# AOP ID and Title:

SNAPSHOT

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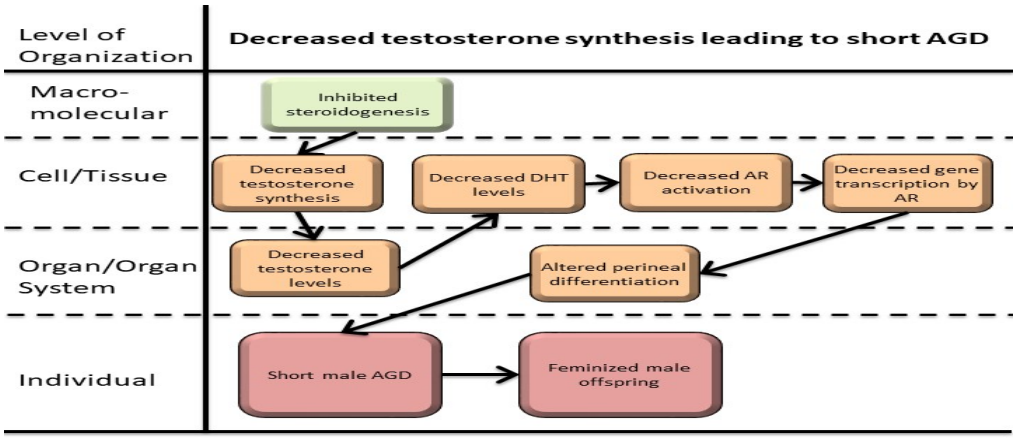
**AOP 307: Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring**

**Short Title: Decreased testosterone synthesis leading to short AGD**

# Graphical Representation

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# Status

| Author status | OECD status | OECD project | SAAOP status |
|---|---|---|---|
| Under development: Not open for comment. Do not cite | | | |

# Abstract

This AOP links decreased testosterone synthesis by fetal Leydig cells with short anogenital distance (AGD) in male offspring. A short AGD around birth is a marker for feminization of male fetuses and is associated with male reproductive disorders, including reduced fertility in adulthood. Although a short AGD is not necessarily ‘adverse’ from a human health perspective, it is considered an ‘adverse outcome’ in OECD test guidelines; AGD measurements are mandatory in specific tests for developmental and reproductive toxicity in chemical risk assessment (TG 443, TG 421/422, TG 414).

Testosterone is primarily synthesized by fetal Leydig cells of the fetal testes by the process of steroidogenesis. The precursor molecule cholesterol is converted to testosterone via several enzymatic steps and includes for instance key CYP enzymes, CYP11 and CYP17. Following synthesis, testosterone is released into the circulation and transported to target tissues and organs where it initiates masculinization processes. Under normal physiological conditions, testosterone produced by the testicles, is converted in peripheral tissues by 5α-reductase into DHT, which in turn binds AR and activates downstream target genes. AR signaling is necessary for masculinization of the developing fetus, including differentiation of the levator ani/bulbocavernosus (LABC) muscle complex in males. The LABC complex does not develop in the absence, or low levels of, androgen signaling, as in female fetuses.

The key events in this pathway is inhibition of testosterone synthesis in the fetal Leydig cells. In turn, this results in reduced circulating testosterone levels and less DHT (converted by 5α-reductase). Low DHT fails to properly activate AR in target tissues, including the developing perineal region, which leads to failure to properly masculinize the perineum/LABC complex and ultimately a short AGD.

# Summary of the AOP

## Events

### Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| | | | | |
| | KE | 413 | Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/events/413) | Reduction, Testosterone synthesis in Leydig cells |
| | KE | 1690 | reduction, testosterone levels (https://aopwiki.org/events/1690) | reduction, testosterone levels |
| | KE | 1613 | Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/events/1613) | Decrease, DHT level |
| | KE | 1614 | Decrease, androgen receptors (AR) activation (https://aopwiki.org/events/1614) | Decrease, AR activation |
| | KE | 286 | Decreased, Transcription of genes by AR (https://aopwiki.org/events/286) | Decreased, Transcription of genes by AR |
| | | | | |
| | AO | 1688 | decrease, male anogenital distance (https://aopwiki.org/events/1688) | short male AGD |

