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Environmental Protection
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Examination of human relevance of anti-androgenic effects observed following exposure to dibutyl phthalate

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Objective

- Describe an ongoing project in which the Adverse Outcome Pathway (AOP) framework is being utilized to perform a systematic review of DBP-induced male reproductive effects.

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

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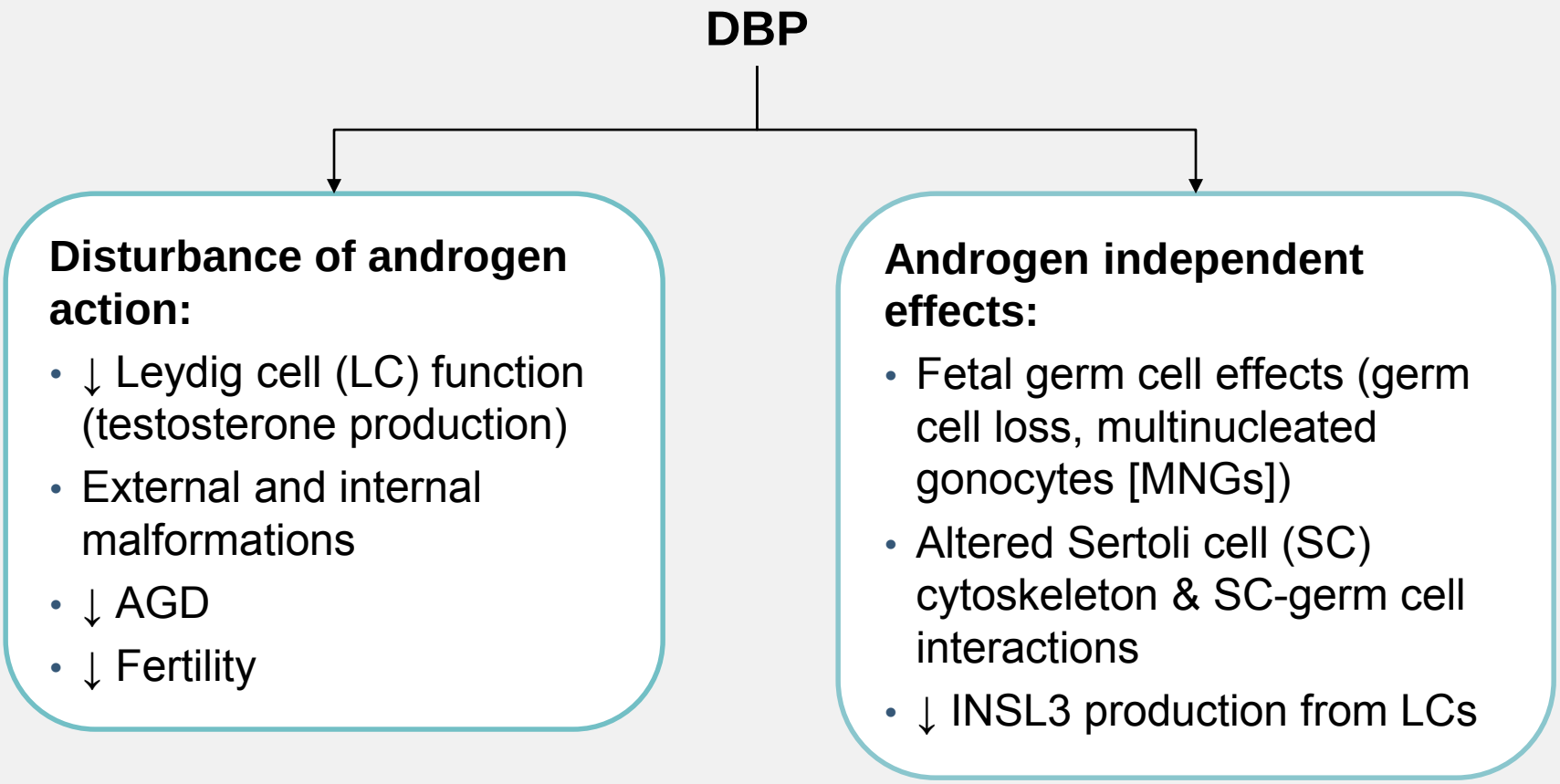
Dibutyl phthalate (DBP)

- DBP is used as a plasticizer in resins, cellulose plastics, adhesives, solvent for dyes, and fixative for perfumes.
- The largest source of exposure to humans is food.
- DBP and other phthalates with side chain lengths between 3 and 9 carbons are known to target the male reproductive system.
- Rat studies on DBP and other phthalates suggest that early life stages (fetal and early postnatal) are the most sensitive to DBP-induced male reproductive toxicity.

