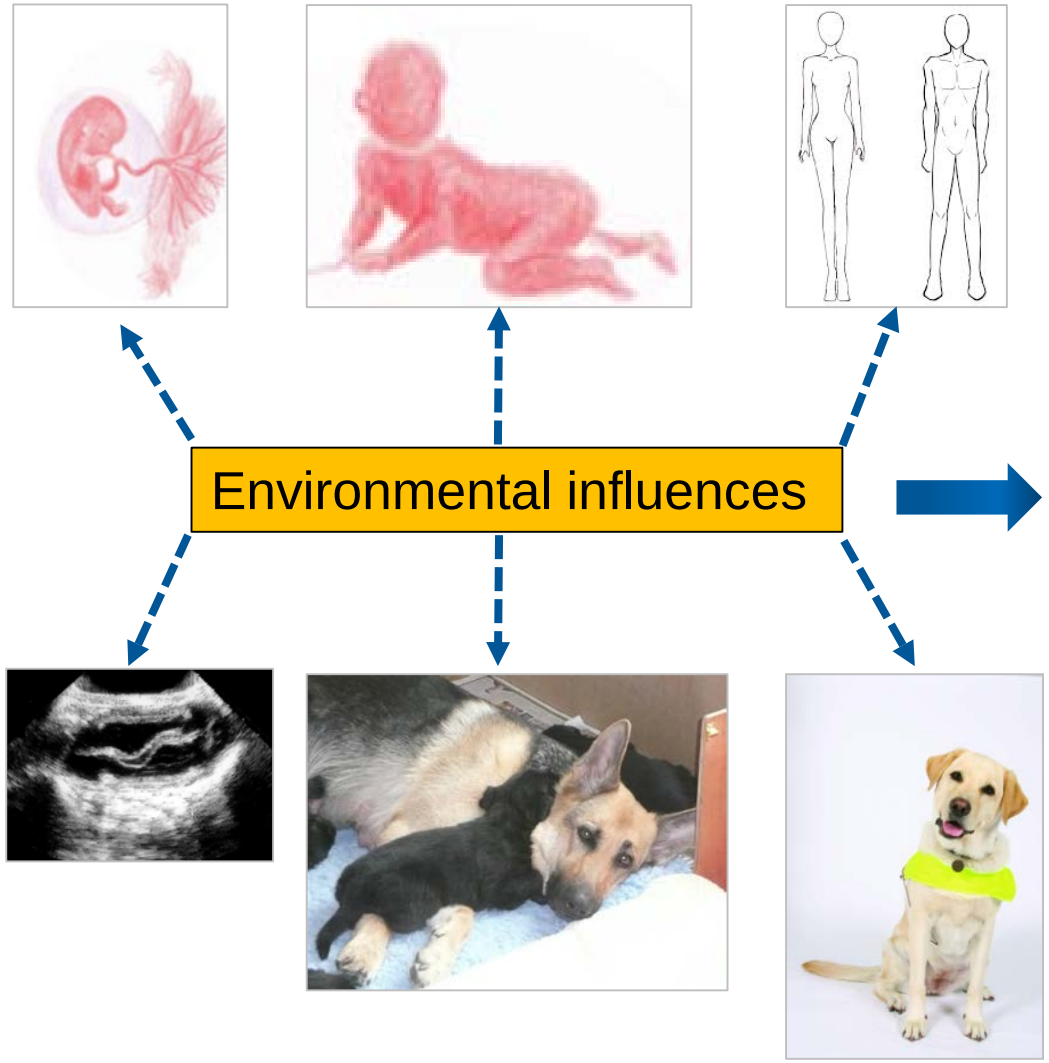
Testicular dysgenesis syndrome

Cryptorchidism

Hypospadias



Testicular dysgenesis syndrome in the dog?



Reproductive function & health in the dog?

- Sperm quality (temporal trends)
- Testicular function/indices of cancer
- Malformations in the pups

Investigating fertility in a population of stud dogs

5 breeds (n=232) 1,925 ejaculates

↓

Routine reproductive examinations
(1988 to 2014)

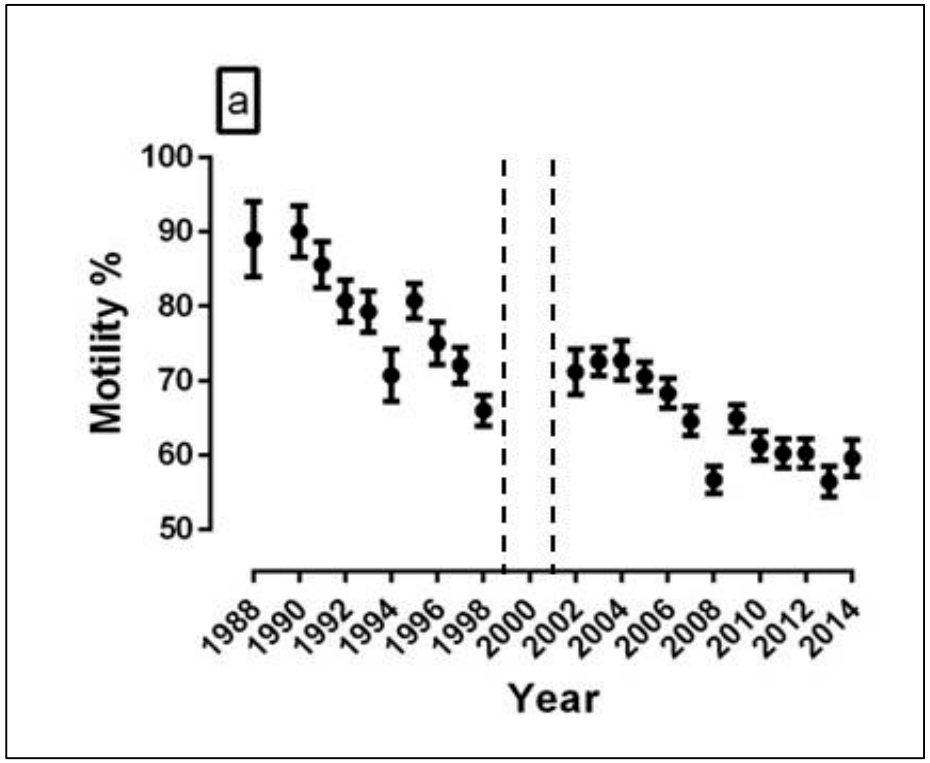
↓

Sperm quality measurements
- % normal motility
- % morphologically normal
- Total sperm output

- Consistent WHO methodology
- Consistent collection by 1 of 3 technical staff
  - All trained by 1 experienced andrologist