### Key Event Relationships

| Upstream Event | Relationship Type | Downstream Event | Evidence | Quantitative Understanding |
|---|---|---|---|---|
| Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/relationships/2125) | adjacent | reduction, testosterone levels | High | Moderate |
| reduction, testosterone levels (https://aopwiki.org/relationships/2126) | adjacent | Decrease, dihydrotestosterone (DHT) level | Moderate | Low |
| Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/relationships/1935) | adjacent | Decrease, androgen receptors (AR) activation | High | Moderate |
| Decrease, androgen receptors (AR) activation (https://aopwiki.org/relationships/2124) | adjacent | Decreased, Transcription of genes by AR | High | Moderate |
| Decreased, Transcription of genes by AR (https://aopwiki.org/relationships/2127) | adjacent | decrease, male anogenital distance | Moderate | Moderate |
| | | | | |
| reduction, testosterone levels (https://aopwiki.org/relationships/2131) | non-adjacent | Decrease, androgen receptors (AR) activation | Moderate | Moderate |

## Stressors

| Name | Evidence |
|---|---|
| Dibutyl phthalate | High |
| Bis(2-ethylhexyl) phthalate | High |

# Overall Assessment of the AOP

## Domain of Applicability

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Foetal | High |
| Pregnancy | High |

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | Moderate | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |
| rat | Rattus norvegicus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116) |
| mouse | Mus musculus | Moderate | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090) |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Male | High |

# References

1. Schwartz CL, Christiansen S, Vinggaard AM, Axelstad M, Hass U and **Svingen T** (2019), Anogenital distance as a toxicological or clinical marker for fetal androgen action and risk for reproductive disorders. *Arch Toxicol* 93: 253-272.

# Appendix 1

## List of Key Events in the AOP

Event: 413: Reduction, Testosterone synthesis in Leydig cells (https://aopwiki.org/events/413)

Short Name: Reduction, Testosterone synthesis in Leydig cells

### Key Event Component

| Process | Object | Action |
|---|---|---|
| testosterone biosynthetic process | testosterone | decreased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:51 - PPARα activation leading to impaired fertility in adult male rodents (https://aopwiki.org/aops/51) | KeyEvent |

| AOP ID and Name | Event Type |
|---|---|
| Aop:18 - PPARα activation in utero leading to impaired fertility in males (https://aopwiki.org/aops/18) | KeyEvent |
| Aop:64 - Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility (https://aopwiki.org/aops/64) | KeyEvent |
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | KeyEvent |

## Biological Context

| Level of Biological Organization |
|---|
| Cellular |

## Cell term

| Cell term |
|---|
| testosterone secreting cell |

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| rat | Rattus norvegicus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116) |
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |
| mice | Mus sp. | Low | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10095) |

Key enzymes needed for testosterone production first appear in the common ancestor of amphioxus and vertebrates (Baker 2011). Consequently, this key event is applicable to most vertebrates, including humans.

## Key Event Description

**Biological state**

Testosterone is a steroid hormone from the androgen group and is found in humans and other vertebrates.

**Biological compartments**

In humans and other mammals, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females and other steroidogenic tissues (e.g., brain, adipose). It either acts locally /or is transported to other tissues via blood circulation. Testosterone synthesis takes place within the mitochondria of Leydig cells, the testosterone-producing cells of the testis. It is produced upon stimulation of these cells by Luteinizing hormone (LH) that is secreted in pulses into the peripheral circulation by the pituitary gland in response to Gonadotropin-releasing hormone (GnRH) from the hypothalamus. Testosterone and its aromatized product, estradiol, feed back to the hypothalamus and pituitary gland to suppress transiently LH and thus testosterone production. In response to reduced testosterone levels, GnRH and LH are produced. This negative feedback cycle results in pulsatile secretion of LH followed by pulsatile production of testosterone (Ellis, Desjardins, and Fraser 1983), (Chandrashekar and Bartke 1998).

**General role in biology**

Testosterone is the principal male sex hormone and an anabolic steroid. Male sexual differentiation depends on testosterone (T), dihydrotestosterone (DHT), and the expression of androgen receptors by target cells (Manson and Carr 2003). During the development secretion of androgens by Leydig cells is essential for masculinization of the foetus (Nef 2000). The foetal Leydig cells develop in utero. These cells become competent to produce testosterone in rat by gestational day (GD) 15.5, with increasing production thereafter. Peak steroidogenic activity is reached just prior to birth, on GD19 (Chen, Ge, and Zirkin 2009). Testosterone secreted by foetal Leydig cells is required for the differentiation of the male urogenital system late in gestation (Huhtaniemi and Pelliniemi 1992). Foetal Leydig cells also play a role in the scrotal descent of the testis through their synthesis of insulin-like growth factor 3 (Insl3), for review see (Nef 2000).

