The effects of veterinary growth stimulants from cattle feedlots in South Africa on reproductive and thyroid parameters in a rat model

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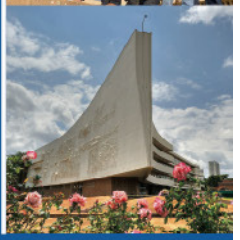
Outline



Introduction

- EDCs
- Complex mixtures

South Africa

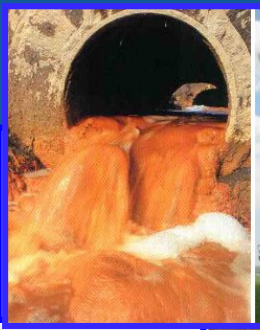
- Veterinary Growth Stimulants

} Water quality
} Semen Quality

Conclusions

Acknowledgements

Think about...










Mechanisms of Toxicity

- Metabolic & Cellular Poison
- Enzyme Induction
- Receptors
- Oxidative Stress & Free Radicals
- Macromolecular Binding & Adduct Formation
- Genotoxicity
- Carcinogenesis
- Apoptosis
- Immunotoxins
- **Reproductive effects**
  - **Endocrine Disruptors**
- Teratogenesis

Levy et al., 2006

Definition

An ED is an exogenous substance or mixture that:

- alters function(s) of the endocrine system

Causes adverse effects at the level of:

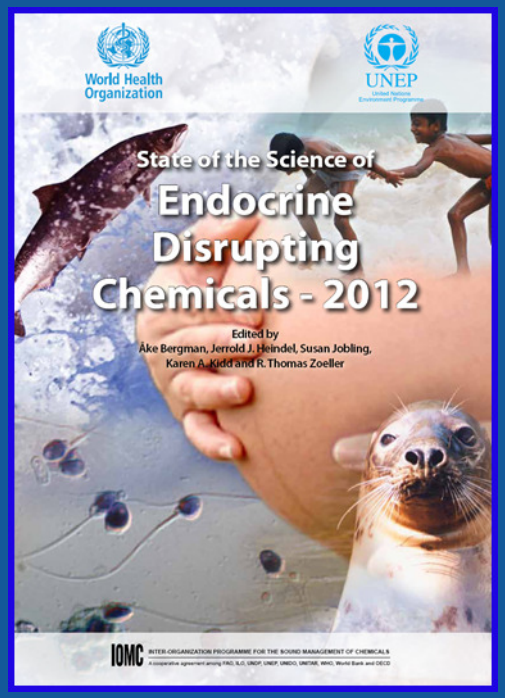
- the organism,
- its progeny,
- populations or subpopulations of organisms

Based on scientific principles, data, weight-of-evidence, and the precautionary principle.

EDSTAC, http://ehp.niehs.nih.gov/who/

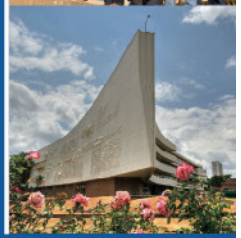
EDCs

- EDCs are not restricted to therapeutic agents
- Appear in several groups of compounds
- Used daily in:
  - Industry
  - Agriculture
  - Workplace
  - Home



Colborn & Clement, 1992;
Toppari et al., 1996

EDC Characteristics



- EDCs are ubiquitous
- Highly persistent & stable
- Resistant to biodegradation
- Lipophylic & bio-accumulate in fat tissue
- Often have an additive or synergistic effect
- Accumulate up the food chain

"The dose makes the poison."
Paracelsus, physician
(1493–1541)

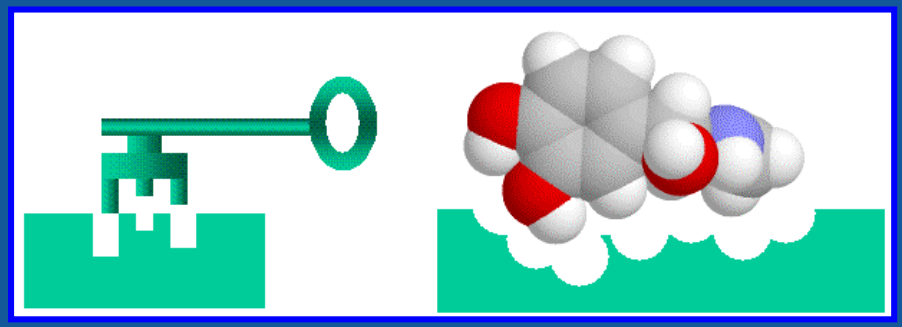
- Traditional Risk Assessment:

estrogenic chemicals - significant underestimations of risk

Genthe et al., 2010

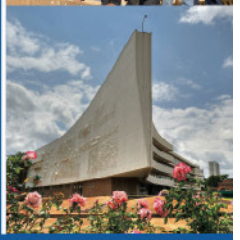
EDCs

- Growing concern about changes in human & wildlife health & fecundity
- Associated with disruption of hormonal systems by environmental chemicals or contaminants
  - (anti-)estrogen
  - (anti-)androgen
  - thyroid hormone

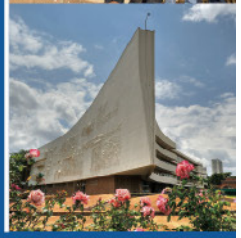


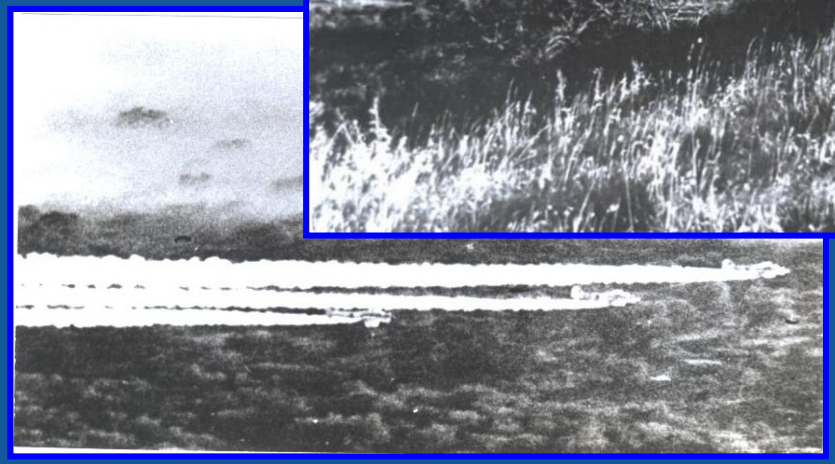
Groups of EDCs

- Organochlorine pesticides (DDT, DDD, DDE; Lindane; etc)
- Polychlorinated biphenyls (PCBs)
- Alkylphenols (p-NP; OP)
- Phthalates
- Bisphenol-A
- Dioxins and furans
- Phytoestrogens
- Cigarette Smoke
- Other