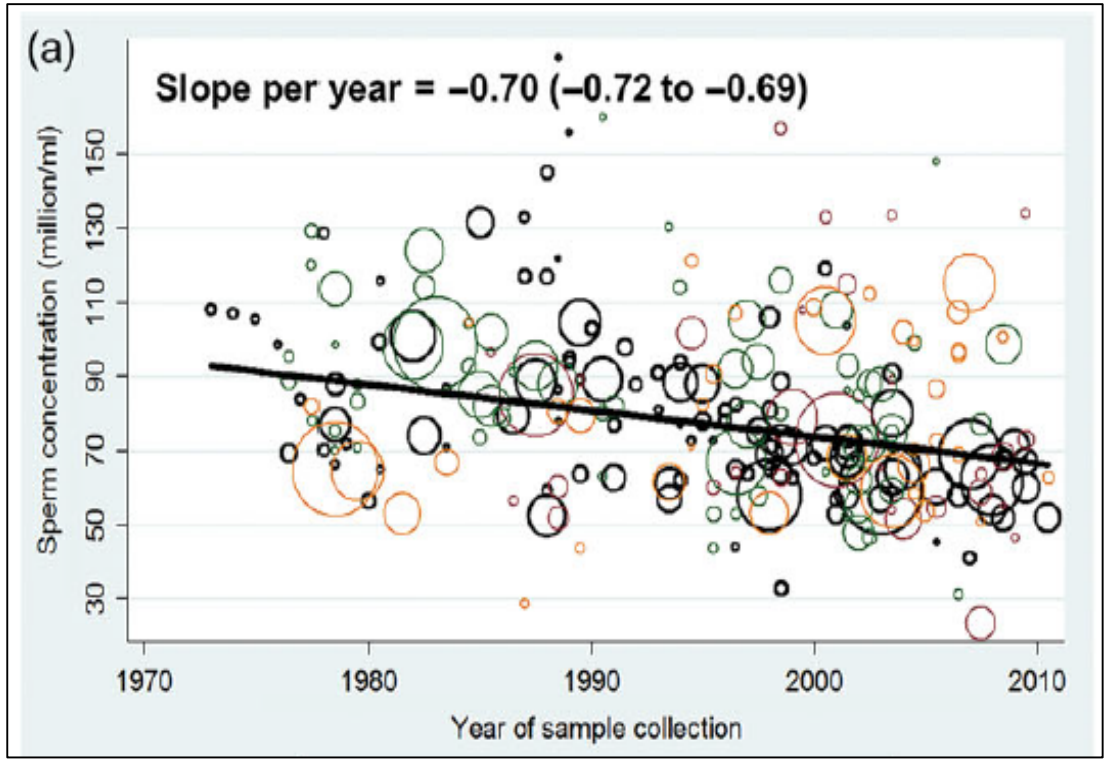
Temporal trends in dog semen quality

Canine semen motility



Consistent methodology
Same laboratory
Three technical staff

Human sperm counts



Controversial:
Methods, training,
Quality assurance…

Levine et al., 2017

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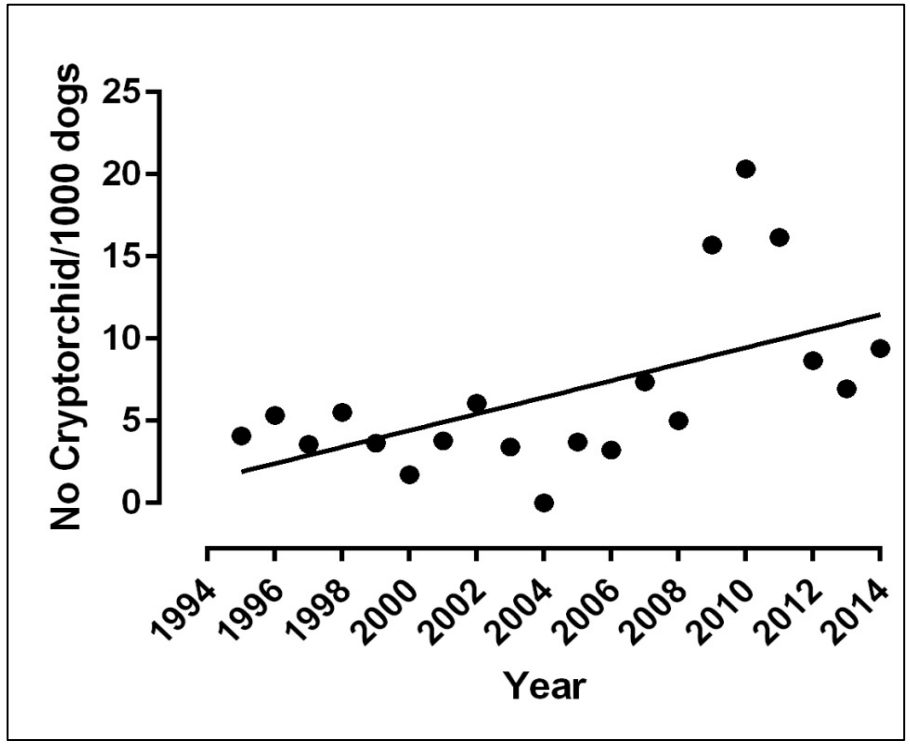
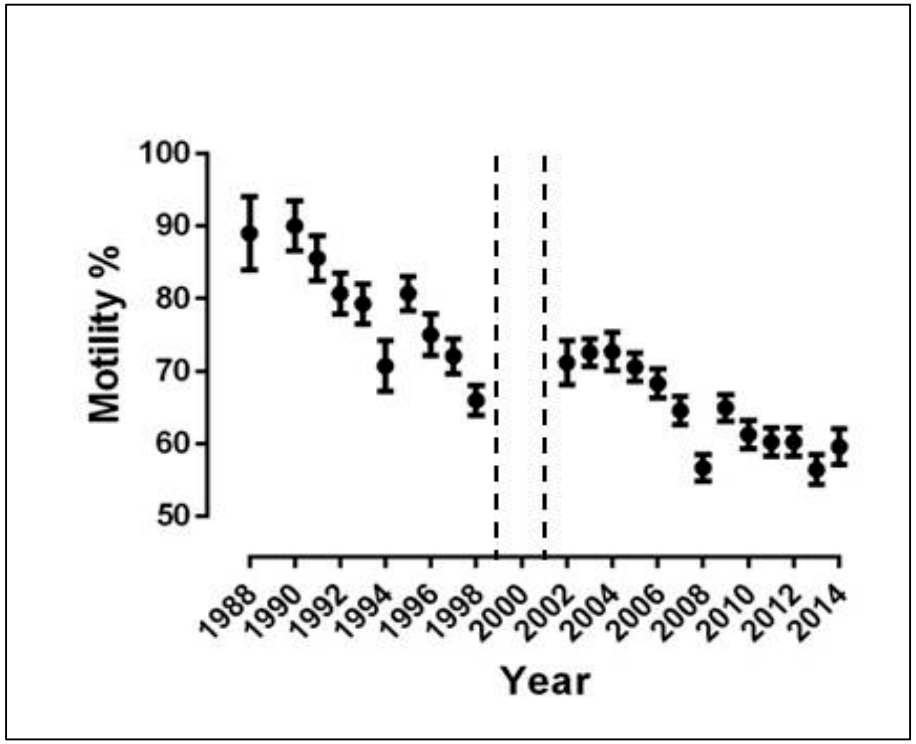
Cryptorchidism in the pups (same population)

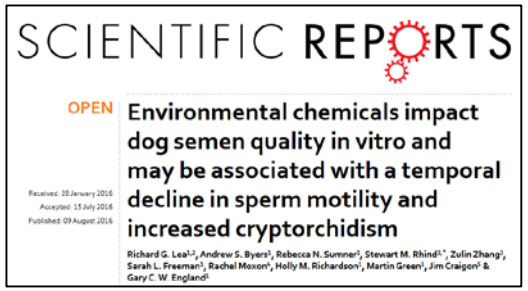
Cryptorchidism



Sperm motility



 # males/year: 651.25 ± 14.79


SCIENTIFIC REPORTS

OPEN

Environmental chemicals impact dog semen quality in vitro and may be associated with a temporal decline in sperm motility and increased cryptorchidism