In humans, the first morphological sign of testicular differentiation is the formation of testicular cords, which can be seen between 6 and 7 weeks of gestation. Steroid-secreting Leydig cells can be seen in the testis at 8 weeks of gestation. At this period, the concentration of androgens in the testicular tissue and blood starts to rise, peaking at 14-16 weeks of gestation. This increase comes with an increase in the number of Leydig cells for review see (Rouiller-Fabre et al. 2009).

Adult Leydig cells, which are distinct from the foetal Leydig cells, form during puberty and supply the testosterone required for the onset of spermatogenesis, among other functions. Distinct stages of adult Leydig cell development have been identified and characterized. The stem Leydig cells are undifferentiated cells that are capable of indefinite self-renewal but also of differentiation to steroidogenic cells. These cells give rise to progenitor Leydig cells, which proliferate, continue to differentiate, and give rise to the immature Leydig cells. Immature Leydig cells synthesize high levels of testosterone metabolites and develop into terminally differentiated adult Leydig cells, which produce high levels of testosterone. With aging, both serum and testicular testosterone concentrations progressively decline, for review see (Nef 2000).

Androgens play a crucial role in the development and maintenance of male reproductive and sexual functions. Low levels of circulating androgens can cause disturbances in male sexual development, resulting in congenital abnormalities of the male reproductive tract. Later in life, this may cause reduced fertility, sexual dysfunction, decreased muscle formation and bone mineralisation, disturbances of fat metabolism, and cognitive dysfunction. Testosterone levels decrease as a process of ageing: signs and symptoms caused by this decline can be considered a normal part of ageing.

### How it is Measured or Detected

OECD TG 456 [1] (http://www.oecd-ilibrary.org/environment/test-no-456-h295r-steroidogenesis-assay_9789264122642-en) is the validated test guideline for an in vitro screen for chemical effects on steroidogenesis, specifically the production of 17ß-estradiol (E2) and testosterone (T). The testosterone syntheis can be measured in vitro cultured Leydig cells. The methods for culturing Leydig cells can be found in the Database Service on Alternative Methods to animal experimentation (DB-ALM): Leydig Cell-enriched Cultures [2] (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_met=232), Testicular Organ and Tissue Culture Systems [3] (http://ecvam-dbalm.jrc.ec.europa.eu/beta/index.cfm/methodsAndProtocols/index?id_met=515).

Testosterone synthesis in vitro cultured cells can be measured indirectly by testosterone radioimmunoassay or analytical methods such as LC-MS.

### References

Chandrashekar, V, and A Bartke. 1998. “The Role of Growth Hormone in the Control of Gonadotropin Secretion in Adult Male Rats.” Endocrinology 139 (3) (March): 1067–74. doi:10.1210/endo.139.3.5816.

Ellis, G B, C Desjardins, and H M Fraser. 1983. “Control of Pulsatile LH Release in Male Rats.” Neuroendocrinology 37 (3) (September): 177–83. Huhtaniemi, I, and L J Pelliniemi. 1992. “Fetal Leydig Cells: Cellular Origin, Morphology, Life Span, and Special Functional Features.” Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.) 201 (2) (November): 125–40.

Manson, Jeanne M, and Michael C Carr. 2003. “Molecular Epidemiology of Hypospadias: Review of Genetic and Environmental Risk Factors.” Birth Defects Research. Part A, Clinical and Molecular Teratology 67 (10) (October): 825–36. doi:10.1002/bdra.10084.

Nef, S. 2000. “Hormones in Male Sexual Development.” Genes & Development 14 (24) (December 15): 3075–3086. doi:10.1101/gad.843800.

Rouiller-Fabre, Virginie, Vincent Muczynski, Romain Lambrot, Charlotte Lécureuil, Hervé Coffigny, Catherine Pairault, Delphine Moison, et al. 2009. “Ontogenesis of Testicular Function in Humans.” Folia Histochemica et Cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society 47 (5) (January): S19–24. doi:10.2478/v10042-009-0065-4.