DDT in South Africa

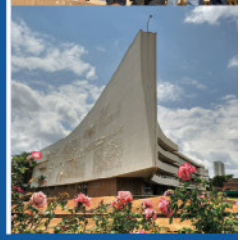




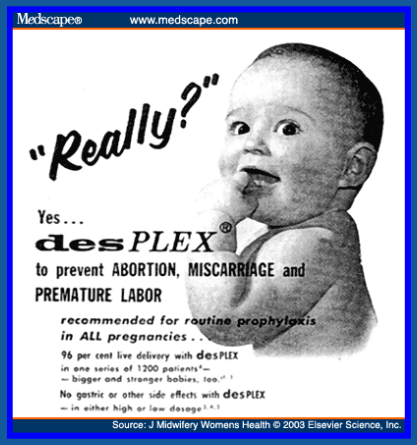
1950-1952

2014

Diethylstilbestrol (DES)

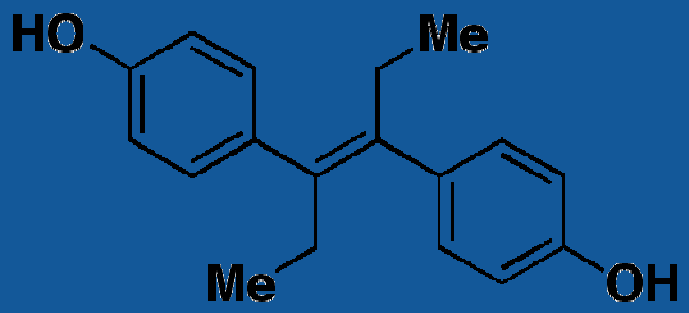


- Late 1940s to 1970s prescribed to pregnant women
  - belief prevent miscarriage
  - worldwide estimates ~ 2 - 8 million exposed
- Still used as growth stimulant – meat industry??



DES Exposed Boys

- Poor sperm quality
- Increased incidence of cryptorchidism
- Sub-fertility/infertility in men and women – 90%
- Increased hypospadias - DES grandsons (> 30 years later)

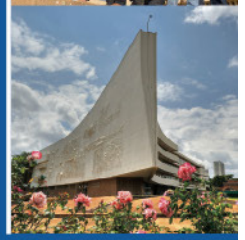


Klip et al., 2002; Newbold, 2004;

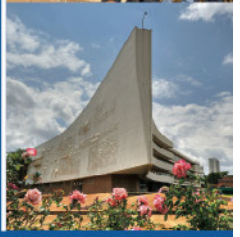
Brouwers et al., 2006

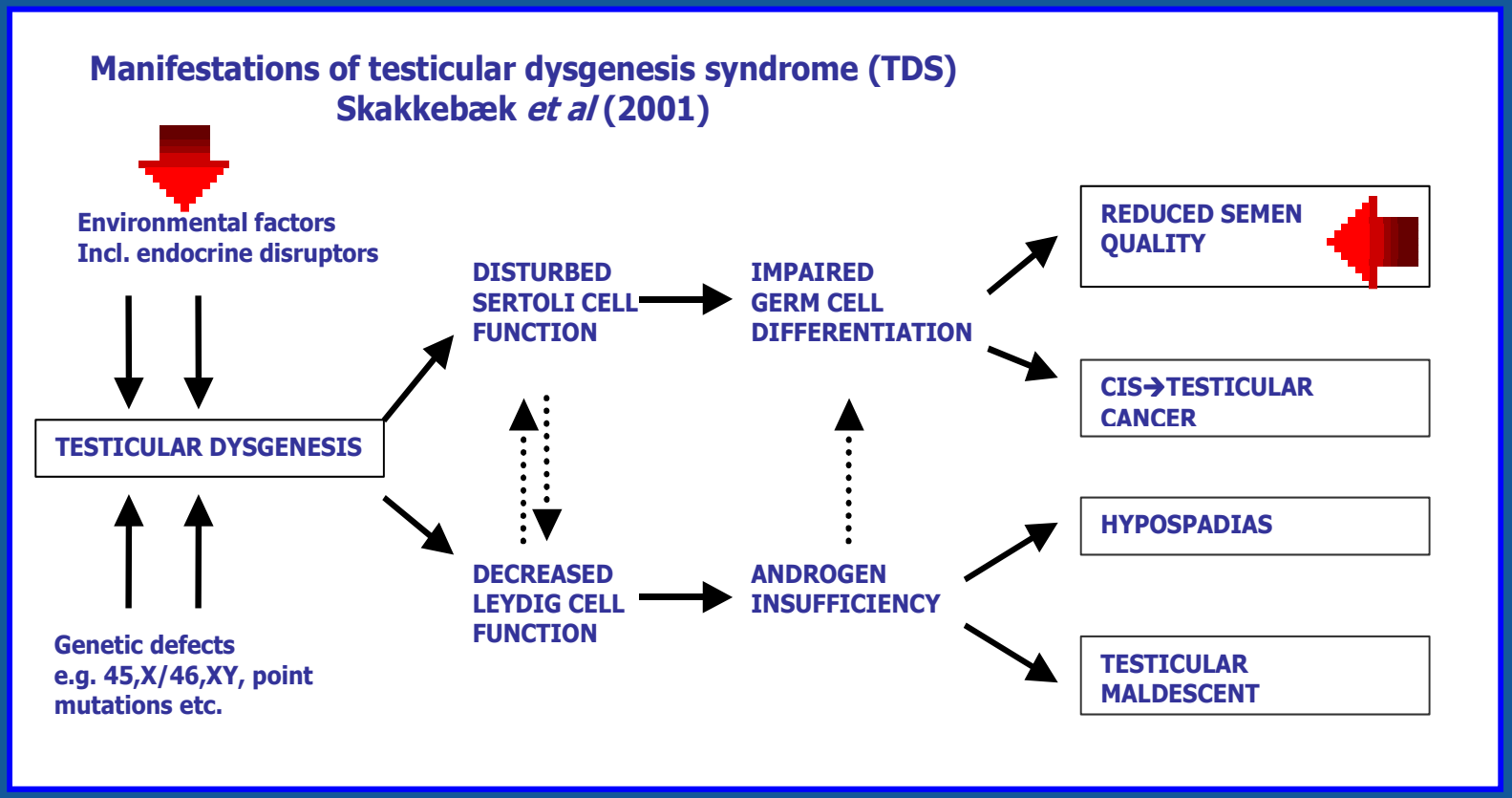
Routes of Exposure

- Diet
  - Meat
  - Dairy products
  - Fish products
  - Pharmaceutical products
- Water
  - Drinking
  - Aquatic sports
- Air
  - Industrial pollution
- Skin/Dermal contact
  - Cosmetics/pharmaceutical products