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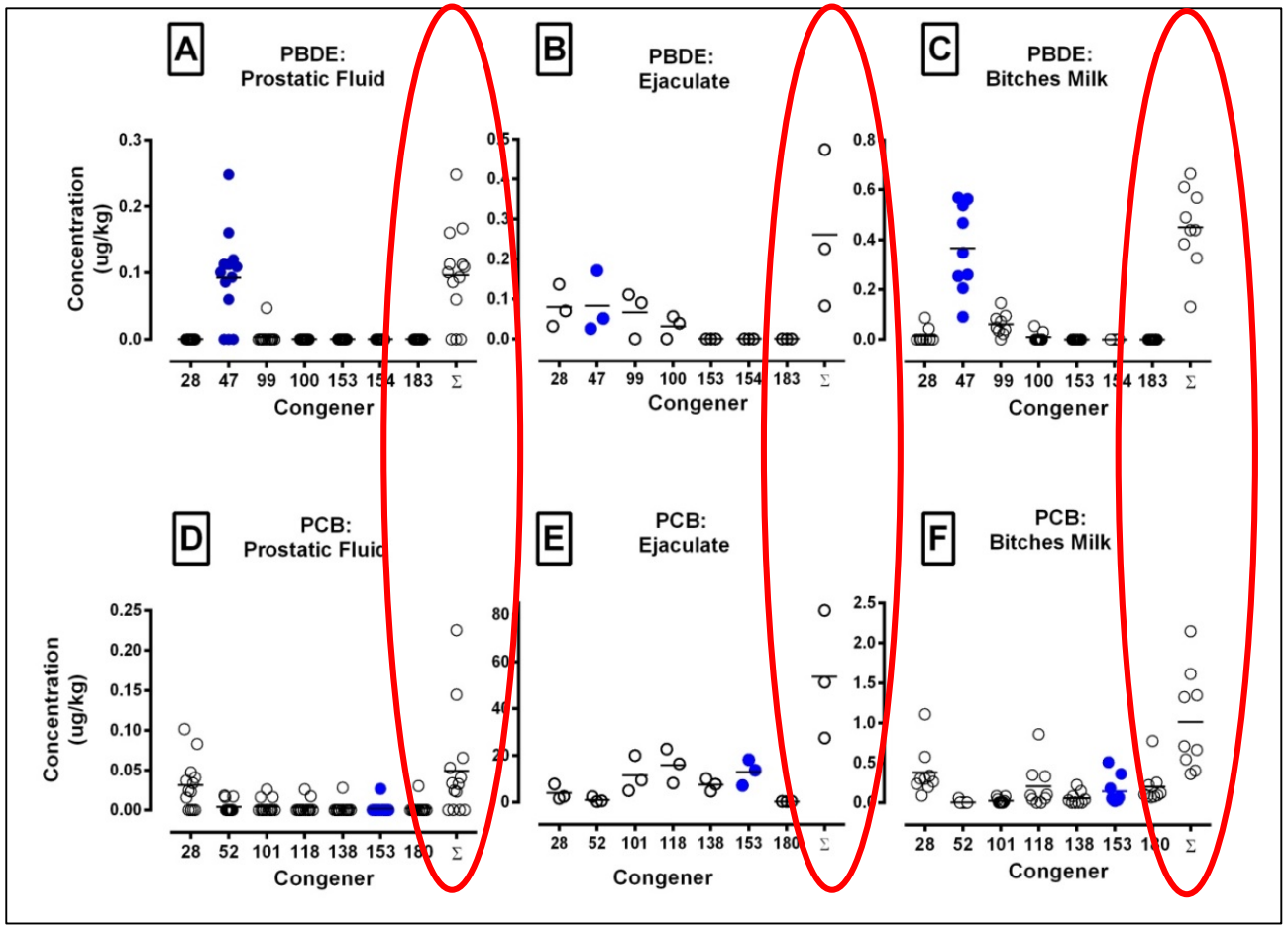
Accepted: 15 July 2016
Published: 09 August 2016

Richard G. Lea[1,2], Andrew S. Byers[1], Rebecca N. Sumner[1], Stewart M. Rhind[1,*], Zulin Zhang[1], Sarah L. Freeman[1], Rachel Moxon[1], Holly M. Richardson[1], Martin Green[1], Jim Craigon[2] & Gary C. W. England[1]

Chemical toxins in dog ejaculate and milk

PBDE congeners
Flame retardants

PCB congeners
Industrial coolants,
Plasticisers…



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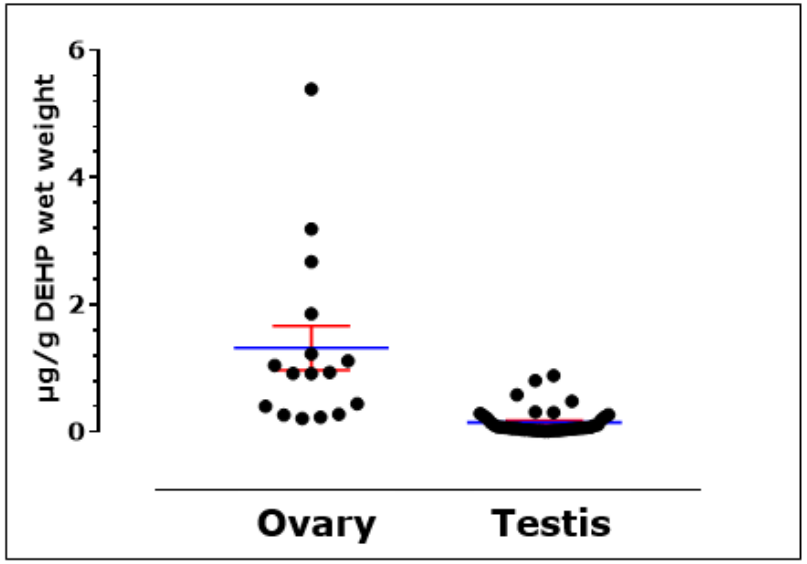
Chemical toxins in dog gonads

	Ovary	Testis	Comparison
ΣPCB congeners *Industrial coolants*	**2.53±0.1µg/kg**	0.334±0.009µg/kg	**Ovary 7.5x > than testis**
ΣPBDE congeners *Flame retardants*	0.431±0.04µg/kg	0.440±0.008µg/kg	Ovary = testis
DEHP *Plasticisers*	**1.318±0.08µg/g**	0.149±0.005µg/g	**Ovary 8.85x >than testis.**

