Environmental/lifestyle effects on (male) reproductive development and its long-term and inter-generational consequences

Richard M Sharpe
E-mail: r.sharpe@ed.ac.uk
Those who did the work

Pablo Hurtado-Gonzales
Rod Mitchell
Sander van den Driesche
Richard Anderson
Afshan Dean
Karen Kilcoyne
Yili Wang
Sheila Macpherson
Chris McKinnell
Tom Chambers
Prevalence data for reproductive disorders in newborn or young adult males

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prevalence</th>
<th>Evidence</th>
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</thead>
<tbody>
<tr>
<td>Cryptorchidism (Testis non-descent)</td>
<td>6-9%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Hypospadias (A penis abnormality)</td>
<td>0.4-0.9%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Low sperm counts</td>
<td>16-20%</td>
<td>Prospective EU studies</td>
</tr>
<tr>
<td>Testis germ cell cancer*</td>
<td>0.45%</td>
<td>Registry data (reliable)</td>
</tr>
<tr>
<td>Primary hypogonadism</td>
<td>~10%</td>
<td>Cross-sectional studies</td>
</tr>
</tbody>
</table>

* accepted to originate from fetal germ cells that failed to differentiate
The commonest reproductive disorders of the developing and young adult male

‘Testicular dysgenesis syndrome’

- Testis germ cell cancer
- Low sperm counts
- Primary hypogonadism

Subnormal Testosterone production or action

Cryptorchidism
Hypospadias

Fetal Period  Birth  Prepubertal Period  Puberty  Adulthood
Male-female difference in anogenital distance (AGD) in humans in the first two years from birth

Population-based data
Means ± 95% CI
N=137-285 per age

From: Thankamony et al 2009 Environ Health Perspect 117:1786-1790
Anogenital distance (AGD) in newborn rats
~Twice as long in males as in females

Provides a life-long read-out of androgen exposure just within the ‘Masculinisation programming window (MPW)’
The ‘masculinisation programming window’ (MPW)

The ‘masculinisation programming window’ (MPW)

- Testosterone production

- DBP Treatment windows
  - Full window (FW): T-↓50-80%
  - Late window (LW): T-↓80%

- MPW: T-↓50%

- Testis differentiation
- Reproductive tract differentiation

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
Effect of DBP-induced reduction in fetal testis testosterone in different time windows: Adult phenotype

- Anogenital distance (AGD)
- Penis length
- Average testis weight (g)
- Adult testis weight (g)

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
Effect of fetal DBP exposure window on phenotype of male rats in adulthood

Incidence of cryptorchidism

Number of animals

Control | FW | MPW | LW

DBP treatment group

Normal | Cryptorchid

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
Effect of fetal DBP exposure window on phenotype of male rats in adulthood

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
Relationship between AGD and genital phenotype (cryptorchidism) in adult male rats

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
AGD in normal boys and boys with cryptorchidism at <2 years of age

From: Thankamony et al (2014) Environ Health Perspect

P<0.0001 for mean AGD SDS in boys with cryptorchidism (○) compared with normal population
Relationship between AGD and genital phenotype (hypospadias) in adult male rats

Van den Driesche, K Kilcoyne et al J Clin Invest Insight
AGD in normal boys and in boys with hypospadias at age <2 years

From: Thankamony et al (2014) Environ Health Perspect

P=0.005 for mean AGD SDS in boys with hypospadias (○) compared with normal population

Lines show population centiles for AGD in normal boys

From: Thankamony et al (2014) Environ Health Perspect
The commonest reproductive disorders of the developing and young adult male
‘Testicular dysgenesis syndrome’

- Testicular dysgenesis syndrome
- Cryptorchidism
- Hypospadias
- Testis germ cell cancer
- Low sperm counts
- Primary hypogonadism

Maternal Diet, Lifestyle, exposures
Subnormal Testosterone production or action

Cryptorchidism Hypospadias

Fetal Period Birth Prepubertal Period Puberty Adulthood
Effect of maternal acetaminophen/painkiller use in pregnancy and cryptorchidism in sons

<table>
<thead>
<tr>
<th>Study authors (year)</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berkowitz &amp; Lapinski (1996)</td>
<td>1.93 (1.03-3.62)</td>
</tr>
<tr>
<td>Jensen et al (2010)</td>
<td>1.38 (1.05-1.83)</td>
</tr>
<tr>
<td>Kristensen et al (2010)</td>
<td>2.78 (1.13-6.84)</td>
</tr>
<tr>
<td>Snijder et al (2012)</td>
<td>2.12 (1.17-3.83)</td>
</tr>
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However, such association studies can never prove cause and effect. And the possibility of confounding is high.