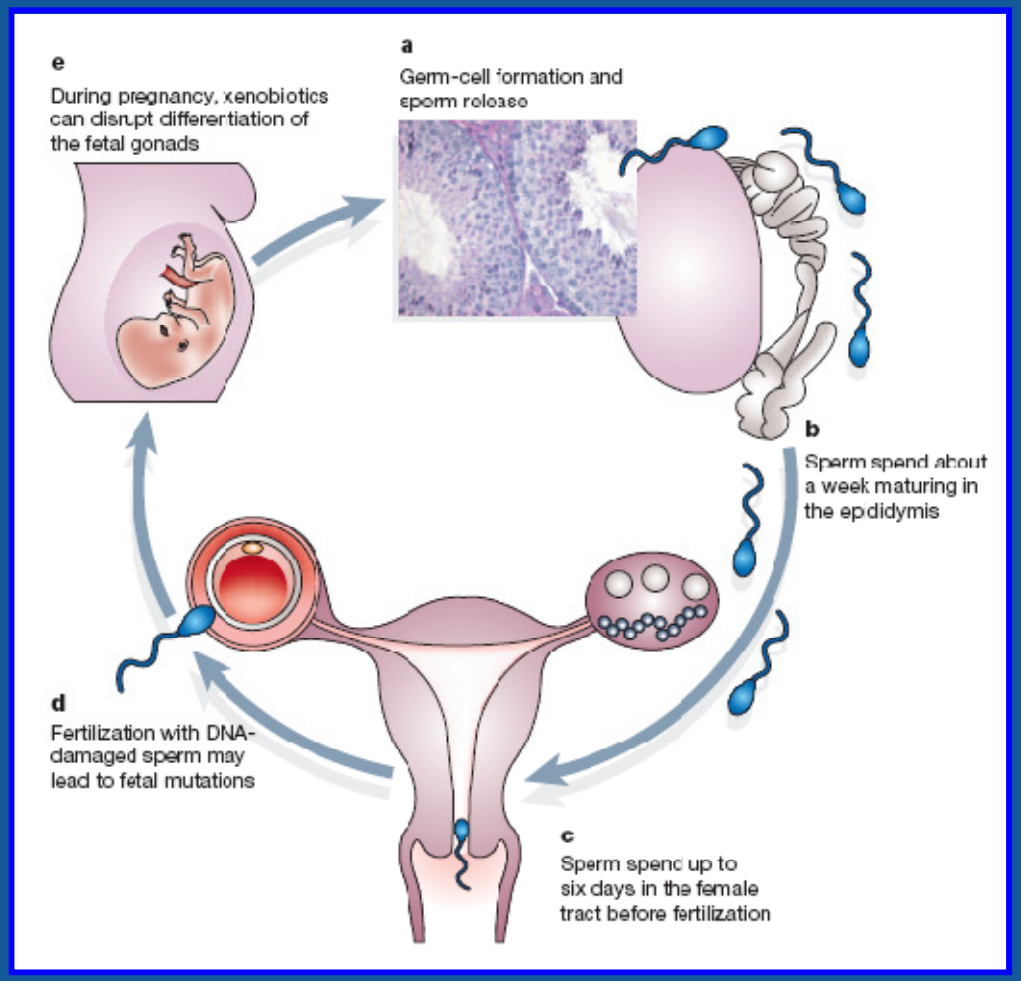
Testicular Dysgenesis Syndrome





Skakkebaek et al., 2001

Vulnerable Stages to EDCs



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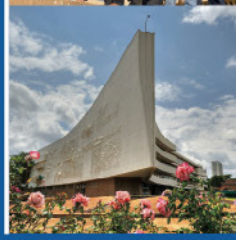
Aitken et al., 2004

Introduction

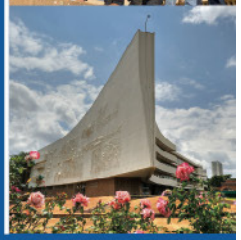


- **Aquatic environment** is the ultimate kitchen sink for man made chemicals

- Most studies have investigated endocrine disrupting chemicals (EDCs) released from **sewage treatment plants** and **industrial effluents**

Jobling & Tyler, 2003; Guillette, 2006; Soyano et al., 2010

Introduction



- Other sources are natural/synthetic hormones released from
  - Animal wastes (fertilise agricultural fields)
  - Feedlot effluents

- In South Africa 75% of all bovine produced, stems from the feedlot production system

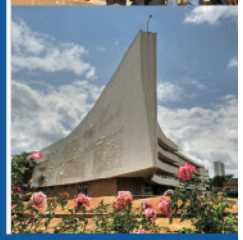
Orlando et al., 2004; Soto et al., 2004; Taljaard, 2009

Veterinary growth stimulants

- Growth stimulants used in the feedlot industry are environmentally stable compounds or metabolites
  - **Testosterone, trenbolone acetate (TBA), methyltestosterone**
  - **17β-estradiol, zeranol, diethylstibestrol, zilpaterol**
  - **Progesterone, melengestrol acetate (MGA)**
- The ultimate fate of many excreted anabolic agents is unknown
- Measurable amounts are released from farm animals and reach the environment
  - For example TBA remains in manure piles for more than 270 days

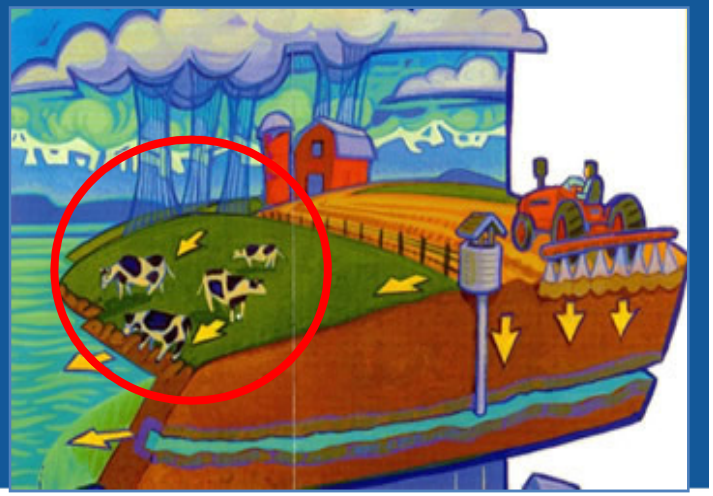
Lange et al., 2001; Soto et al., 2004

Veterinary growth stimulants



- The **excretions** from these animals are not treated and land up in the **local aquatic system**