Van der Mescht et al., in preparation



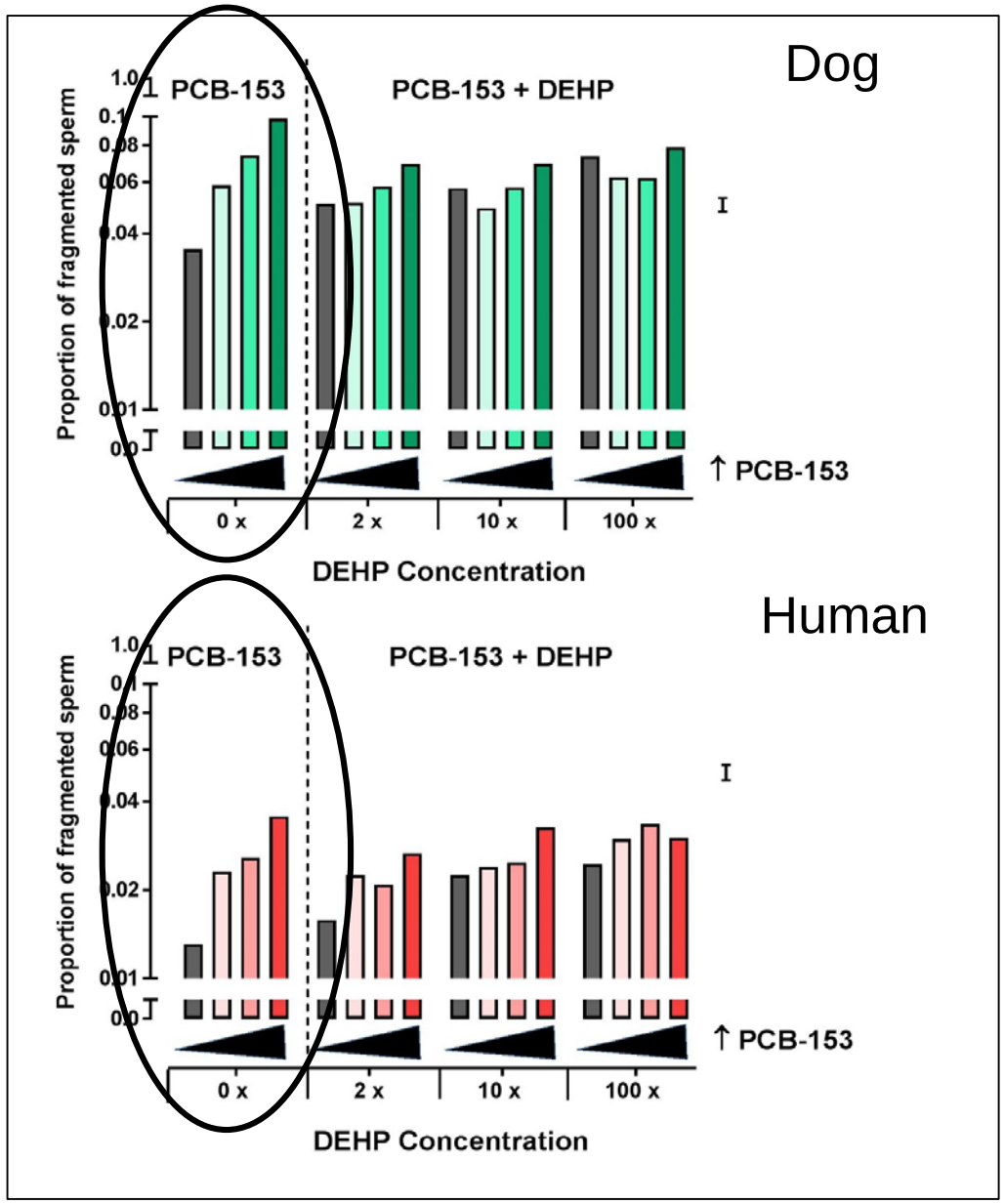
Chemical effects on dog and human sperm: causality?

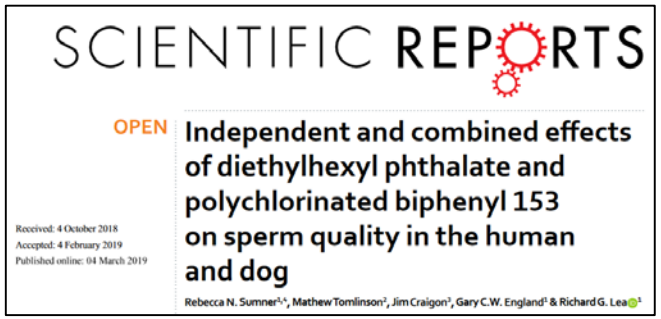
Chemicals:
Industrial: polychlorinated biphenyl congener
Plasticiser: phthalate

Parameter: DNA fragmentation

		DEHP			
		0 x	2 x	10 x	100 x
PCB-153	0 x	•	•	•	•
	2 x	•	•	•	•
	10 x	•	•	•	•
	100 x	•	•	•	•

Note: Chemical concentrations relevant to detected levels in dog testis:
2x, 10x, 100x testis concentration





Testicular cancer in humans and dogs: causality?

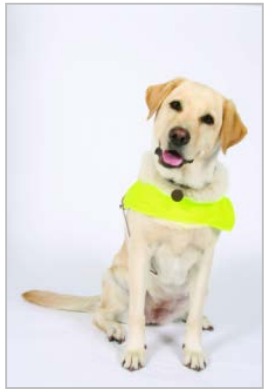
Human:

- Temporal changes & regional differences established
- Evidence base suggests environmental
- **But** exposure and effect not demonstrated

Dog:

- Few studies since many dogs neutered
- Increased from 16% (1962) to 27% (2007)
  - *Greco et al., J Comp Pathol.,2008*
- Precursors of cancer cells (GCNIS) cells detected in testis
  - *Greco et al., Theriogenology, 2008*

Chemical profiles and pathologies in dog testes: influence of geographical location.



Testes
- Routine neutering
- Standardised processes

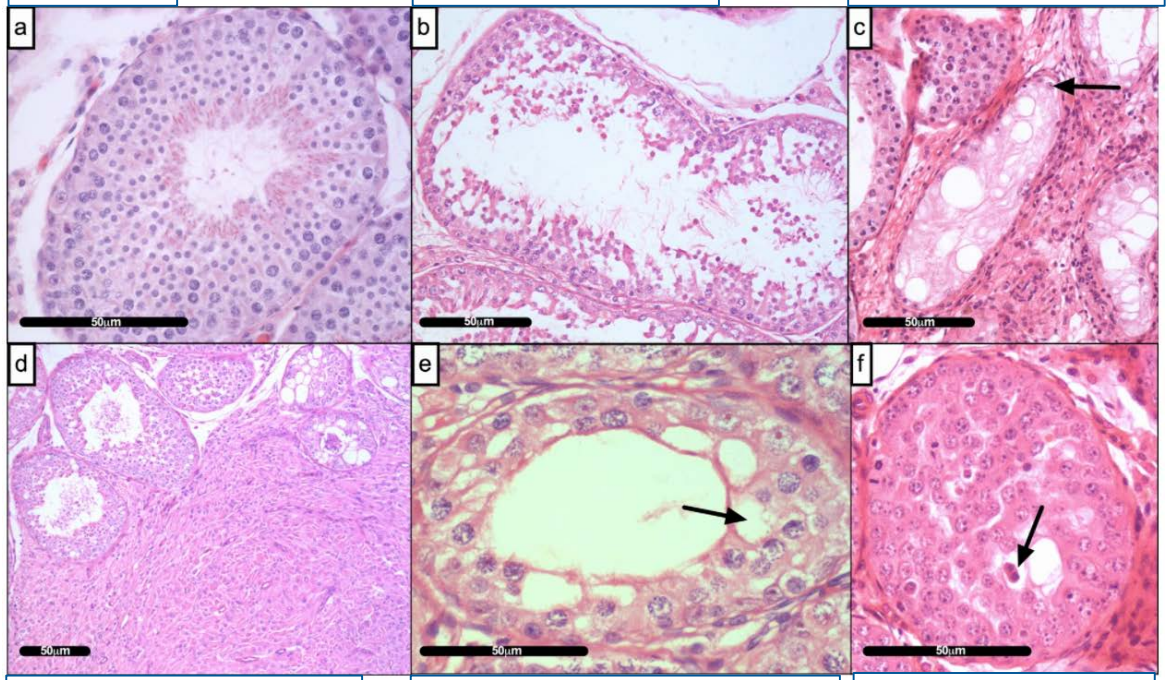
UK: 3 regions
- West Midlands
- South East
- East Midlands

Finland
- Vantaa

Denmark
- Copenhagen

Regional differences?
- Pathology
- Cell markers
- Chemical profiles

Dog testis pathology evaluation



Testicular histopathology parameters

- Sub-gross appearance
- Tubule atrophy and/or degeneration
- Cell changes: Sertoli and germ
- Interstitial area