DBP-induced effects in the male reproductive system after gestational exposure

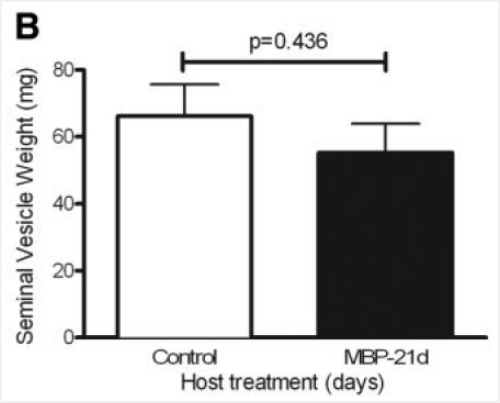


Adapted from NRC, 2008

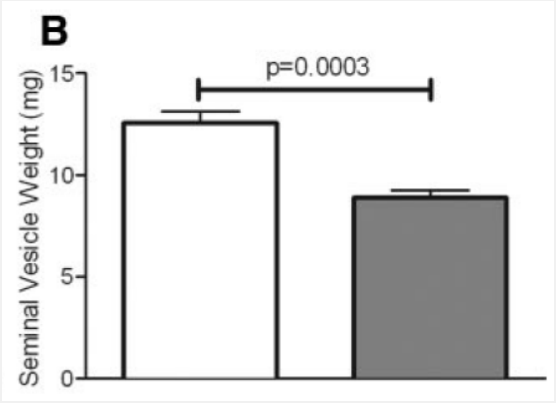
Human relevance of evidence from experimental studies

- Studies using ex-vivo human tissue culture preparations, or rodent and human testicular tissue xenografts report that human fetal testes are less sensitive to DBP-induced disruption of testosterone production (reviewed: WHO, 2012; Albert and Jégou 2014; Johnson et al 2012).