- In South Africa no research has been done on the estrogenic activity in water associated with the use of growth stimulants

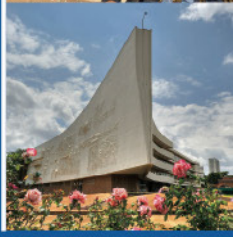


Aims

- To do target **chemical analyses** and to **screen water** sources from cattle feedlots for estrogenic activity using the Recombinant Yeast Screen (YES) and T47D-KBluc bioassays

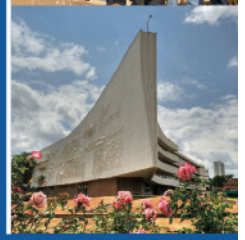
- To determine the **effects** of selected veterinary growth stimulants (GS) found in cattle feedlots **on male reproductive health and thyroid function**, using Sprague Dawley rats

Methods

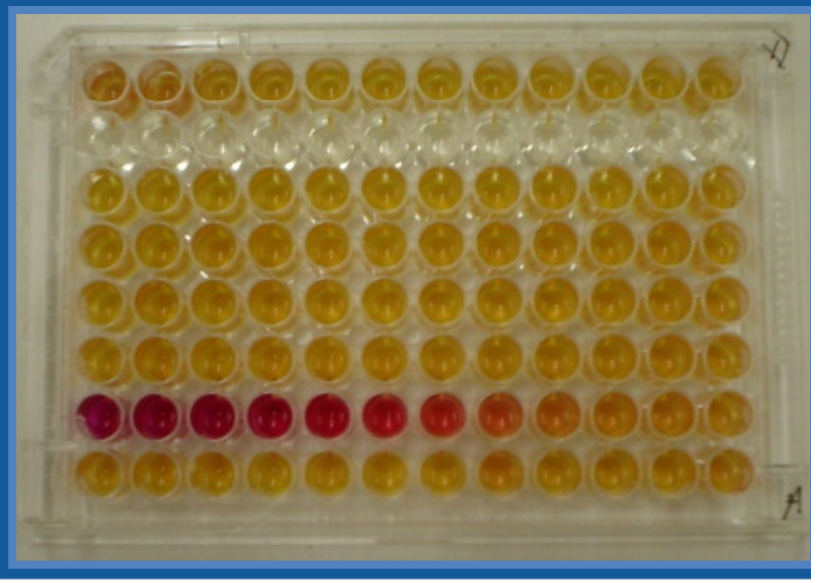


- Various water sources (n=44) were identified in and around 3 feedlots, collected 3 times per year.
- Water samples were collected
  in 1L - glass Schott bottles
  over a period of a year
- Bio-assays were done on
  11 selected samples from
  2 feedlots

Yeast Estrogen Screen: YES



- *Saccharomyces cerevisiae* (yeast)
- Genetically modified to contain the human estrogen receptor (ERα)
- Colour reaction occurs in a dose dependent manner (yellow to red)



- 17ß-estradiol – positive control
- Ethanol (solvent) – negative control

- Detection limit of 2-3nM (0.6-2.7 ng/L) for 17β-estradiol

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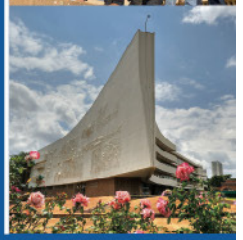
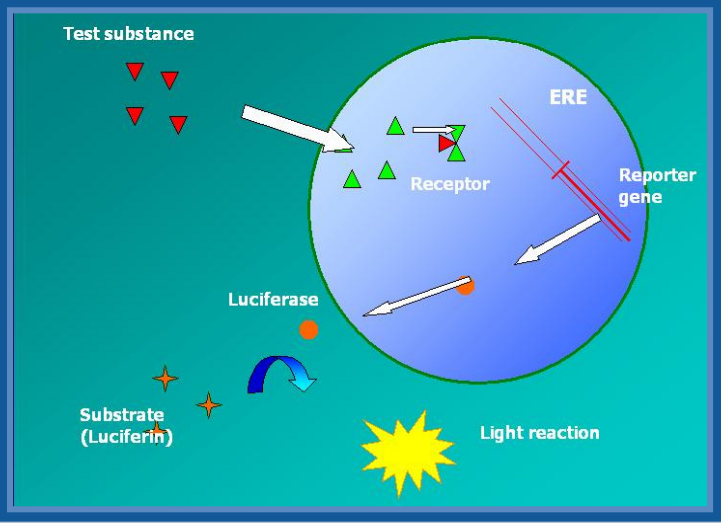
Routledge & Sumpter, 1996; Aneck-Hahn, 2003

Reporter gene assay: T47D-KBluc



- T47D human breast adenocarcinoma cells (luciferase reporter gene construct)
- Contains ERα and ERβ
- Compound enters cell
- Binds to the ER – activates luciferase reporter gene construct ⟶ luciferase
- Luciferin and appropriate co-factors are added (Chemiluminescence)
- The light produced is relative to the degree of estrogenic activity
- Detection limit: 0.06- 1.3ng/L for 17 β-E



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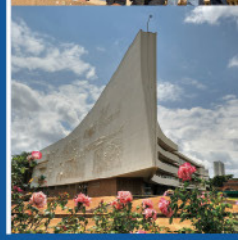
Wilson et al., 2004; WRC Project: K5-1816