Scored on a 5 point scale
0 = 0%
1 = 1-25%
2 = 26-50%
3 = 51-75%
4 = 76-100%

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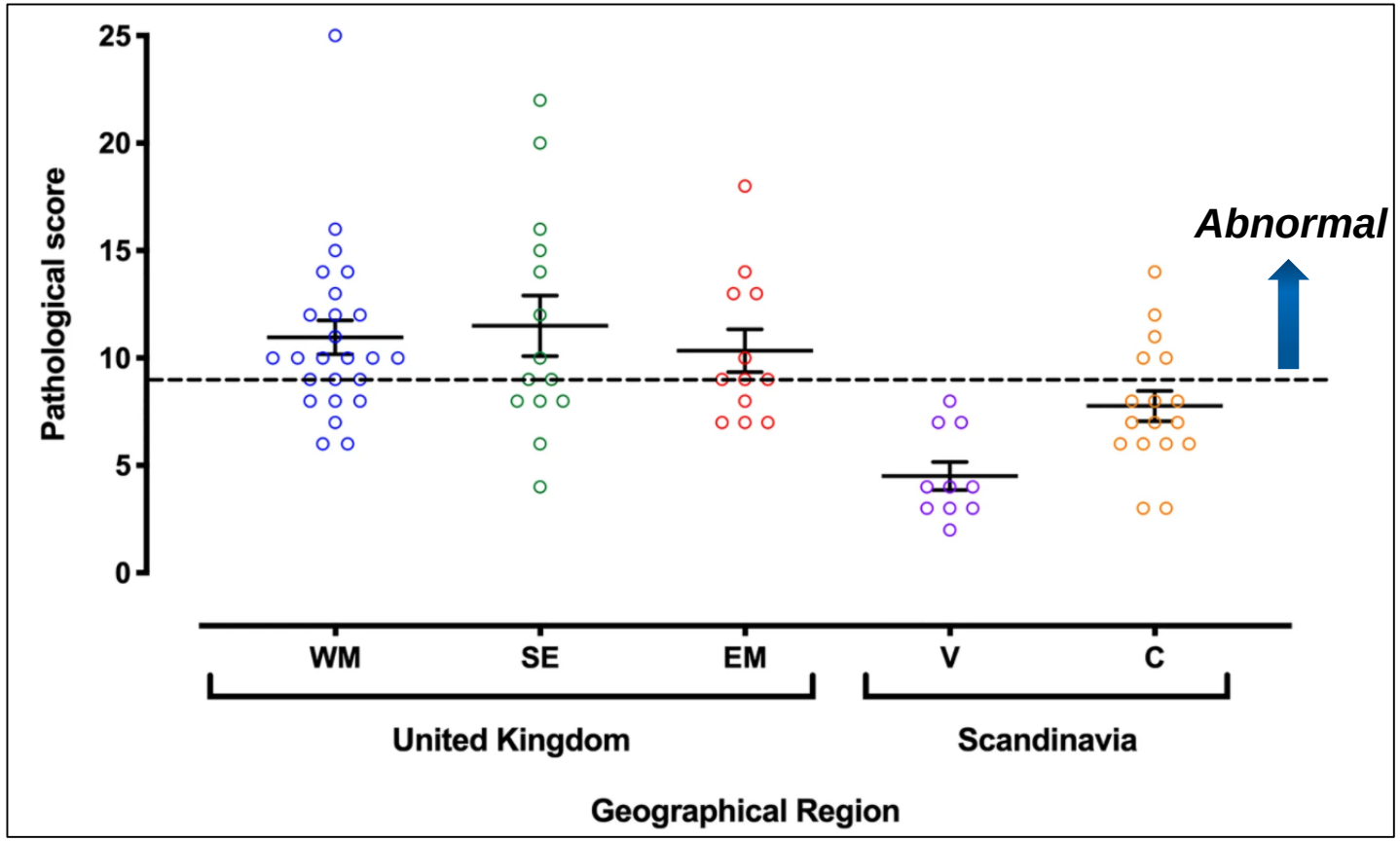
Regional differences in histopathology score



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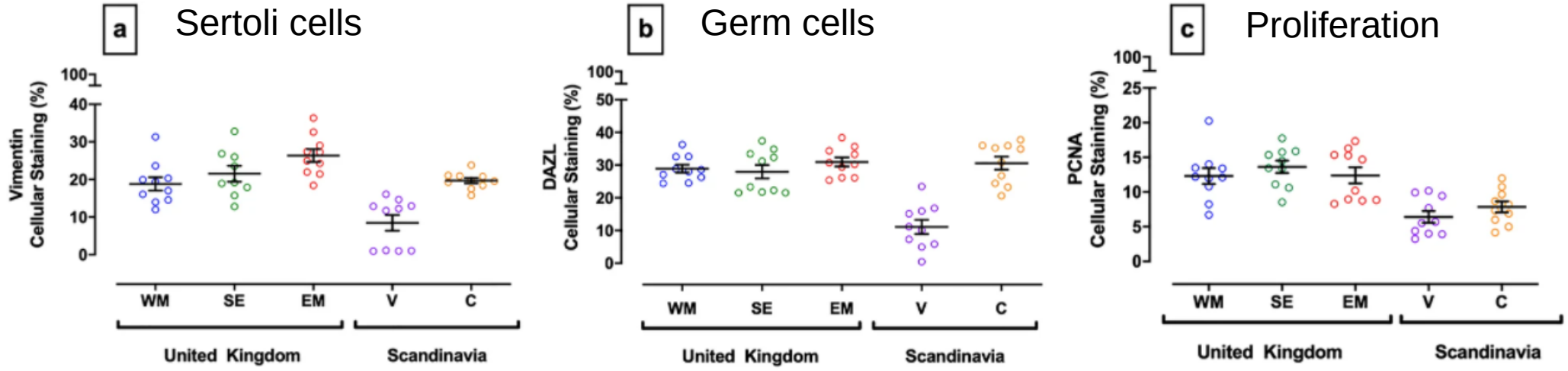
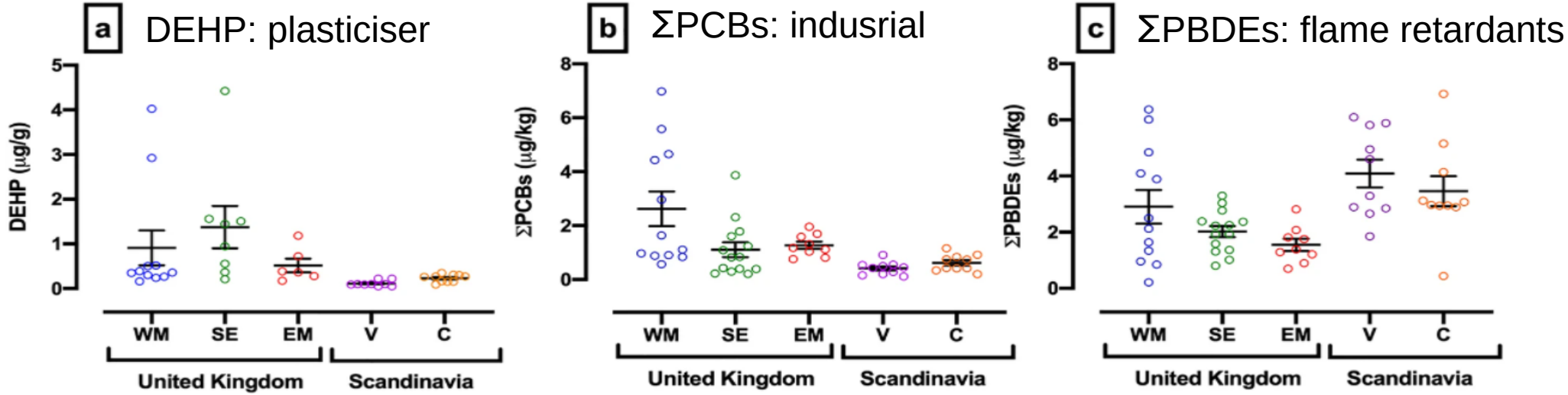
Testis cell types and chemical pollutants

Testis cells



Testis chemicals



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Concluding statements

Causality between exposure and effect (TDS: dog vs human)

- Temporal changes
- Geographical variation
- Chemical *in vitro* effects on dog and human sperm similar