Human fetal xenografts

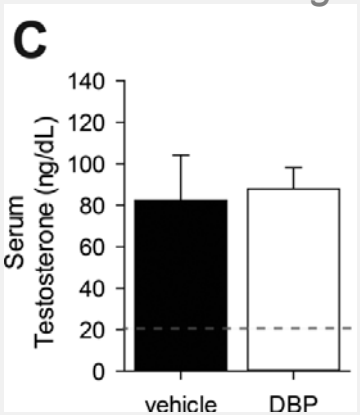


Rat fetal xenografts



From *Mitchell et al 2012*

Human fetal xenografts



From *Spade et al 2014*

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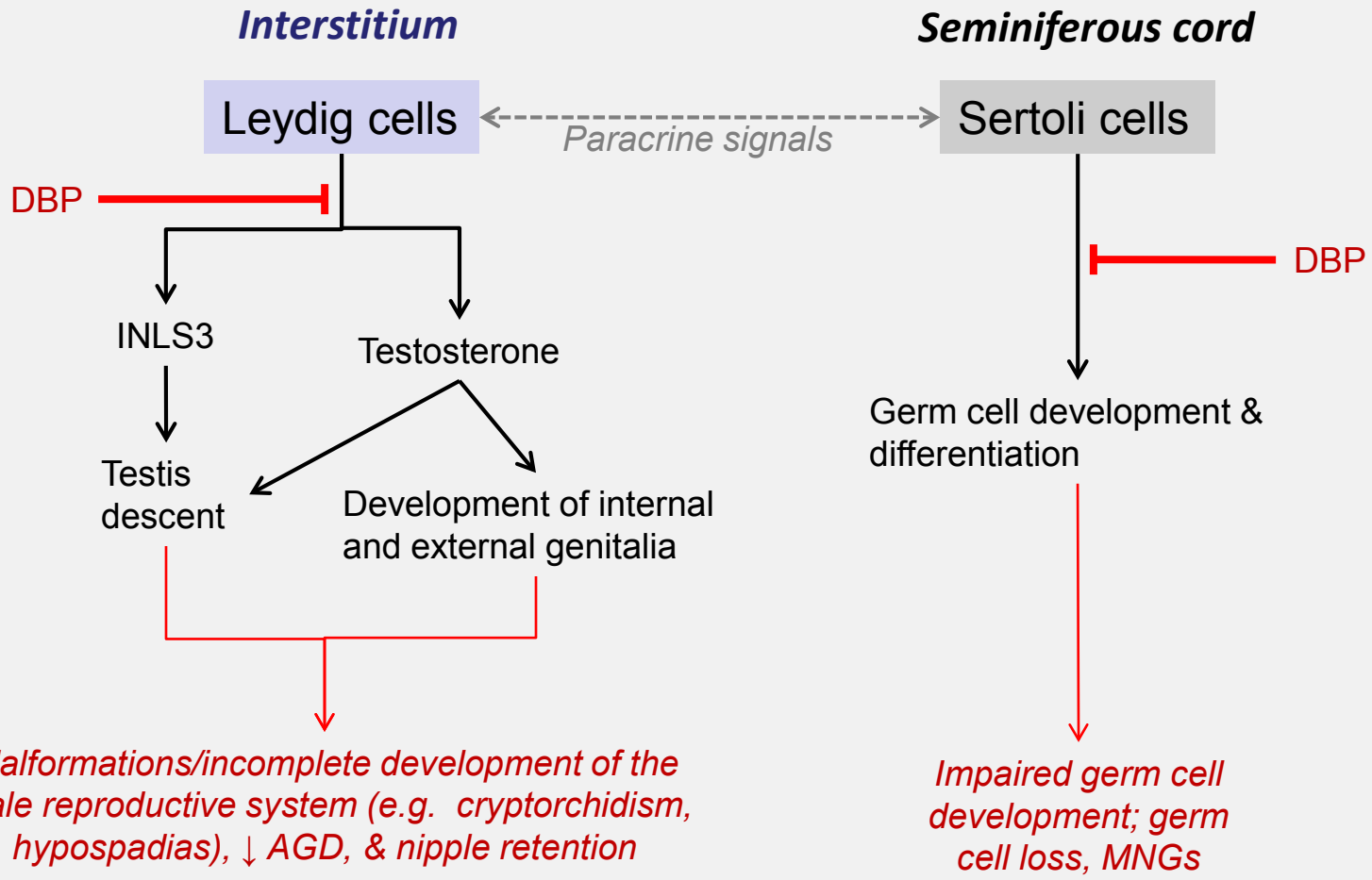
Scope and objectives