## Event: 1690: reduction, testosterone levels (https://aopwiki.org/events/1690)

Short Name: reduction, testosterone levels

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | KeyEvent |

### Biological Context

| Level of Biological Organization |
|---|
| Tissue |

## Event: 1613: Decrease, dihydrotestosterone (DHT) level (https://aopwiki.org/events/1613)

Short Name: Decrease, DHT level

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:288 - Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288) | KeyEvent |
| Aop:289 - Inhibition of 5α-reductase leading to impaired fecundity in female fish (https://aopwiki.org/aops/289) | KeyEvent |
| Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305) | KeyEvent |
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | KeyEvent |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Key Event Description

Reduction in DHT synthesis leads to a reduction in DHT circulating levels. [1][2]

### How it is Measured or Detected

DHT levels in a sample can be measured by (High Performance) Liquid Chromatography. After sample fractionation, DHT can be identify by comparison with internal standards spectrum. Quantification of DHT levels can be performed using hormones measurements kits (ELISA), instrumental techniques (LC-MS) or liquid scintillation spectrometry (after radiolabeling).[3]

### References

[1] Miller Walter L. (1988) Molecular Biology of Steroid Hormone Synthesis. Endocrine Reviews, 9(3): 295-318.https://doi.org/10.1210/edrv-9-3-295 (https://www.google.com/url?q=https://doi.org/10.1210/edrv-9-3-295&sa=D&ust=1554891396614000)

[2] Miller W.L. and Auchus R.J. (2011) The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. Endocrine Reviews, 32(1): 81-151.https://doi.org/10.1210/er.2010-0013 (https://www.google.com/url?q=https://doi.org/10.1210/er.2010-0013&sa=D&ust=1554891396616000)

[3] Shiraishi S., Lee P.W., Leung A., Goh V.H., Swerdloff R.S. and Wang C. (2008) Simultaneous measurement of serum testosterone and dihydrotestosterone by liquid chromatography-tandem mass spectrometry. Clinical chemistry, 54(11): 1855-63.https://doi.org/10.1373/clinchem.2008.103846 (https://www.google.com/url?q=https://doi.org/10.1373/clinchem.2008.103846&sa=D&ust=1554891396617000)

## Event: 1614: Decrease, androgen receptors (AR) activation (https://aopwiki.org/events/1614)

Short Name: Decrease, AR activation

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:288 - Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288) | KeyEvent |
| Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305) | KeyEvent |

| AOP ID and Name | Event Type |
|---|---|
| Aop:306 - Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306) | KeyEvent |
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | KeyEvent |
| Aop:344 - Androgen receptor (AR) antagonism leading to nipple retention (NR) in male (mammalian) offspring (https://aopwiki.org/aops/344) | KeyEvent |

## Biological Context

| Level of Biological Organization |
|---|
| Cellular |

## Key Event Description

Androgen receptor activation is regulated by the binding of androgens. AR activity can be decreased by either a lack of steroidal ligands (testosterone, DHT) or the presence of antagonist compounds. [1][2]

## How it is Measured or Detected

Significance of AR signaling in fetal development can be studied through a conditional deletion of the androgen receptor using a Cre/loxP approach. The recommended animal model for reproductive study is the mouse.[3]

Also, epidemiological case-studies following mouse or humans expressing a complete androgen insensitivity allow to directly assess the effects of a lack of AR activation on the development.[4]

Enzyme immunoassay (ELISA) kits for in vitro quantitative measurement of AR activity are available. Androgen receptors activity can be measured using bioassay such as the (Anti-)Androgen Receptor CALUX reporter gene assay.[5]

## References

[1] Davey R.A and Grossmann M. (2016) Androgen Receptor Structure, Function and Biology: From Bench to Bedside. Clinical Biochemist Reviews, 37(1): 3-15. PCM4810760

[2] Gao W., Bohl C.E. and Dalton J.T. (2005) Chemistry and Structural Biology of Androgen Receptor. Chemical Reviews 105(9): 3352-3370https://doi.org/10.1021/cr020456u (https://www.google.com/url?q=https://doi.org/10.1021/cr020456u&sa=D&ust=1554891396627000)

[3] Kaftanovskaya E.M., Huang Z., Barbara A.M., De Gendt K., Verhoeven G., Ivan P. Gorlov, and Agoulnik A.I. (2012) Cryptorchidism in Mice with an Androgen Receptor Ablation in Gubernaculum Testis. Molecular Endocrinology, 26(4): 598-607.https://doi.org/10.1210/me.2011-1283 (https://www.google.com/url?q=https://doi.org/10.1210/me.2011-1283&sa=D&ust=1554891396628000)

[4] Hutson J.M. (1985) A biphasic model for the hormonal control of testicular descent. Lancet, 24;2(8452): 419-21http://dx.doi.org/10.1016/S0140-6736(85)92739-4 (https://www.google.com/url?q=http://dx.doi.org/10.1016/S0140-6736(85)92739-4&sa=D&ust=1554891396629000)