Potential data and sample sources

- Reproductive history: dog breeding centres, **GRLS?**
- **Diet linkages**
- Testicular pathology & reproductive health
- Monitoring chemical exposures, milk, semen, gonads, **silicone tags?**
- Laboratory: *in vitro* culture of Sertoli & interstitial cells

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- *Zulin Zhang*

University of Copenhagen

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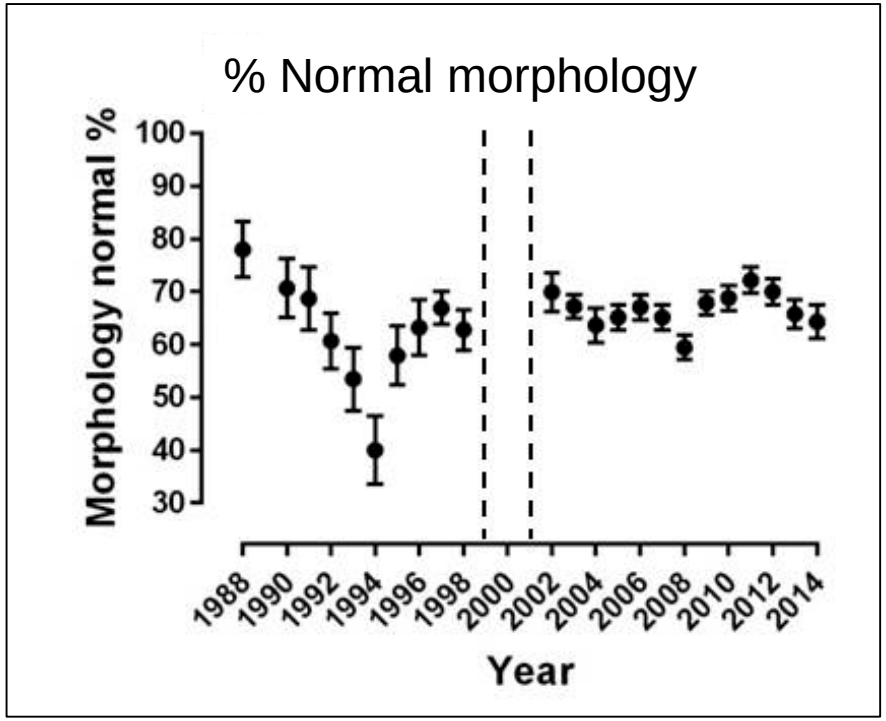
University of Helsinki