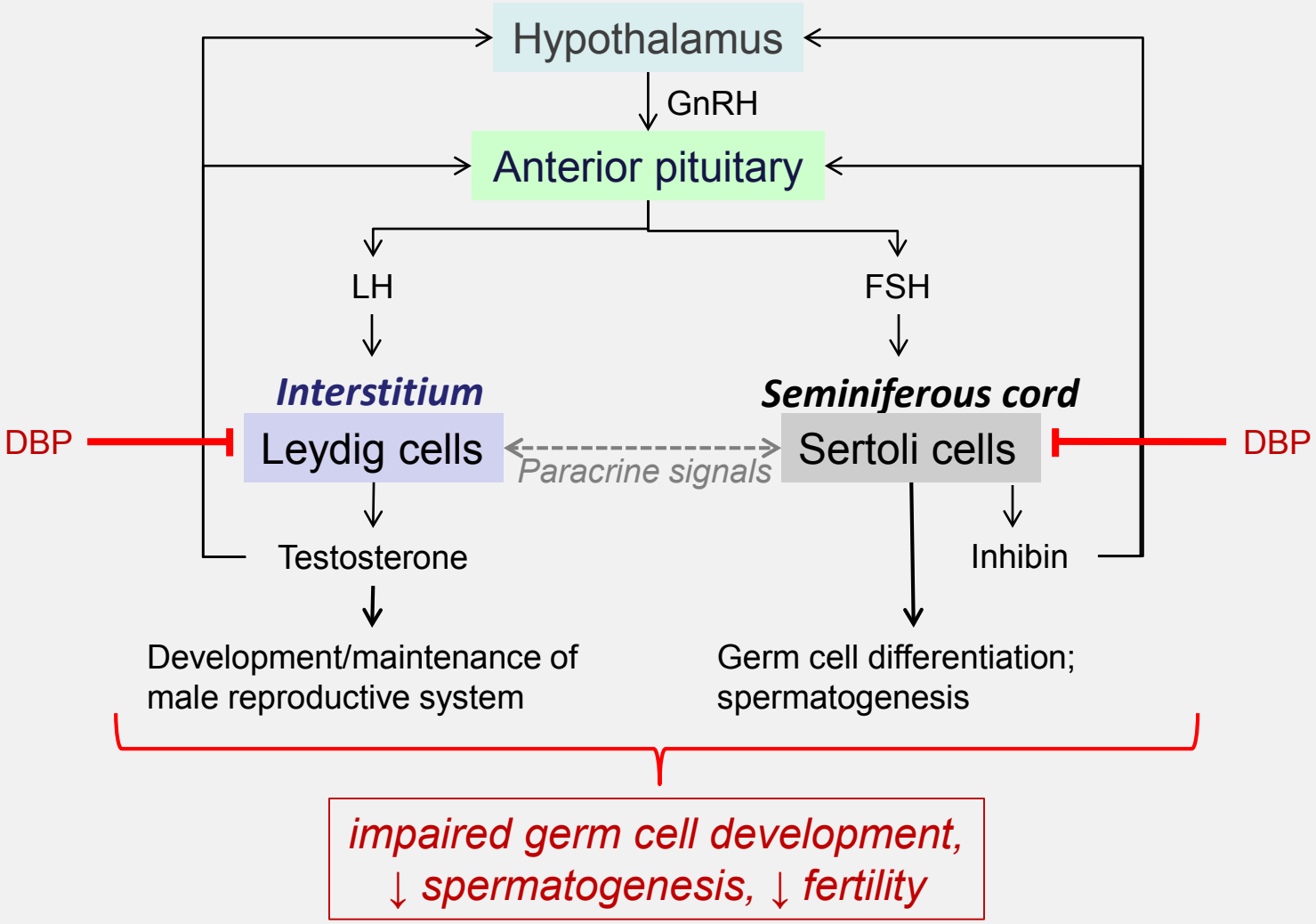
- "The identification of common molecular, cellular or/and phenotypic targets in both rat and human models should precede the choice of a toxicological endpoint in the rat to accurately assess the safety threshold of any ED in humans" (Habert et al 2014).
- The AOP framework was considered to be a useful tool to integrate information from a variety of experimental models and levels of biological organization.

Mechanism for DBP-induced male reproductive effects (gestational exposure)



Adapted from Martino-Andrade & Chahoud 2010; Foster 2006; Sharpe & Skakkebaek, 2008; EPA 2009

Mechanism for DBP-induced male reproductive effects (early post-natal lifestage exposure)



Adapted from: Foster and Gray 2013; Hu et al 2009

Literature search and identification of studies

Literature search strategy developed for IRIS DBP Tox Review → Identification of toxicological and mechanistic studies → Title/abstract review (studies informative on potential reproductive effects)

Study citations and secondary literature review → Study selection and information extraction

- **In vivo studies [~40]:** Gestation &/or post natal exposures
- **In vitro studies [10]:** Cell lines, or ex-vivo tissue culture
- **Xenograft studies [5]:** Rodent models, humans and non-human primates

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Considerations-criteria used to evaluate experimental and mechanistic evidence

- Lifestage: Due to biochemical, physiological, and endocrine differences during development, evidence was organized according to the lifestage of exposure.
  - Gestational; masculinization programming window
  - Puberty (before, during)
  - Sexually mature
- Reporting: Species and strain of animals, dosing, and exposure duration.
- Exposure route: oral, inhalation, dermal exposures, and cell culture.