[5] van der Burg B., Winter R., Man HY., Vangenechten C., Berckmans P., Weimer M., Witters M. and van der Linden S. (2010) Optimization and prevalidation of the in vitro AR CALUX method to test androgenic and antiandrogenic activity of compounds. Reproductive Toxicology, 30(1):18-24 https://doi.org/0.1016/j.reprotox.2010.04.012 (https://www.google.com/url?q=https://doi.org/0.1016/j.reprotox.2010.04.012&sa=D&ust=1554891396630000)

## Event: 286: Decreased, Transcription of genes by AR (https://aopwiki.org/events/286)

Short Name: Decreased, Transcription of genes by AR

### Key Event Component

| Process | Object | Action |
|---|---|---|
| regulation of gene expression | androgen receptor | decreased |

AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:19 - Androgen receptor antagonism leading to adverse effects in the male foetus (mammals) (https://aopwiki.org/aops/19) | KeyEvent |
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | KeyEvent |
| Aop:344 - Androgen receptor (AR) antagonism leading to nipple retention (NR) in male (mammalian) offspring (https://aopwiki.org/aops/344) | KeyEvent |
| Aop:345 - Androgen receptor (AR) antagonism leading to decreased fertility in females (https://aopwiki.org/aops/345) | KeyEvent |

Stressors

| Name |
|---|
| Bicalutamide |
| Cyproterone acetate |
| Epoxiconazole |
| Flutamide |
| Flusilazole |
| Prochloraz |
| Propiconazole |
| Stressor:286 Tebuconazole |
| Triticonazole |
| Vinclozalin |

Biological Context

| Level of Biological Organization |
|---|
| Cellular |

Cell term

| Cell term |
|---|
| eukaryotic cell |

## Evidence for Perturbation by Stressor

### Bicalutamide

Using analysis of androgen-regulated gene expression in the LNCaP prostate cancer cell line (Ngan et al. 2009).

### Cyproterone acetate

Using analysis of androgen-regulated gene expression in the LNCaP prostate cancer cell line (Ngan et al. 2009) and using the AR-CALUX reporter assay in antagonism mode, cyproterone acetate showed an IC50 of 7.1 nM (Sonneveld et al. 2005).

### Epoxiconazole

Using transiently AR-transfected CHO cells, epoxiconazole showed a LOEC of 1.6 mM and an IC50 of 10 mM (Kjærstad et al. 2010).

### Flutamide

Analysis of androgen-regulated gene expression in the LNCaP prostate cancer cell line (Ngan et al. 2009) and using the AR-CALUX reporter assay in antagonism mode, flutamide showed an IC50 of 1.3 uM (Sonneveld et al. 2005).

### Flusilazole

Using hAR-EcoScreen Assay, triticonazole showed a LOEC for antagonisms of 0.8 mM and an IC50 of 2.8 (±0.1) mM (Draskau et al. 2019)

### Prochloraz

Using gene expression analysis of the androgen-regulated genes ornithine decarboxylase, prostatic binding protein C3 as well as insulin-like growth factor I. Gene expression levels were reduced in ventral prostates of male Wistar pups at postnatal day 16 following *in utero* and lactational exposure from maternal perinatal dosing with prochloraz (50 and 150 mg/kg/day) from gestational day 7 to postnatal day 16 (Laier et al. 2006). Also, using transiently AR-transfected CHO cells, prochloraz showed a LOEC of 6.3 mM and an IC50 of 13 mM (Kjærstad et al. 2010).

### Propiconazole

Using transiently AR-transfected CHO cells, propiconazole showed a LOEC of 12.5 mM and an IC50 of 18 mM (Kjærstad et al. 2010).

### Stressor:286 Tebuconazole

Using transiently AR-transfected CHO cells, tebuconazole showed a LOEC of 3.1 mM and an IC50 of 8.1 mM (Kjærstad et al. 2010).

### Triticonazole

Using hAR-EcoScreen Assay, triticonazole showed a LOEC for antagonisms of 0.2 mM and an IC50 of 0.3 (±0.01) mM (Draskau et al. 2019).

### Vinclozalin

Using the AR-CALUX reporter assay in antagonism mode, vinclozolin showed an IC50of 1.0 uM (Sonneveld et al. 2005).

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |
| rat | Rattus norvegicus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116) |
| mouse | Mus musculus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Foetal | High |
| Adult, reproductively mature | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

Both the DNA-binding and ligand-binding domains of the AR are highly evolutionary conserved, whereas the transactivation domain show more divergence, which may affect AR-mediated gene regulation across species (Davey and Grossmann 2016). Despite certain inter-species differences, AR function mediated through gene expression is highly conserved, with mutation studies from both humans and rodents showing strong correlation for AR-dependent development and function (Walters et al. 2010).

