2016

Causal Factors of Cryptorchidism and Endocrine Disrupting Chemicals Such as Prenatal Maternal Cigarette Smoke: A Narrative Review

Andrew R. Morrissey
University of Central Florida

CAUSAL FACTORS OF CRYPTORCHIDISM AND ENDOCRINE DISRUPTING CHEMICALS SUCH AS PRENATAL MATERNAL CIGARETTE SMOKE: A NARRATIVE REVIEW

by

ANDREW R. MORRISSEY

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Health Science
in the College of Health and Public Affairs
and in The Burnett Honors College
at the University of Central Florida
Orlando, Florida

Spring Term, 2016

Thesis Chair: Dr. Michael J. Rovito, Ph.D.

ABSTRACT

Cryptorchidism is a male congenital disorder with an unspecified, multifactorial etiology. This review evaluated the strength of select factors in the development of cryptorchidism to better understand its etiology. The strength of relationship between factors and their respective functions during testicular descent was evaluated. Factors evaluated in the causal pathway include the signaling mechanisms Desert Hedgehog (DHH), Insulin-like Hormone 3 (INSL3) and Platelet-Derived Growth Factor (PDGF), as well as sex hormone regulation (androgen: estrogen ratio, aromatase expression).

Articles supporting a factor in testicular descent were evaluated and scored. These scores were summed to create the "Step Score" for each step in the causal pathway. An arrow system was developed which ranked the strength of each pathway step as either "weak", "moderate" or "strong". Thus, step scores and the strength of factors in the pathological pathway were determined: DHH (15-moderate), PDGF (10-weak), INSL3 (24-strong) and Androgen: Estrogen ratio, Aromatase (23-strong). The pathological pathway produced by this review represents a literature based perspective of the research regarding cryptorchidism etiology.

Literature indicates that prenatal exposure to endocrine disrupting chemicals in animals and humans may lead to abnormal genital development. Recently, prenatal maternal cigarette smoke was demonstrated to be a risk factor for cryptorchidism. This controversial finding was explored in the context of endocrine disrupting chemicals. However, literature has provided very little evidence in support of this hypothesis and more research is needed to better evaluate prenatal maternal smoking as a risk factor for undescended testis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

- DDT(dichlorodiphenyltrichloroethane)
- DBP(di(n-butyl) phthalate)
- DES(Diethylstilbestrol)
- BPA(Bisphenol A)
- E2(Estradiol)

INTRODUCTION

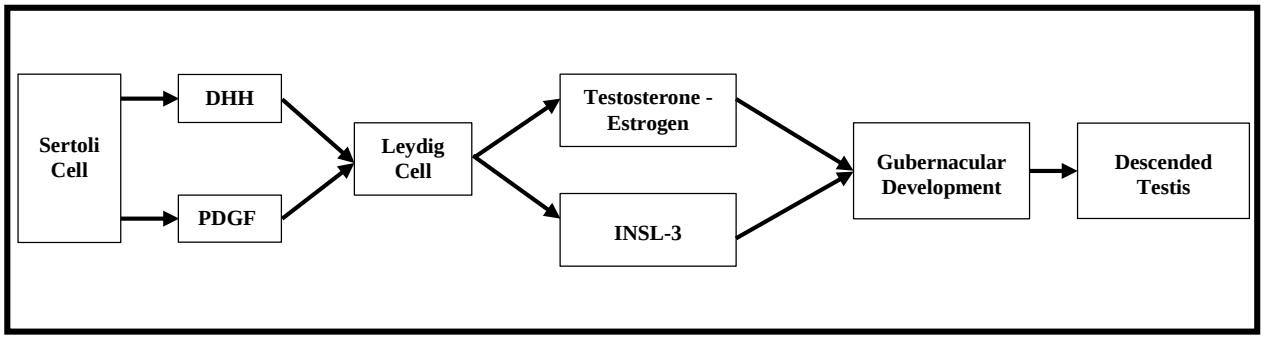
Cryptorchidism (CO), the failure of testis to properly descend, is estimated to affect between 2-8% of newborn boys (Virtanen et al., 2007). Although literature demonstrates heterogeneity in regards to effect, CO is one of the few known causes of testicular cancer (TC). For example, Lip et al. (2013) meta-analysis of TC indicated a 190% increased risk of TC in study participants with a history of CO. This increased risk of TC, as well as the increased risk of infertility among males with CO (Trsinar & Muravec, 2009) underscores the clinical significance of identifying the causal pathway of the disease.