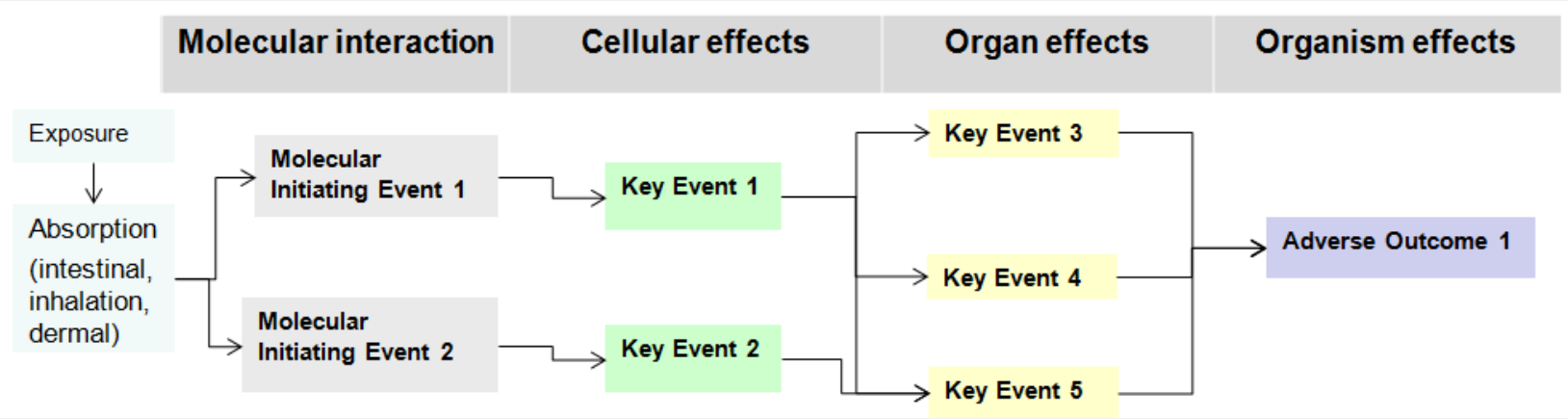
Database-inventory created for analysis of DBP-induced male reproductive effects

Study information captured in database:

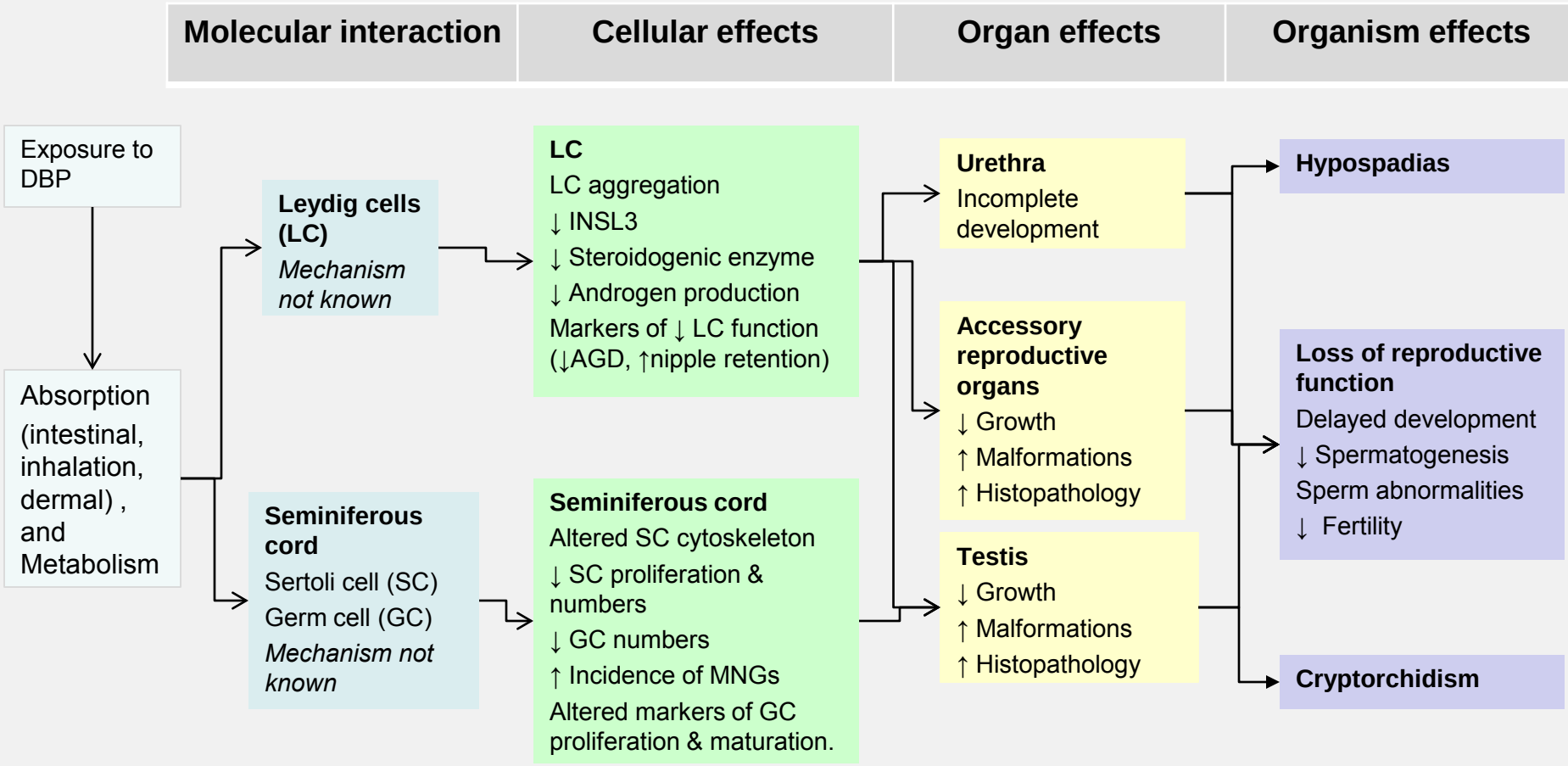
- HERO ID & reference.
- Species, strain, & age/life stage of test model.
- Test compound, dose, exposure route & duration.
- Target organ categories (e.g. organ wt, hormone levels, histopathology, mechanistic, etc).
- Reported outcomes for individual effects.
- Corresponding key event or adverse outcome.