Methods: Reproductive Toxicology study

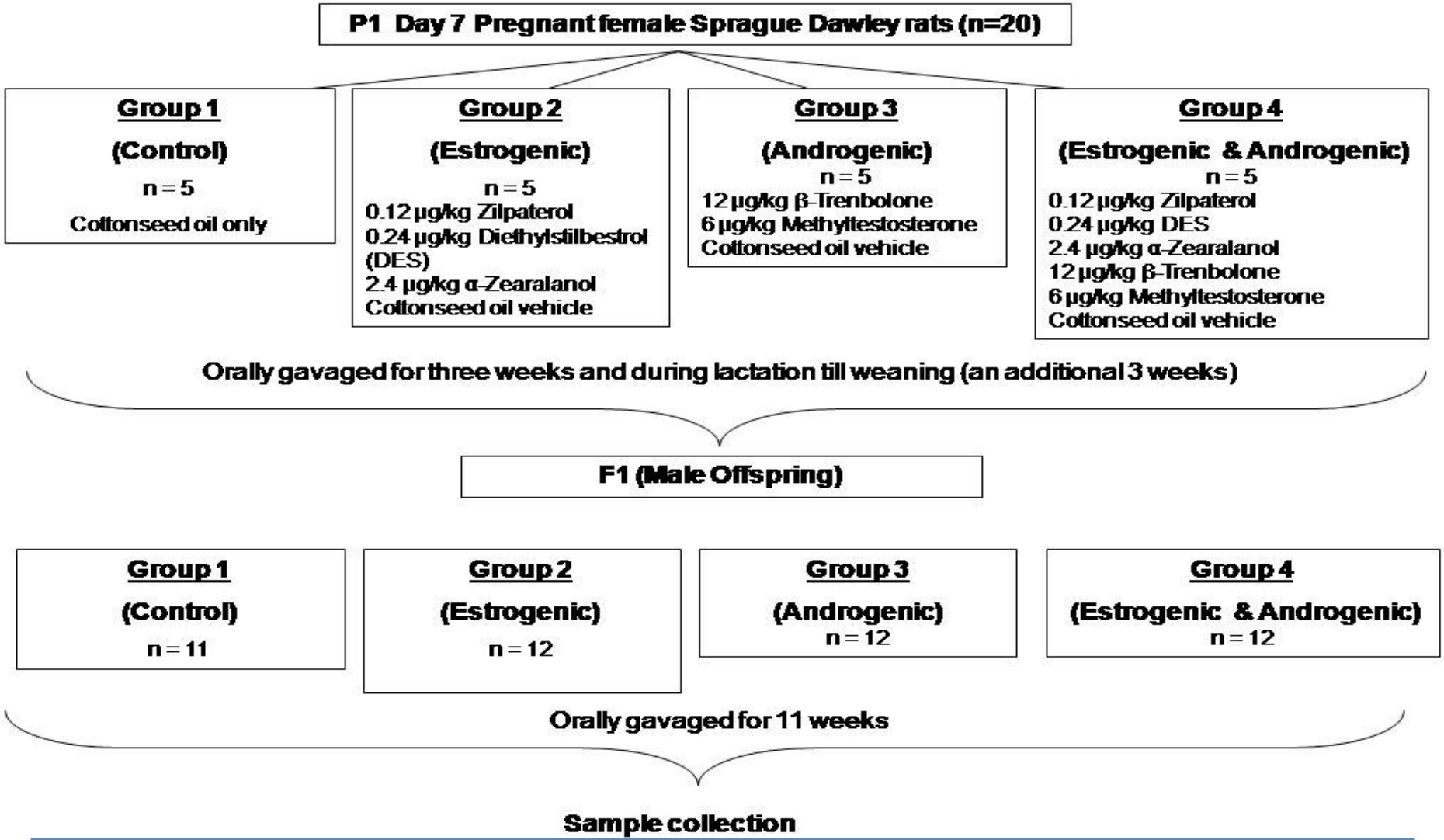


- Ethical approval obtained from the UP Animal Use and Care Committee (AUCC) [Project no: H031-07]
- The OECD 415 Reproductive Toxicity Study protocol was modified to accommodate a control group and 3 experimental groups
- Compounds and concentrations were used at environmental levels, as found in the runoff water of a local feedlot

OECD 415,1983; 1-8; www.oecd.org)

Results: Chemical analyses

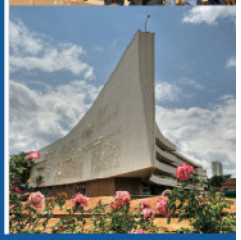
- Estrogenic compounds
  - Zilpaterol
  - DES
  - α-Zeralenol
- Androgenic compounds
  - β-Trenbelone
  - Methyltestosterone



Sample collection

1. Ano-genital distance (AGD)
2. Total sperm count
3. Organs weights: testis, epididymis, prostate, seminal vesicles
4. Histological evaluation of the testes
5. Thyroid function

Methods: Thyroid



- Blood was collected by cardiac puncture.
- Clotted blood was then spun down to collect serum for:

  TSH, T_3 and T_4.
- Kits were used:
  - Coat-A-Count Canine TSH IRMA (PIIK9T-5, 2006-12-29; Cat no IK 9T1);
  - Coat-A-Count Total T_3 (PITKT3-5, 2006-12-29; Cat no TKT31);
  - Coat-A-Count Canine T_4 (PITKC4-5, 2006-12-29, Cat no TKC41).

Results: Bioassays

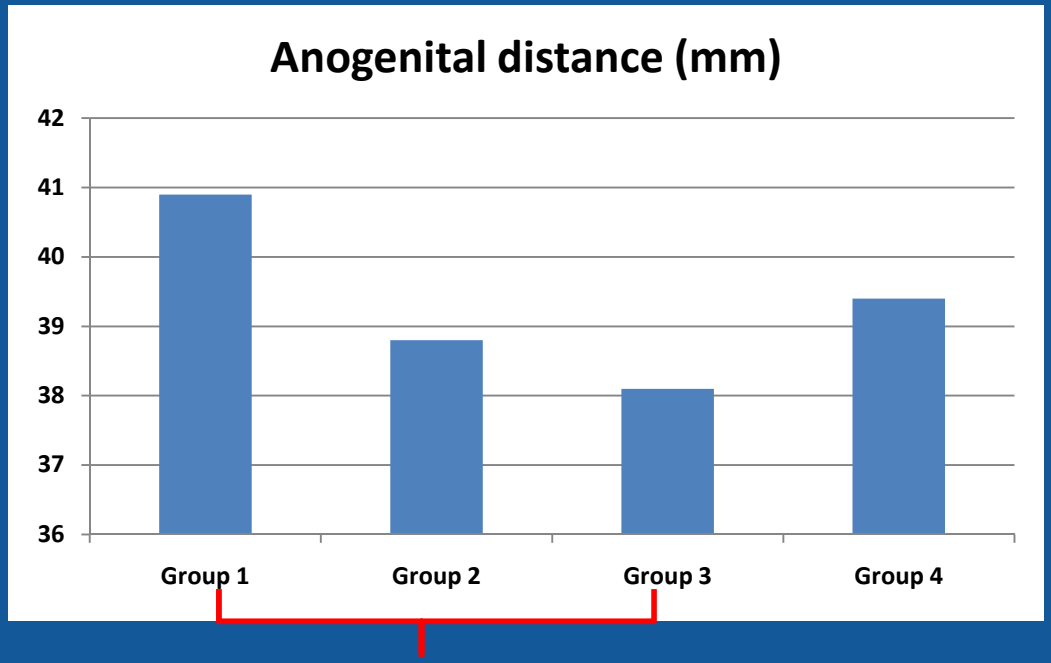
Site description	YES: EEq (ng/L)	T47D-KBluc: EEq (ng/L)
Borehole 150m upstream	☠	☠
Settling dam	☠	2.57 ± 0.39
Influent feedlot dam	0.38 ± 0.15 ☠	0.32 ± 0.04 ☠
Borehole in feedlot 1a	☠	0.13 ± 0.03
Feeding cradle	n/q ☠	0.02 ± 0.004
Borehole in feedlot 2	n/q ☠	0.14 ± 0.02
Downstream from feedlot	n/q ☠	0.94 ± 0.67 ☠
Borehole in feedlot 1b	<dl	<dl
Borehole downstream	n/q	0.47 ± 0.01
Reservoir water	<dl	0.25 ± 0.14
4km downstream	<dl	0.04 ± 0.007