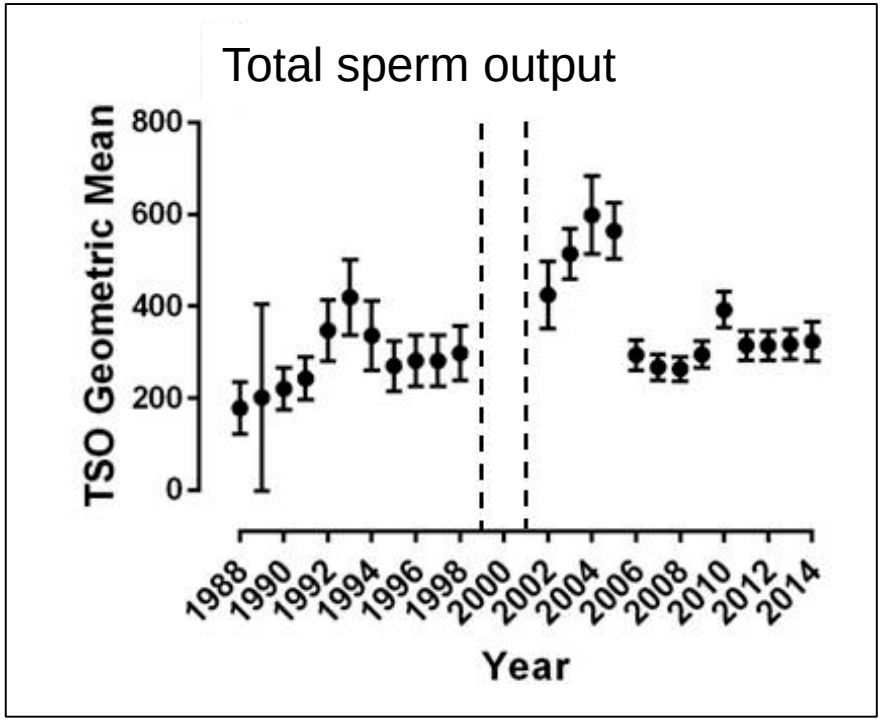
- *Lena Lindh*

Support dog breeding centre

- *Rachel Moxon*
- *Natasha White*

Further temporal trends in canine sperm: 1988-2014

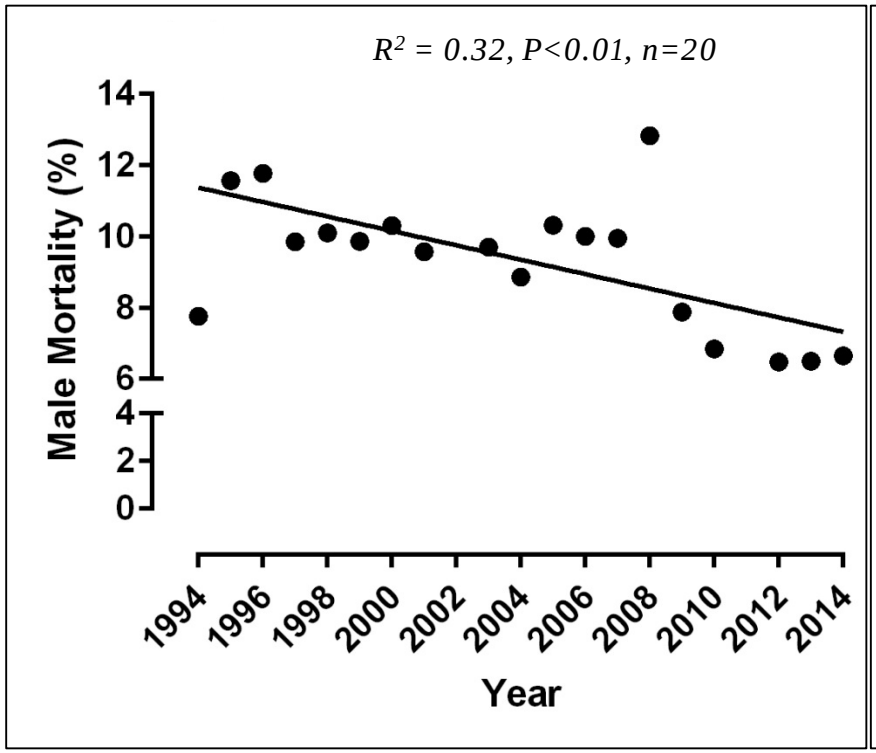




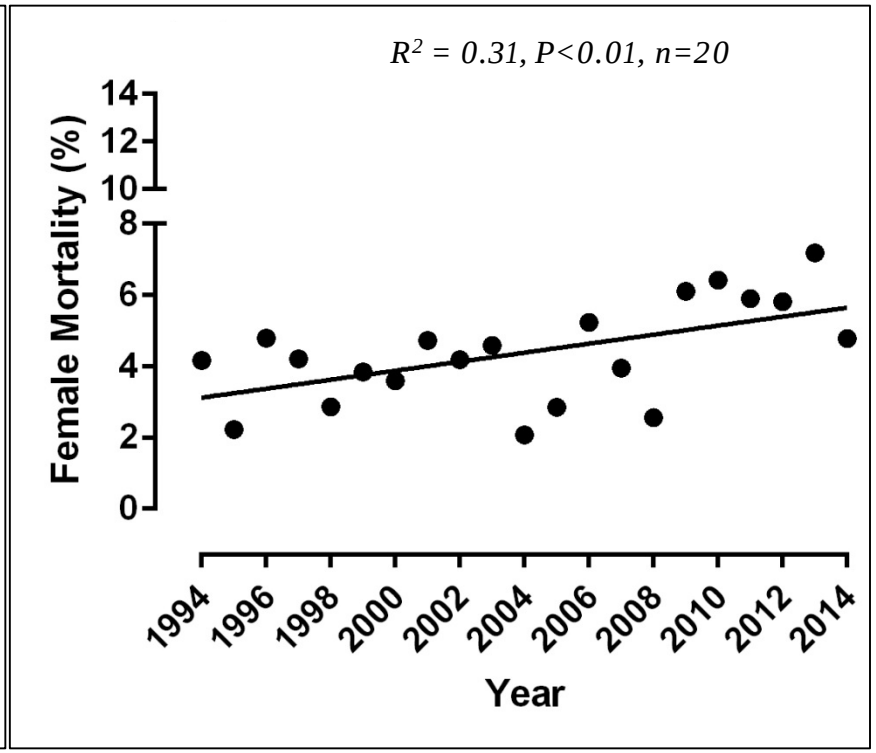
Selection bias for fertility?
Sertoli cell numbers?

Stillbirths & Pup mortality

Male mortality



Female mortality



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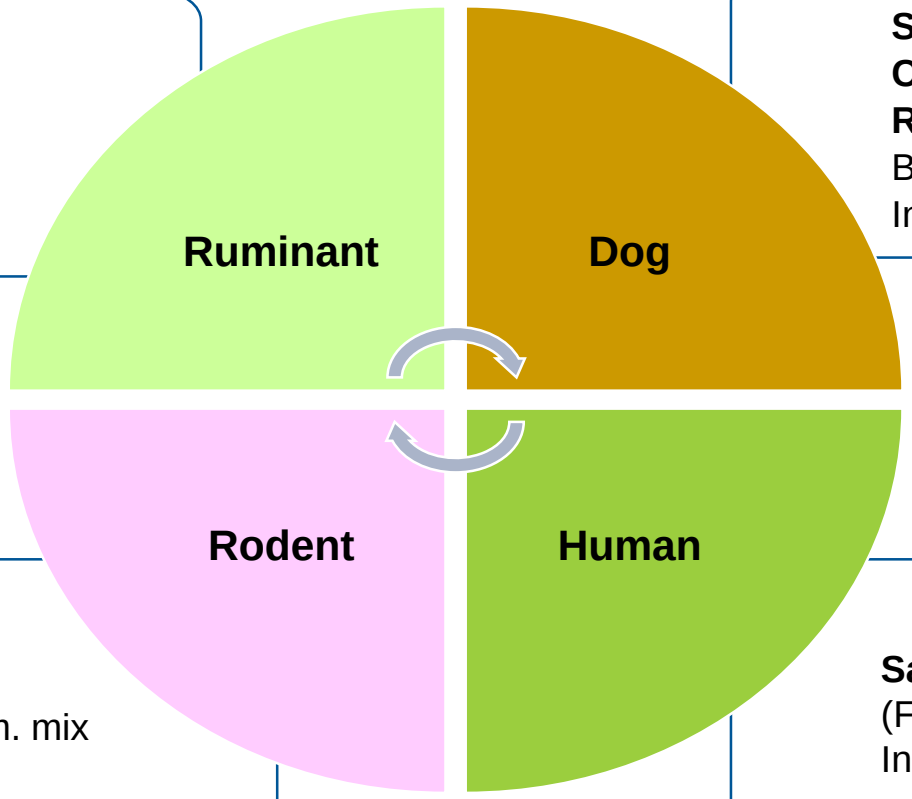
Animal models & sentinels: the global picture!

Real life model
Herbivore
Develop. stages
In vitro models
Transgenerational

Sentinel
Carnivore
Real life exposure
Biomonitoring
In vitro models



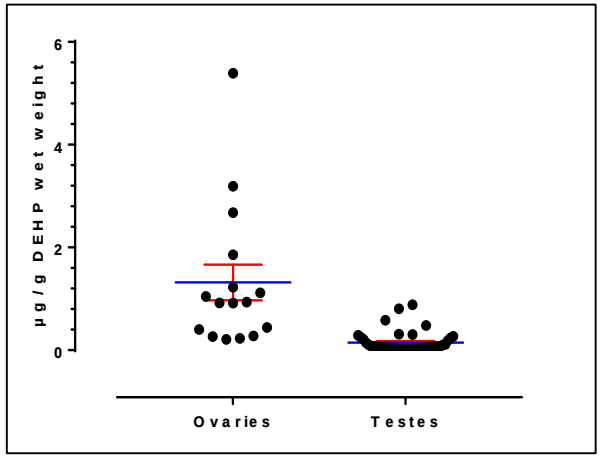
TDS models
Single to limited chem. mix
Transgenerational