Types of experimental studies captured in database:

- In-vivo
- In-vitro (i.e. cell culture)
- Ex-vivo
- Xenograft



Pathway for DBP-induced male reproductive effects after gestational exposure during MPW



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Preliminary observations; gestational exposure

- Overall, the available evidence suggests that DBP exposure during gestation may alter development of the male reproductive system.
- Fetal rats appear more sensitive to DBP-induced anti-androgenic effects than are mice and may be more sensitive than other rodent species, non-human primates, and human fetal testis xenografts and ex-vivo tissue cultures.
- DBP-induced androgen-independent effects are conserved among most mammalian models (rats, rabbits and mice) and human xenografts.

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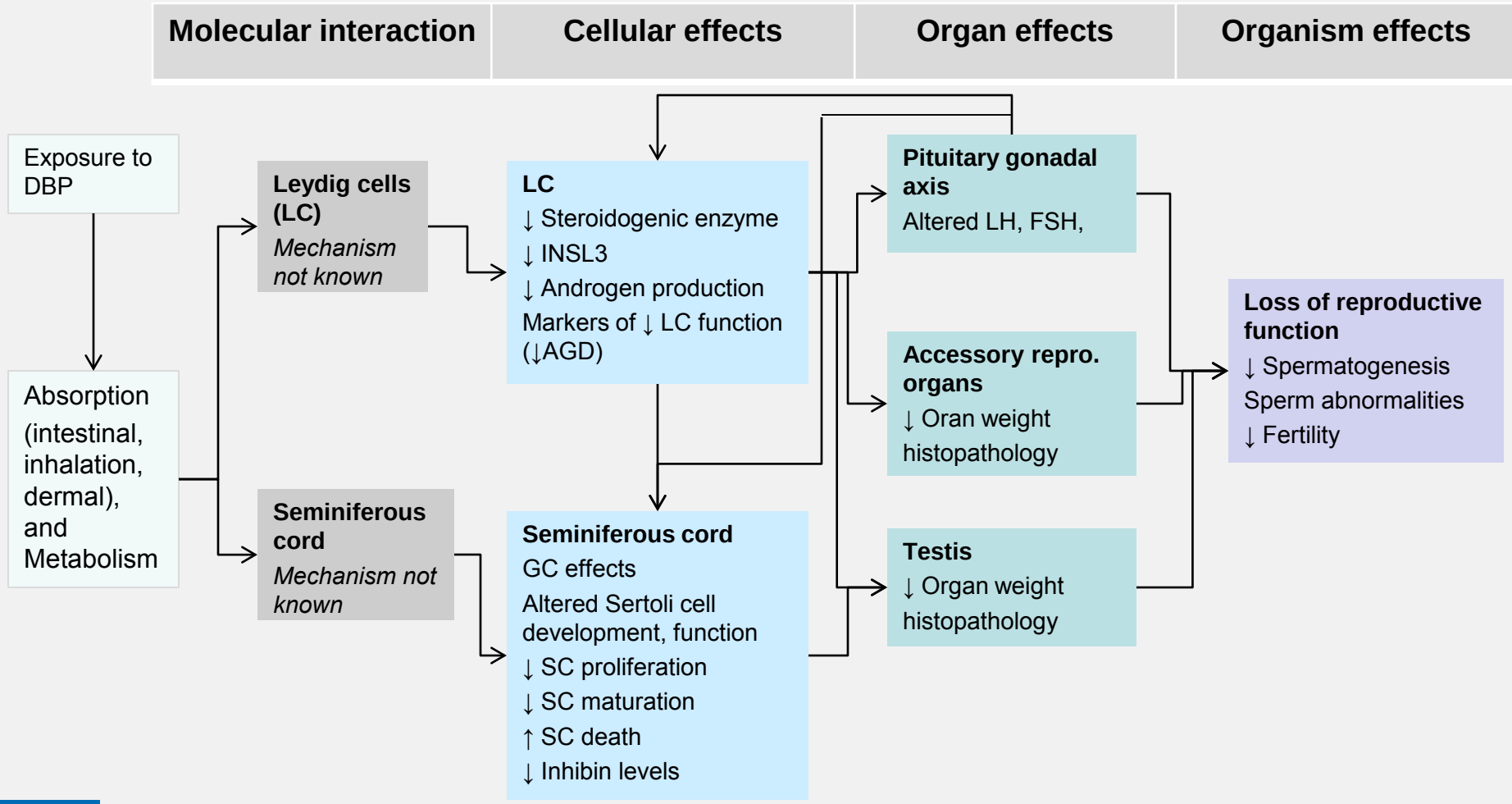
Pathway for DBP-induced male reproductive effects in early post natal life stages