<dl: below detection limit of assay; n/q: positive but not quantifiable; ☠ cytotoxicity

YES: 7/11 – cytotoxicity **T47-KBluc:** 9/11 – pos (0.02-2.57ng/L); 3/11 - cytotoxicity

Results

Anogenital distance



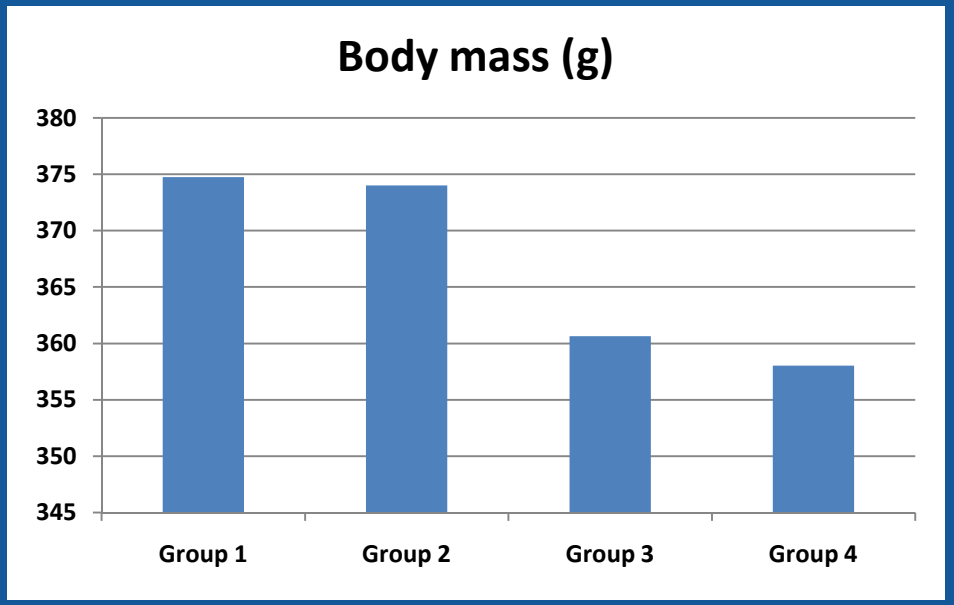
Control group and Group 3 (Androgenic)

- Ano-genital distance was significantly decreased **(p = 0.0117)**

Results

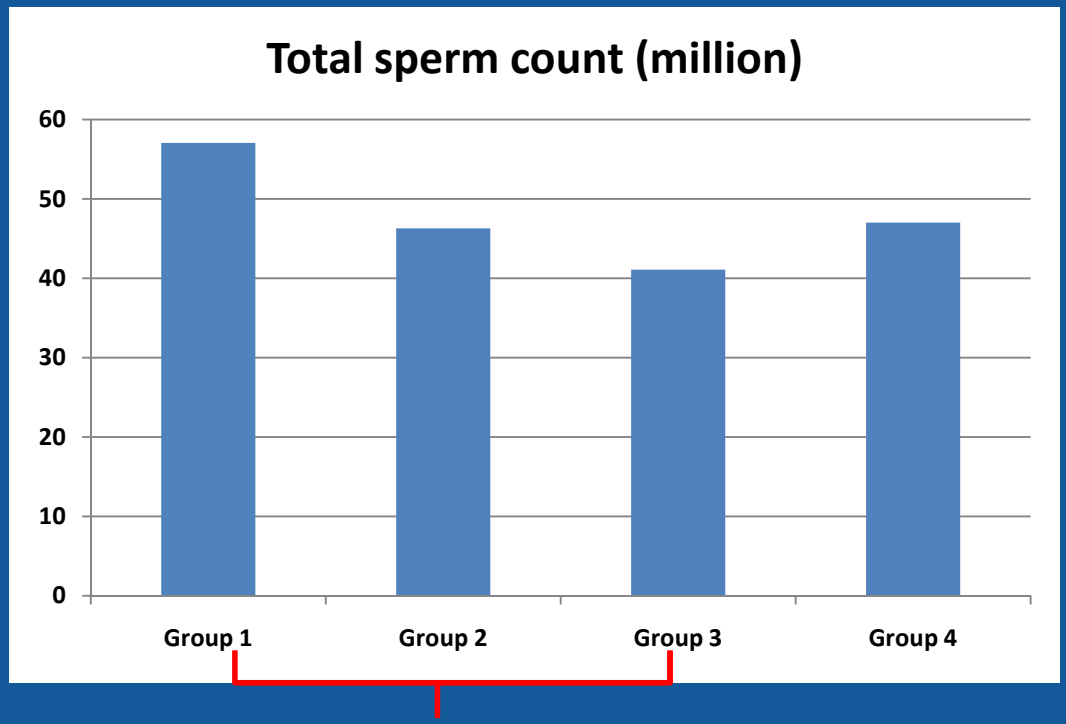
No statistical differences between the control group and experimental groups for:

- mean body mass
- total testicular mass
- mean epididymal mass



Results

Total sperm count

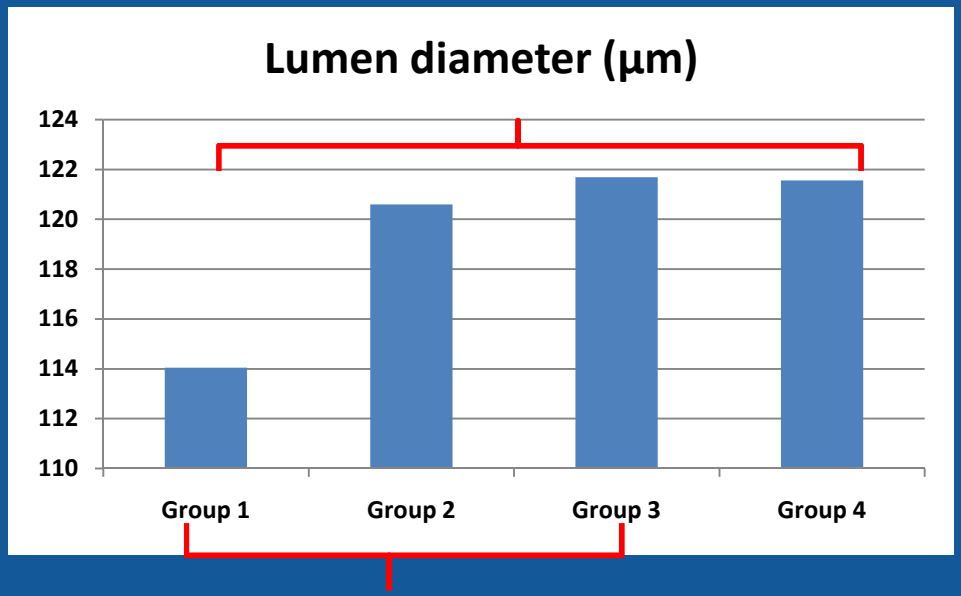


Control group and Group 3 (Androgenic)

- Lower total sperm count **(p = 0.0337)**

Results

Lumen diameter



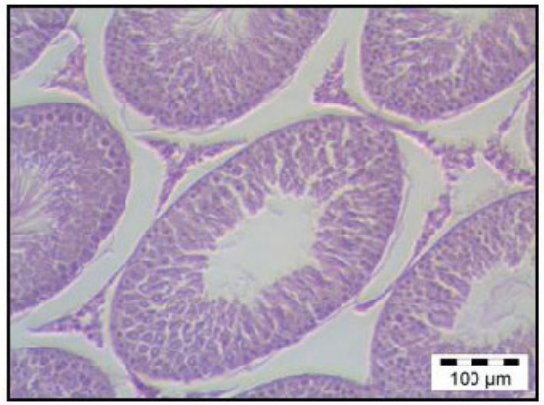
Control group and Group 3 (Androgenic)

- Histologically: Larger total lumen diameter **(p = 0.0455)**

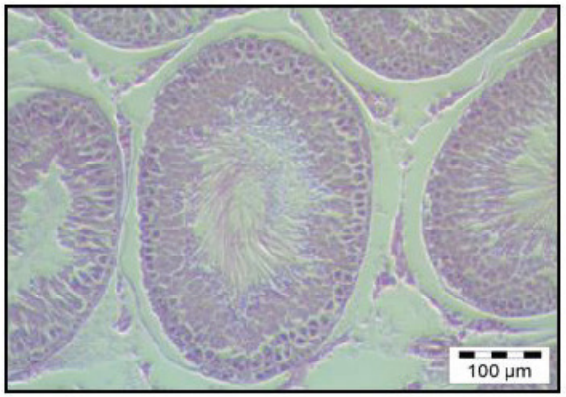
Control group and Group 4 (Estrogenic & Androgenic)

- Histologically: Larger total lumen diameter **(p = 0.0289)**

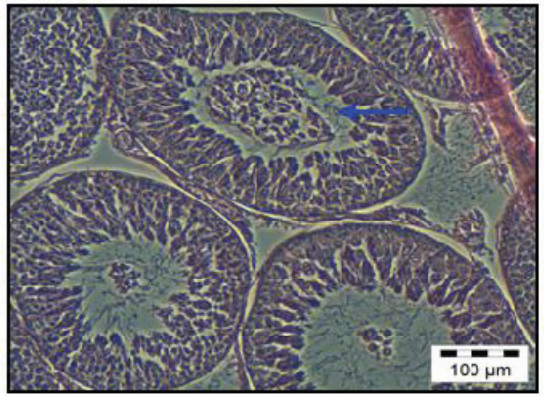
Results



a)



b)

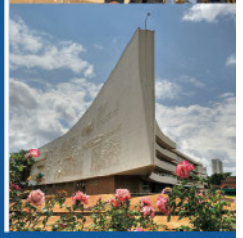


c)