Sampling - Biomonitoring
(Fetal, adult)
In vitro models

Chemical effects on neonatal mouse ovaries in vitro

Phthalates: DEHP vs MEHP
Polybrominated diphenyl ethers: PBDEs

Exposure based on dog Mean Gonad Concn. MGC



PND4 gonads

Ovaries
Bursa removed

Testes

Culture: 1 day
medium

Culture: 5 days
Chemical
Relative to MGC
e.g. 0.6, 6, 60

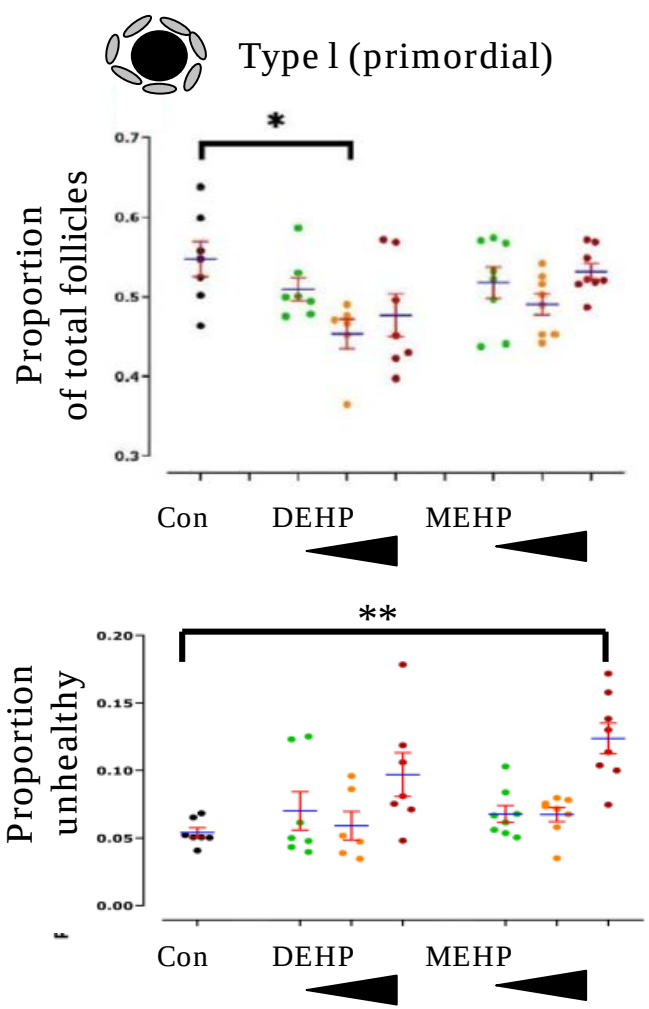
Bouins fixation

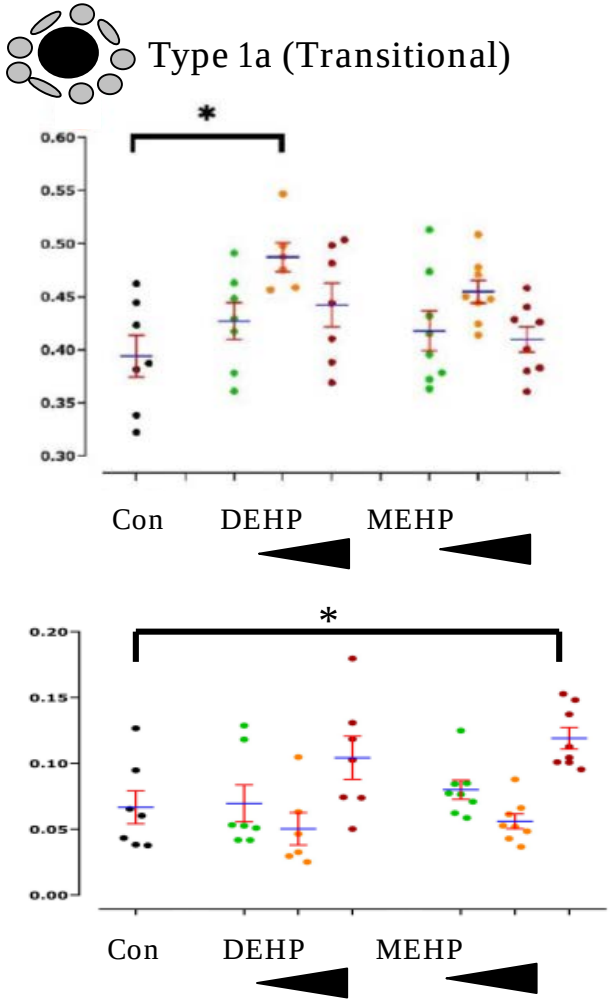
RNA later

Histology
- Follicle counts adjusted (Abercrombie)
- Follicle health
- Functional & developmental markers

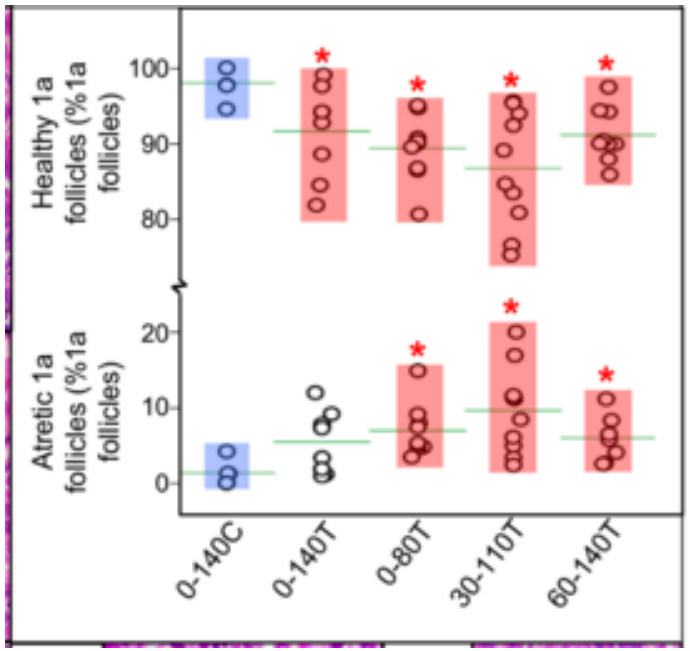
Molecular
- Nuclei acid extraction
- Microarray, qPCR
- (NGS)

Phthalate exposure adversely affects early stage follicles



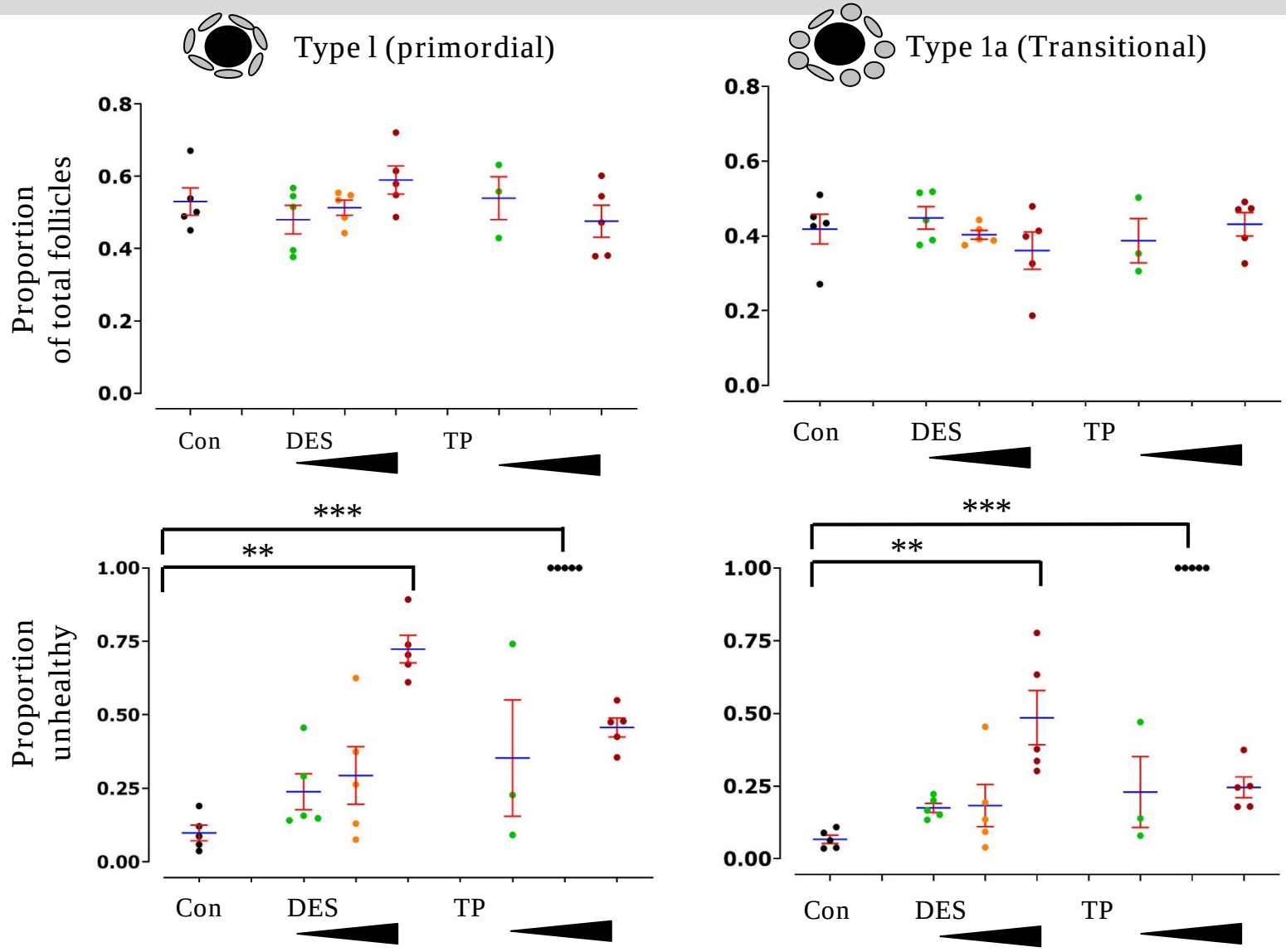


Sheep biosolids model



Van der Mescht et al., in preparation

Oestrogenic vs androgenic



Van der Mescht et al., in preparation