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Preliminary observations early post-natal life stages

- DBP-induced Leydig cell effects are conserved in different mammalian species: (rats, rabbits, mice, gerbils, and guinea pigs, non-human primates [in-vivo and xenografts]).
- DBP-induced effects in the seminiferous cord (SC & GC) are also conserved among most mammalian models (rats, mice, and non-human primate [xenograft]).

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Utility and challenges of applying an AOP framework for this evaluation

- The AOP framework is a useful tool to gather, organize, and analyze mechanistic and toxicological information from a variety of experimental models, and levels of biological organization.
- Temporal considerations (e.g. timing of exposure and outcome evaluation) facilitates analysis of types of effects and related modes of action after exposure during specific lifestages.
- Challenges: large number of available studies, diversity of experimental models and designs, reporting gaps.

Acknowledgements:

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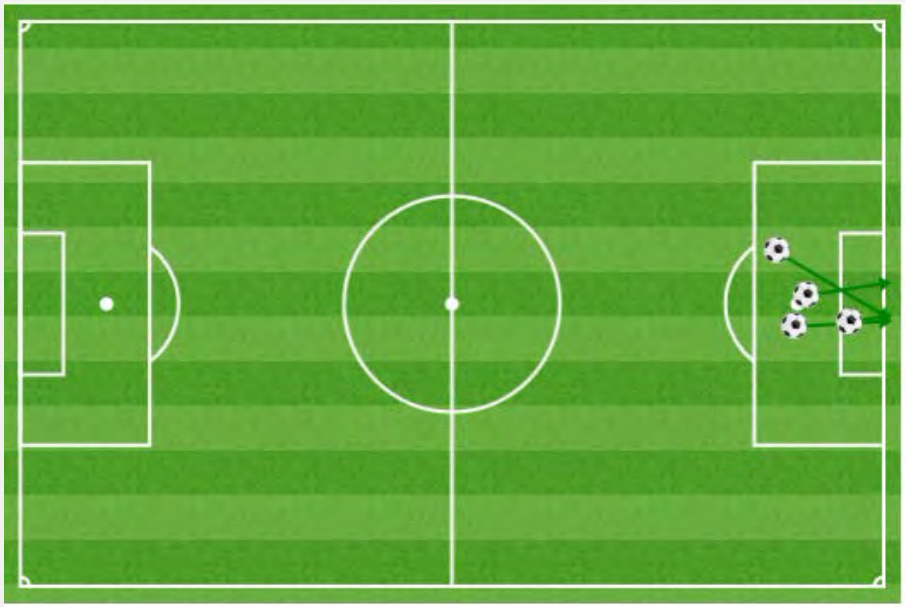
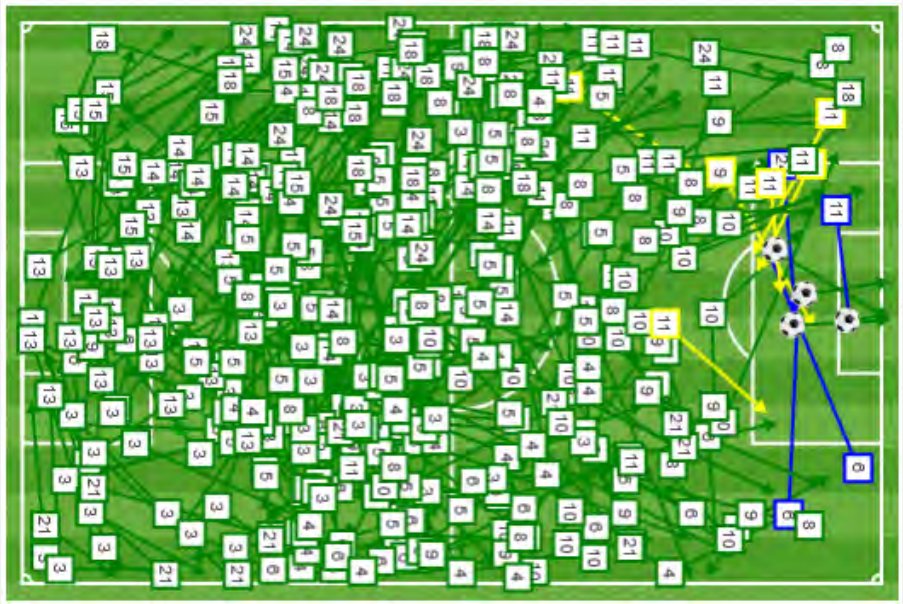
- Jason Fritz
- Ravi Subramaniam
- Bob Sonawane