Literature has demonstrated that the cause of CO remains largely unknown and suggests that the etiology of CO may be multifactorial in nature (Barthold et al., 2015). Recently, prenatal maternal smoking was identified as a risk factor for CO (Jensen, Toft, Thulstrup, Bonde, & Olsen, 2007; Thorup, Cortes, & Petersen, 2006). This association is not without controversy however as other studies have not been in accordance (Damgaard et al., 2008). Despite this inconsistency, the unknown etiology of CO and percent of women in the United States who smoke during pregnancy (10.7%) (Tong et al., 2013) present a public health risk: women who smoke during pregnancy may be increasing their child's likelihood of CO.

In order to investigate this risk and better understand CO etiology, this review created a physiological pathway (Figure 1) related to CO development which includes the following factors: Insulin-like Hormone 3 (INSL3), Desert Hedgehog (DHH) and Platelet Derived Growth Factor (PDGF) signaling, as well as Androgen-Estrogen Ratio and Aromatase expression. A corresponding pathological pathway (Figure 2) was also created in which the aforementioned

factors were presented based on strength of relationship. The studies included in the pathological pathway illustrate the importance of their physiological function. This review suggests malfunction of these factors leads to CO. Additional factors associated with CO pathogenesis were not included in the study evaluation and were therefore not included in the pathological pathway. Such associated factors include prenatal exposure to endocrine disrupting chemicals (EDCs), genetic susceptibility and aryl hydrocarbon receptor (AhR) function. These topics are reviewed here and provide an insightful backdrop for the factors evaluated in the pathological pathway.

Figure 1: Factors in the Physiological Pathway of Cryptorchidism



BACKGROUND

Endocrine Disrupting Chemicals

The endocrine system regulates many physiological processes, including sexual development of genital organs through the action of hormones. Furthermore, the specific hormone and quantity produced are determined by the interaction of various biomolecular and physiological pathways. There are many factors that determine the cellular response to a hormone, primarily the concentration of hormone versus the availability of unoccupied receptors (Gore et al., 2009). Here, the hypothesis that EDCs contribute to the development of CO is discussed and prenatal maternal cigarette smoking is presented as a carrier of such chemicals.

EDCs are exogenous chemicals that alter the function of the endocrine system, causing adverse health effects in the organism, its progeny or subpopulation (National Institute of Environmental Health Sciences, 2015). EDCs are pervasive in the environment and can be found in many commercial products including pesticides, plastics, flame retardants and cosmetics. Even the combustion of hydrocarbon fuels may produce EDCs (Annamalai & Namasivayam, 2015).

The adverse health effects caused by exposure to EDCs are of particular significance given that approximately 154,000 tonnes of alkylphenols are produced in the United States each year (Knez, 2013). Importantly, the EDC Bisphenol A was found in the urine of 92.6% of American males (Calafat, Xiaoyun, Lee-Yang, Reidy, & Needham, 2008). It is noted that studies have reported humans quickly metabolize BPA into inactive forms. However, fetal metabolism

of BPA is decreased compared to adults (Gore et al., 2015) and exposure to multiple EDCs simultaneously may greatly increase the risk of adverse health effects.

Overall, there is much evidence that associates EDCs with adverse reproductive health outcomes, including reduced age of menarche in females, as well as low sperm count and genital malformations in males (Buttke, Sircar, & Martin, 2012). When rodents were exposed to multiple EDCs, each far below their danger thresholds, severe negative health effects were produced, including disorders of sexual differentiation (Jacobsen, Christiansen, Boberg, Nellemann & Hass, 2010).