This KE is applicable for both sexes, across developmental stages into adulthood, in numerous cells and tissues and across taxa.

## Key Event Description

The Androgen Receptor and its function

Androgens act by binding to the Androgen receptor (AR) in androgen-responsive tissues (Davey and Grossmann 2016). Human AR mutations and mouse knockout models have established the fundamental role of AR in masculinization and spermatogenesis (Maclean et al.; Walters et al. 2010; Rana et al. 2014). The AR is also expressed in many other tissues such as bone, muscles, ovaries and within the immune system (Rana et al. 2014).

Decreased transcription of genes by the AR as a Key Event

The AR belongs to the steroid hormone nuclear receptor family. It is a ligand-activated transcription factor with three domains; the N-terminal domain, the DNA-binding domain, and the ligand-binding domain with the latter being the most evolutionary conserved (Davey and Grossmann 2016). Upon activation by ligand-binding, the AR translocate from the cytoplasm to the cell nucleus, dimerizes, binds to androgen response elements in the DNA to modulate gene transcription (Davey and Grossmann 2016). The transcriptional targets varies between different cells and tissues, as well as with developmental stages and is, for instance, dependent on available co-regulators (Bevan and Parker 1999; Heemers and Tindall 2007).

Several known and proposed target genes of AR canonical signaling have been identified by analysis of gene expression following treatments with AR agonists (Bolton et al. 2007; Ngan et al. 2009) and can for instance be found in the Androgen-Responsive Gene Database (Jiang et al. 2009).

## How it is Measured or Detected

*In vitro*

Decreased transcription of genes by the AR can be measured by measuring the transcription level of known downstream target genes by RT-qPCR or other transcription analyses approaches, eg transcriptomics.

Indirect approaches include the use of transient or stable transactivation assays including the validated OECD test guideline assay, Test No. 458: *Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals* (OECD 2016). The stably transfected AR-EcoScreenTM cell line is freely available for the Japanese Collection of Research Bioresources (JCRB) Cell Bank under reference number JCRB1328. These cell-based transcriptional activation assays are typically used to detect AR agonists and antagonists. However, these types of assays are well suited to measure this KE as what they measure is exactly AR transcriptional activity. Other assays along this line include the AR-CALUX reporter gene assay that is derived from human U2-OS cells stably transfected with the human AR and an AR responsive reporter gene (van der Burg et al. 2010).

*In vivo*

Known downstream target gene transcription level can be measured in tissues by RT-qPCR or other gene expression analyses approaches.

## References

Bevan C, Parker M (1999) The role of coactivators in steroid hormone action. Exp. Cell Res. 253:349–356

Bolton EC, So AY, Chaivorapol C, et al (2007) Cell- and gene-specific regulation of primary target genes by the androgen receptor. Genes Dev 21:2005–2017. doi: 10.1101/gad.1564207

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## List of Adverse Outcomes in this AOP

### Event: 1688: decrease, male anogenital distance (https://aopwiki.org/events/1688)

Short Name: short male AGD

#### Key Event Component

| Process | Object | Action |
|---|---|---|
| androgen receptor signaling pathway | Musculature of male perineum | disrupted |

#### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:305 - 5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305) | AdverseOutcome |
| Aop:306 - Androgen receptor (AR) antagonism leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/306) | AdverseOutcome |
| Aop:307 - Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307) | AdverseOutcome |

#### Stressors

| Name |
|---|
| Butylparaben |
| p,p'-DDE |
| Bis(2-ethylhexyl) phthalate |
| Dexamethasone |
| Fenitrothion |
| Finasteride |
| Flutamide |
| Ketoconazole |
| Linuron |
| Prochloraz |

| Name |
| --- |
| |
| Procymidone |
| Triticonazole |
| Vinclozolin |
| di-n-hexyl phthalate |
| Dicyclohexyl phthalate |
| butyl benzyl phthalate |
| monobenzyl phthalate |
| di-n-heptyl phthalate |

Biological Context

| Level of Biological Organization |
| --- |
| Tissue |

Organ term

| Organ term |
| --- |
| perineum |

## Evidence for Perturbation by Stressor

### Butylparaben

Butylparaben has been shown to cause decreased male AGD in rats following intrauterine exposure to 500 and 1000 mg/kg bw/day (Boberg et al, 2016; Zhang et al, 2014). A separate study using 600 mg/kg bw/day did not see an effect on male AGD (Boberg et al, 2008).