AOPs are similar to… fútbol
E.g. El Clasico: Real Madrid 0-4 Barcelona





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Summary

- Overall, the available evidence suggests that phthalate exposure during gestation and early post-natal life stages alters development of the male reproductive system.
  - Rats appear to be more sensitive to DBP-induced anti-androgenic effects during gestation.
  - DBP-induced androgen-independent responses during gestation appear to be conserved across species.
  - DBP-induced post-natal responses (LCs, GCs, SCs) also appear conserved across species.
- The AOP framework is a useful tool to integrate information from diverse experimental models, and levels of biological organization.

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Preliminary cross-species coherence analysis for gestational effects

Event	Evidence in animals	Evidence in humans (ex-vivo & xenograft)
Leydig cells (LCs)	No evidence	No evidence
Sertoli cells (SCs), germ cells (GCs)	No evidence	No evidence
LCs	+ Rat [10] & rabbits [1] - Marmosets [1] & mice [3]	- Human xenografts [3] - Human ex-vivo [1]
SCs, GCs	+ SC and GC effects in rats [12] + GC effects in rabbits [1] mice [3] - Marmoset [1]	+ Human xenografts [4]
Urethra	+ Rats [2] - Marmosets [1]	No evidence
Accessory reproductive organs	+ Rats [5] & rabbits [1] - Marmoset [1]	- Host seminiferous vesicle weight [2], prostate and LABC weight [1]
Testis	+ Rats [5] & rabbits [1] - Marmoset [1]	No evidence
Organism effects: reproductive functions	+ Rats [3] & rabbits [1] - Marmoset [1]	No evidence

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Preliminary cross-species coherence analysis for effects in pubertal and sexually mature animals

Event	Evidence in animals	Evidence in humans (ex-vivo & xenograft)
Leydig cells (LCs)	No evidence	No evidence
Sertoli cells (SCs), Germ cells (GCs)	No evidence	No evidence
LCs	+ rats [7], mice [1], rabbits [1], non-human primates [1], + LC culture models (mouse [2] & dog [1] cells), + non-human primate xenografts [1]	No evidence
SCs, GCs	+ rats (in vivo [7] and cell culture[4]), mice [2], & non-human primates xenografts [1] - non-human primates (in-vivo) [1]	No evidence
Pituitary gonadal axis	Inconsistent effects in rats [3] - Rabbits [1] or mice [1]	No evidence
Accessory reproductive organs	+ rats [3], rabbits [1], gerbils [1], & non-human primates xenograft [1] - Mice [1]	No evidence
Testis	+ rats [8], rabbits [1], mice [2], & guinea pigs [1]. - Syrian hamsters [1] mice [1].	No evidence
Reproductive functions	+ rats [4], rabbits [1], mice [1], & guinea pigs [1] - mouse [1]	No evidence.