In a recent human study, cord blood INSL3 levels were negatively correlated to cord blood free bisphenol A, which is indirect evidence that EDCs can impact the production of INSL3 by the Leydig cell (Chevalier et al., 2015). Furthermore, Xenoestrogens (XEs), which are a type of EDC, imitate naturally occurring estrogen and can disrupt normal endocrine function in a variety of ways, even at low dosages (Zhang, Luo, Zhang, Cai, & Pan, 2014). For example, the exposure of male human fetuses to several organic pesticides simultaneously via breast milk has been associated with CO (Damgaard et al., 2006). These studies illustrate the dynamic effects of EDCs and underscore the importance of researching their effects on the body.

One particular source of EDCs is the smoke produced by tobacco cigarettes. Cigarette smoke contains many different chemicals, including the pesticide DDT (Smith & Hansch, 2000), which is a known EDC (Diamanti-Kandarakis et al., 2009). Molecules that are lipophilic can accumulate in the body and exert long term negative health effects, as is known to occur with DDT exposure in humans (Smith & Hansch, 2000). The chemicals found in cigarette smoke also

include Polycyclic Aromatic Hydrocarbons (PAH) (Rodgman & Green, 2014), which cross the placenta into the fetal bloodstream (Fowler et al., 2008).

Exposure to PAHs may negatively impact biochemical signaling via the AhR, as discussed in the next section. The chemicals found in cigarette smoke also lead to alterations in DNA methylation of replicating cells and this alteration may influence the offspring of mothers who smoke (Lee et al., 2015). In addition, exposure to XEs during pregnancy was associated with reduced DNA methylation (.84%) of the placenta in male births (Vilahur et al., 2014). Therefore, this review considers cigarette smoke to be a source of EDCs and is examined as a potential risk factor for CO.

Genetic Expression and Aryl Hydrocarbon Receptors

Despite the unknown etiology of CO, there is evidence that altered genetic expression may be involved in undescended testis. An overarching hypothesis, termed Testicular Dysgenesis Syndrome (TDS) has been proposed (Skakkebaek, Meyts, & Main, 2001) to describe a cluster of male reproductive abnormalities with a unifying etiology. Through this lens CO, hypospadias, altered spermatogenesis and TC are viewed as symptoms of an undefined cause of disease (Skakkebaek, Meyts, & Main, 2001b; Jorgensen, Meyts, Main, & Skakkebaek, 2010). TDS may serve to organize the research of male genital disorders, as CO may yet be a result of greater developmental dysfunction (Toppari, Rodprasert & Virtanen, 2014). It is important to note that non-syndromic CO and syndromic CO cases may not share the same causal pathway and that genetic variation may play a role in this difference (Lip, Murchison, Cullis, Govan, & Carachi, 2013).

Genetic Mutation and Cryptorchidism

There have been few studies that implicate specific genetic etiologies for CO. Mutations of two genes in particular have demonstrated that variation in gene expression is a causal factor of CO. The genes INSL3 and RXFP2 play an important role in testicular descent; however, mutations in these genes were found to only account for 2-3 % of nonsyndromic CO cases. In addition, studies of other genetic variations such as Estrogen Receptor I (ER1), Androgen Receptor (AR) and Steroidogenic Factor 1 (SF-1) have produced inconsistent results regarding genetic factors of CO (Barthold et al., 2015).

Aryl Hydrocarbon Receptor and Cryptorchidism Susceptibility

AhRs are receptor proteins that are expressed in the germ cells of human fetal testis and may act as receptors for exogenous ligands, such as the chemicals found in cigarette smoke (Coutts, Fulton, & Anderson, 2007). The Aryl Hydrocarbon Receptor Nuclear Translocator Gene (ARNT2) is a gene that has been investigated recently for its role in CO. The ARNT2 gene belongs to a family of transcription factors that are known to regulate physiological response to environmental contaminants, among other things (Sasaki et al., 2003; Watanabe et al., 2007; Willingham & Baskin, 2007).

In one case control study, Japanese patients with a specific genotype (AA) involving the single nucleotide polymorphism SNP rs5000770 of the ARNT2 gene had a significant increase in risk of CO (OR: 4.0, CI:1.9-8.5). In addition, synergistic interactions involving the rs5000770 SNP of ARNT2 and four other genes were also observed, introducing the possibility that susceptibility to CO may be conferred (Qin et al., 2012). However, there is little evidence

regarding the effect of ARNT2 mutations on male genital development (Qin et al., 2012). Due to the lack of research involving ARNT2 effects on male genital development and the small, ethnic sample size studied, these findings must be interpreted with caution.