Direct evaluation of fetal human testis effects would be a far more desirable approach.
Human Fetal Testis Xenograft Model
Developed by Rod Mitchell

Elective terminations → Fetus → Testes → Grafts

Host Mice → Castrate Host (no testosterone)

Treatments of the host mouse

- hCG (pregnancy hormone)
- Control
- Acetaminophen

Measure Testosterone

Seminal Vesicle weight

Blood Testosterone

Rod Mitchell
Effect of xenograft (host) exposure to acetaminophen on fetal human testis T production

Effect of xenograft (host) exposure to acetaminophen on fetal human testis T production

Paracetamol therapeutic dose – 7-days of treatment

Effect of xenograft (host) exposure to acetaminophen on fetal human testis T production

Paracetamol therapeutic dose – 7-days of treatment

Seminal vesicle weight = biomarker of androgen action

Maternal analgesic use also has potential to impact the fetal germ cell epigenome.

Analgesic exposure of rats in pregnancy results in:

- Reduced GC number in male and female offspring resulting in reduced female adult fertility
- Impaired ovarian function in grand-daughters
- The grand-daughter effects were transmitted via paternal as well as maternal lines

Translatable to humans, they raise concerns that analgesic use in pregnancy could potentially affect fertility of resulting daughters and grand-daughters.
Studying intergenerational ‘effects’
Effect of in utero exposure of rats to analgesics
F1 male offspring phenotype

Effect of in utero exposure of rats to analgesics
F1 female offspring phenotype

Mating of F1 analgesic-exposed offspring

Indomethacin- or Paracetamol-exposed as a fetus

Grow to adults

F1 Male \( \times \) Control female → F2 males

F1 Female \( \times \) Control male → F2 females

\( \text{F1 Male} \times \text{Control female} \)→ F2 males and F2 females

\( \text{F1 Female} \times \text{Control male} \)→ F2 males and F2 females
Effect of in utero exposure of rats to analgesics.
F2 female offspring phenotype.

Effect of in utero exposure of rats to analgesics on F2 female offspring phenotype (Pnd25)

Mating of F1 analgesic-exposed offspring

Indomethacin- or Paracetamol-exposed as a fetus

Grow to adults

F1 Male X Control female

F2 males

F2 females

F1 Female X Control male

F2 males

F2 females
Time-course for changes to the fetal germ cell ‘epigenome’ in the mouse

?? ‘Window of opportunity’ for dietary/environmental signals to modify the epigenome?

H3K27me3 = repressive histone methylation mark
Altered fetal environment causes epigenetic changes to male germ cells which lead to inter-generational effects.

Maternal under-nutrition during fetal male germ cell remethylation alters sperm DNA methylation in adulthood (F1)

This is the period in male fetuses when genome remethylation occurs

Effect of (grand)maternal diet in pregnancy on ovarian reserves of grand-daughters (F2)

3 days after birth of offspring, F1 females were maintained on same diet as mothers or cross-fostered to mums on control diet (= recuperated), then at weaning all females fed on control diet until mated at 12 weeks of age to generate F2 offspring.

Developmental (in utero+lactational) exposure to an obesogenic diet reduces ovarian reserves in F1 females

Control diet = 7% simple sugars 3% fat

Obesogenic diet = 10% simple sugars 20% animal fat +sweetened condensed milk (55% sugars+8% fat)

Altered histone methylation in male fetal germ cells has inter-generational consequences

**RESEARCH ARTICLE SUMMARY**

**EPIGENETICS**

Disruption of histone methylation in developing sperm impairs offspring health transgenerationally

Keith Siklenka, Serap Erkek, Maren Godmann, Romain Lambrot, Serge McGraw, Christine Lafleur, Tamara Cohen, Jianguo Xia, Matthew Suderman, Michael Hallett, Jacquetta Trasler, Antoine H. F. M. Peters,* Sarah Kimmins*

Altered histone methylation in male fetal germ cells has inter-generational consequences

**Paternal epigenetic transmission**

- F₀ germ line (sperm)  
  - Altered histone methylation and RNA content in sperm

**Intergenerational effects**
- Reduced survivability
- Increased abnormal development

**Transgenerational effects**
- Increased abnormal development

Disruption of histone methylation in developing sperm by exposure to the KDM1A transgene in one generation severely impaired development and survivability of offspring. These defects were transgenerational and occurred in non-TG descendants in the absence of KDM1A germline expression. Developmental defects in offspring and embryos were associated with altered RNA expression in sperm and embryos.

Conclusions

- Fetal development is a critical period for shaping future reproductive health of that individual.

- Maternal diet, lifestyle and exposures during (?early) pregnancy has clear potential to impact developmental processes within the fetal gonads of both sexes.

- Such effects on the fetal germ cells has potential to alter their ‘epigenetic status’ which may lead to effects (adverse or beneficial?) in the next generation (grand-children).