### p,p'-DDE

p,p,DDE has been shown to cause decreased male AGD in rats following intrauterine exposure to 100-200 mg/kg bw/day (Loeffler & Peterson, 1999; Wolf et al, 1999).

### Bis(2-ethylhexyl) phthalate

DEHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 300-1500 mg/kg bw/day (Christiansen et al, 2010; Gray et al, 2000; Howdeshell et al, 2007; Jarfelt et al, 2005; Kita et al, 2016; Li et al, 2013; Lin et al, 2009; Moore et al, 2001; Nardelli et al, 2017; Saillenfait et al, 2009; Wolf et al, 1999).

### Dexamethasone

Dexamethasone has been shown to cause decreased male AGD in rats following intrauterine exposure to 0.1 mg/kg bw/day (Van den Driesche et al, 2012).

### Fenitrothion

Fenitrothion has been shown to cause decreased male AGD in rats following intrauterine exposure to 25 mg/kg bw/day (Turner et al, 2002).

### Finasteride

Finasteride has been shown to cause decreased male AGD in rats following intrauterine exposure to 100 mg/kg bw/day (Bowman et al, 2003).

### Flutamide

Flutamide has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 16-100 mg/kg bw/day (Foster & Harris, 2005; Hass et al, 2007; Kita et al, 2016; McIntyre et al, 2001; Mylchreest et al, 1999; Scott et al, 2007; Welsh et al, 2007).

### Ketoconazole

Ketoconazole has been shown to cause decreased male AGD in rats following intrauterine exposure to 50 mg/kg bw/day in one study (Taxvig et al, 2008), but no effect in another study using same dose (Wolf et al, 1999).

### Linuron

Linuron has been shown to cause decreased male AGD in rats following intrauterine exposure to 50-100 mg/kg bw/day (Hotchkiss et al, 2004; McIntyre et al, 2002; Wolf et al, 1999).

### Prochloraz

Prochloraz has been shown to cause decreased male AGD in rats following intrauterine exposure to 150-250 mg/kg bw/day (Laier et al, 2006; Noriega et al, 2005).

### Procymidone

Procymidone has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 50-150 mg/kg bw/day (Hass et al, 2012; Hass et al, 2007; Wolf et al, 1999).

### Triticonazole

Triticonazole has been shown to cause decreased male AGD in rats following intrauterine exposure to 150 and 450 mg/kg bw/day (Draskau et al, 2019).

### Vinclozolin

Vinclozolin has been shown to cause decreased male AGD in rats following intrauterine exposure to doses between 50-200 mg/kg bw/day (Christiansen et al, 2009; Gray et al, 1994; Hass et al, 2007; Matsuura et al, 2005; Ostby et al, 1999; Schneider et al, 2011; Wolf et al, 2004).

### di-n-hexyl phthalate

DnHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 500-750 mg/kg bw/day (Saillenfait et al, 2009a; Saillenfait et al, 2009b).

### Dicyclohexyl phthalate

DCHP has been shown to cause decreased male AGD in rats following intrauterine exposure to 350-750 mg/kg bw/day (Aydoğan Ahbab & Barlas, 2015; Hoshino et al, 2005; Saillenfait et al, 2009a).

### butyl benzyl phthalate

BBP has been shown to cause decreased male AGD in rats following intrauterine exposure to 500-1000 mg/kg bw/day (Ema & Miyawaki, 2002; Gray et al, 2000; Hotchkiss et al, 2004; Nagao et al, 2000; Tyl et al, 2004).

### monobenzyl phthalate

MBeP has been shown to cause decreased male AGD in rats following intrauterine exposure to 375 mg/kg bw/day (Ema et al, 2003).

### di-n-heptyl phthalate

DHPP has been shown to cause decreased male AGD in rats following intrauterine exposure to 1000 mg/kg bw/day (Saillenfait et al, 2011).

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | Moderate | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606) |
| rat | Rattus norvegicus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116) |
| mouse | Mus musculus | High | NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090) |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Foetal | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Male | High |

A short AGD in male offspring is a marker of insufficient androgen action during critical fetal developmental stages (Schwartz et al, 2019; Welsh et al, 2008). A short AGD is thus a sign of undervirilization, which is also associated with a series of male reproductive disorders, including genital malformations and infertility in humans (Juul et al, 2014; Skakkebaek et al, 2001).