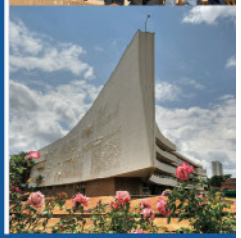
a) Control Group

b) Estrogenic Group

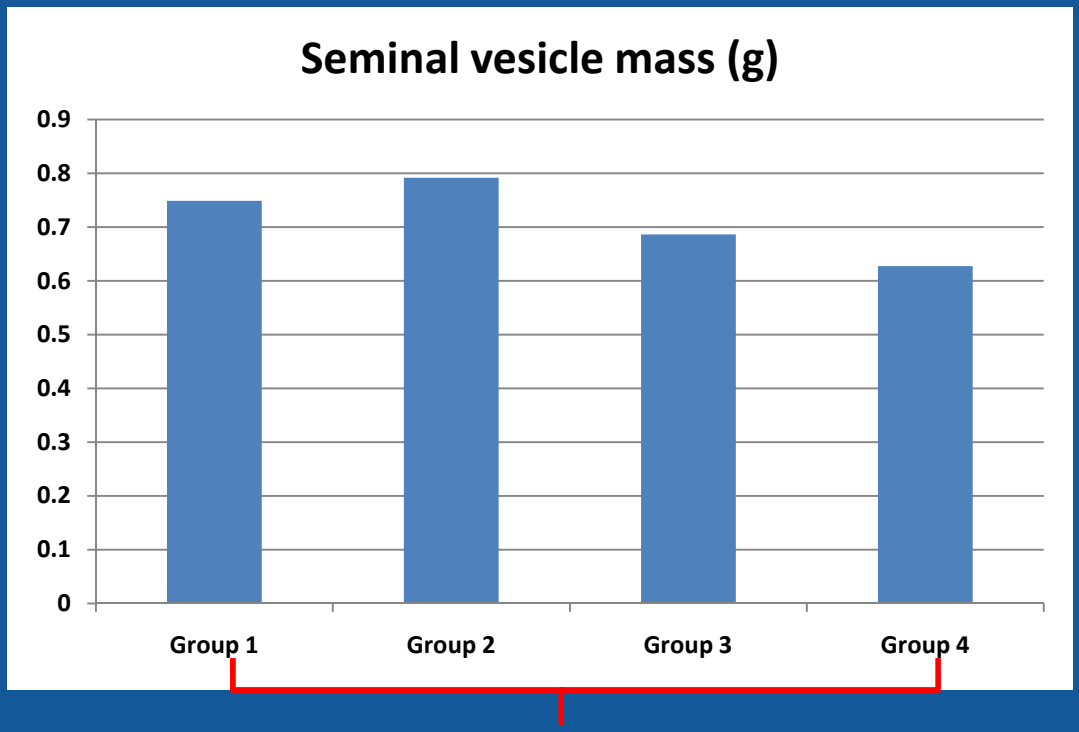
c) Androgenic Group



Results



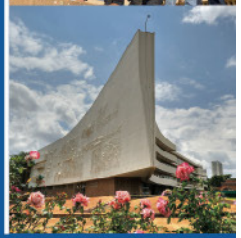
Seminal vesicle mass



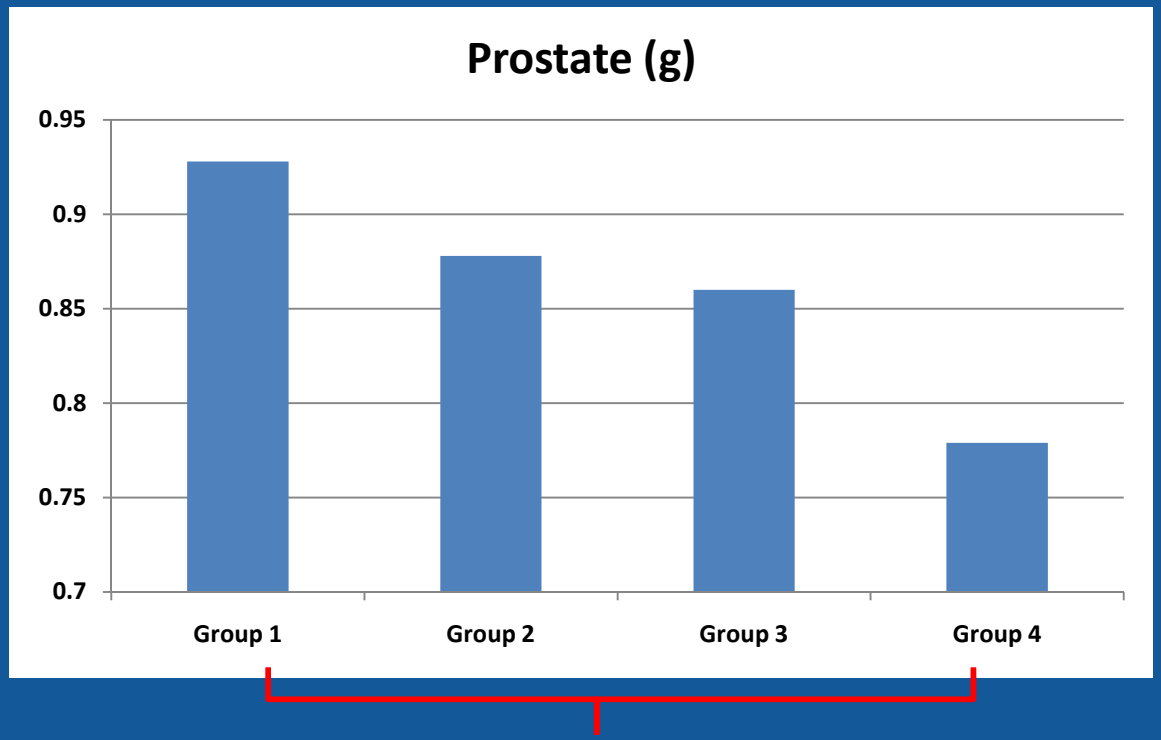
Control group and Group 4 (Estrogenic & Androgenic)

- Lower mean seminal vesicle mass **(p = 0.0074)**

Results



Prostate



Control group and Group 4 (Estrogenic & Androgenic)

- Lower mean prostate mass **(p = 0.0151)**

Results

- **T3** did not differ significantly between Control and Experimental Groups.
- **T4** was statistically significantly **higher** in Group 2 ($p = 0.009$) and 3 ($p= 0.021$) compared to the Control.
- TSH – lack of sensitivity in the kit used for rats.

Discussion: Estrogenic activity

- Estrogenic activity is present in water samples from feedlots:
  - 0.02 – 2.57 ng/L estradiol equivalents (EEqs)
- Long-term exposure to EEqs in excess of 1ng/L result in:
  - Ovotestis
  - Estrogen-induced intersex in catfish

Matthiessen et al. (2006)

Discussion: AGD

- Methyltestosterone is converted to 17α-methylestradiol which after exposure had **estrogenic effects**

- This may have contributed to the mixture which resulted in the decreased **AGD**, lower **seminal vesicle** and **prostate mass**

Hornung et al., 2004; Kishner & Svec, 2008; Phillips & Foster, 2008

Discussion: Histology

- **Seminiferous tubule fluid** produced by the Sertoli cell is **androgen** dependent
- Alterations to any of these functions may be reflected by **tubular lumen dilation** or contraction
- In this study **dilation** was observed in Groups 3 and 4
- In Group 3, rats showed **apical sloughing** of the immature germ cells

Sharpe, 1989; Creasy 2001

Discussion: Sperm counts

- AR agonists and **estrogenic** compounds can cause a reduction in testosterone production from the testes
- Together with a reduced release of gonadotropins, LH and FSH from the pituitary (negative feedback)
- ➔ reduced spermatogenesis

  The sperm counts were lowered across the groups (significant in Group 3)

Wason et al., 2003; Kilian et al., 2007

Conclusions

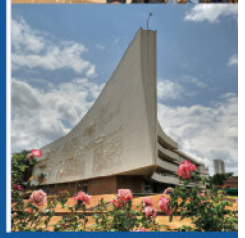
- Feedlots appear to contribute to the aquatic burden of EDCs
- This might add to a **complex mixture of EDCs** in the environment, including DDT used for malaria vector control
- Preliminary results warrant further field studies on the potential biological impact on aquatic life and mammal species

Conclusions

- Low doses of EDCs may exert more potent effects than higher doses
- Particularly if exposure occurs during a critical developmental window
- Current evidence suggests that mammals are more susceptible to EDCs during fetal and post-natal life than in adulthood

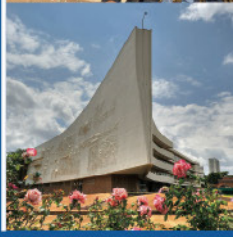
Sheenan et al., 1999; Sweeney, 2002; Diamanti-Kandarakis et al., 2009

Conclusions



- Exposure to environmentally relevant concentrations of veterinary growth stimulants had an effect on the reproductive health of maternally and direct exposed male rats
- Environmental contaminants that alter thyroid hormone signaling, particularly during the critical neonatal period, could have permanent effects on testicular development
- This might have serious implications for human reproductive health

Acknowledgements



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