There are numerous human epidemiological studies showing associations with intrauterine exposure to anti-androgenic chemicals and short AGD in newborn boys alongside other reproductive disorders (Schwartz et al, 2019). This underscores the human relevance of this AO. However, in reproductive toxicity studies and chemical risk assessment, rodents (rats and mice) are what is tested on. The list of chemicals inducing short male AGD in male rat offspring is extensive, as evidenced by the 'stressor' list and reviewed by (Schwartz et al, 2019).

## Key Event Description

The anogenital distance (AGD) refers to the distance between anus and the external genitalia. In rodents and humans, the male AGD is approximately twice the length as the female AGD (Salazar-Martinez et al, 2004; Schwartz et al, 2019). This sexual dimorphisms is a consequence of sex hormone-dependent development of secondary sexual characteristics (Schwartz et al, 2019). In males, it is believed that androgens (primarily DHT) activate AR-positive cells in non-myotic cells in the fetal perineum region to initiate differentiation of the perineal *levator ani* and *bulbocavernosus* (LABC) muscle complex (Ipulan et al, 2014). This AR-dependent process occurs within a critical window of development, around gestational days 15-18 in rats (MacLeod et al, 2010). In females, the absence of DHT prevents this masculinization effect from occurring.

The involvement of androgens in masculinization of the male fetus, including the perineum, has been known for a very long time (Jost, 1953), and AGD has historically been used to, for instance, sex newborn kittens. It is now well established that the AGD in newborns is a proxy readout for the intrauterine sex hormone milieu the fetus was developing. Too low androgen levels in XY fetuses makes the male AGD shorter, whereas excess (ectopic) androgen levels in XX fetuses makes the female AGD longer, in humans and rodents (Schwartz et al, 2019).

## How it is Measured or Detected

The AGD is a morphometric measurement carried out by trained technicians (rodents) or medical staff (humans).

In rodent studies AGD is assessed as the distance between the genital papilla and the anus, and measured using a stereomicroscope with a micrometer eyepiece. The AGD index (AGDi) is often calculated by dividing AGD by the cube root of the body weight. It is important in statistical analysis to use litter as the statistical unit. This is done when more than one pup from each litter is examined. Statistical analyses is adjusted using litter as an independent, random and nested factor. AGD are analysed using body weight as covariate as recommended in Guidance Document 151 (OECD, 2013).

## Regulatory Significance of the AO

In regulatory toxicology, the AGD is mandatory inclusions in OECD test guidelines used to test for developmental and reproductive toxicity of chemicals. Guidelines include 'TG 443 extended one-generation study', 'TG 421/422 reproductive toxicity screening studies' and 'TG 414 developmental toxicity study'.

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# Appendix 2

# List of Key Event Relationships in the AOP

## List of Adjacent Key Event Relationships

Relationship: 2125: Reduction, Testosterone synthesis in Leydig cells leads to reduction, testosterone levels (https://aopwiki.org/relationships/2125)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **adjacent** | **High** | **Moderate** |

Relationship: 2126: reduction, testosterone levels leads to Decrease, DHT level (https://aopwiki.org/relationships/2126)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **adjacent** | **Moderate** | **Low** |

Relationship: 1935: Decrease, DHT level leads to Decrease, AR activation (https://aopwiki.org/relationships/1935)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Inhibition of 17α-hydrolase/C 10,20-lyase (Cyp17A1) activity leads to birth reproductive defects (cryptorchidism) in male (mammals) (https://aopwiki.org/aops/288)** | **adjacent** | **High** | **High** |
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **adjacent** | **High** | **Moderate** |
| **5α-reductase inhibition leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/305)** | **adjacent** | **High** | **Moderate** |

Relationship: 2124: Decrease, AR activation leads to Decreased, Transcription of genes by AR (https://aopwiki.org/relationships/2124)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **adjacent** | **High** | **Moderate** |
| **Androgen receptor (AR) antagonism leading to nipple retention (NR) in male (mammalian) offspring (https://aopwiki.org/aops/344)** | **adjacent** | **Moderate** | **Moderate** |

Relationship: 2127: Decreased, Transcription of genes by AR leads to short male AGD (https://aopwiki.org/relationships/2127)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **adjacent** | **Moderate** | **Moderate** |

## List of Non Adjacent Key Event Relationships

Relationship: 2131: reduction, testosterone levels leads to Decrease, AR activation (https://aopwiki.org/relationships/2131)

AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| **Decreased testosterone synthesis leading to short anogenital distance (AGD) in male (mammalian) offspring (https://aopwiki.org/aops/307)** | **non-adjacent** | **Moderate** | **Moderate** |