Interestingly, EDCs found in cigarette smoke may act via the AhR (Ohtake et al., 2007; Kitamura & Kasai, 2007). A complication involving these receptors is that ERs and AhRs communicate via "cross talk" and may play a critical role in regulatory processes (Beischlag, Morales, Hollingshead, & Perdrew, 2008). In a recent human study involving the female fetus, AhR activation was stimulated by prenatal maternal cigarette smoking. In addition, the genes involved in ovary development were altered and estrogen exposure was increased (Fowler et al., 2014). While these results were obtained by investigating the female fetus, they may provide analogous evidence and guidance for future research regarding the role of EDCs and genetic basis of CO.

While factors such as EDC exposure and genetic mutation are implicated in CO etiology, other factors are better supported in the literature. These factors include INSL3, DHH and PDGF signaling, as well as sex hormone regulation. This narrative review evaluated these factors and presented them in a pathological pathway (Figure 2). A deeper understanding of the strength of relationship between these factors and their corresponding functions is critical to uncovering CO etiology. Therefore, the pathological pathway was constructed and the strength of the factors included in it were evaluated based on specific evaluation criteria.

METHODS

Study Design

A narrative review was performed in order to investigate the strength of evidence within the causal pathway of CO. An objective of this study was to catalogue a significant portion of the literature regarding both established and suggested factors in the development of CO. The evidence was evaluated and presented in a simple diagram that indicates the strength of relationship between pathway elements (Figure 2). The aim of this study was to clarify CO etiology by summarizing the strength of evidence regarding select factors in a meaningful review that may inspire additional research.

Search Strategy

A search of the literature was conducted using University of Central Florida Libraries QuickSearch and Google Scholar. Pathway search terms included*: cryptorchidism and DHH, EDCs and DHH, endocrine disrupting chemicals, estrogen receptor, cryptorchidism aromatase, cryptorchidism genes, cigarette smoking and cryptorchidism, INSL3 and cryptorchidism, PDGF and cryptorchidism, prenatal smoking and cryptorchidism.* Additional search terms were employed to locate supplemental articles. These studies did not meet all search criteria and were subsequently omitted from the pathological pathway. Articles that met all 4 of the following criteria were placed in the pathological pathway, while those publications that did not meet all 4 criteria were referenced in a supplemental capacity. As such, each supplemental article included

in this review has met criteria 1-3, while each pathway article has met all 4 of the following criteria:

1. Full text published in the English language
2. Published during or after 1989
3. Related to the pathogenesis of CO
4. Provides results that were obtained by valid scientific methods and empirical research supporting a relationship between two causal factors of CO; may or may not provide a statistically significant result

Supplemental sources were obtained to further explain physiological relationships, clarify concepts or provide additional information related to CO development. Nineteen articles met all search criteria and were included in the pathological pathway for evaluation.

Primary Measures

This narrative review produced a causal diagram which presents the strength of evidence regarding select factors in the causal pathway of CO and identified steps in the pathway that may be susceptible to EDCs, such as prenatal maternal cigarette smoke. Factors evaluated were included based on literature. Steps of the pathological pathway (Figure 2) are well supported in the literature as factors in the development of CO and were therefore selected for evaluation. In certain cases, such as with PDGF signaling studies, the included studies may not have produced cryptorchid offspring due to the experimental techniques employed. The immediate impact however, remains significant due to the established downstream role in testicular descent of the

factor in question. The system used to evaluate the factors in pathological pathway (Figure 2) is as follows:

1. Assessment of Pathway Articles

   Studies conducted on human subjects were considered to have greater generalizability and were therefore given a score of 2 points. Animal studies were considered less generalizable and were given a score of 1 point. Laboratory studies were considered to have produced stronger relationships between variables. For this reason, laboratory studies were given a score of 2 points, while other studies (i.e.: epidemiological) were given a score of 1 point. Studies that published statistically significant data directly related to the relationship between two causal factors were given 1 point, while those studies without were not assessed any points in this category.

   a. Each article was scored based on:

      i. Subject of study

         1. Human, Animal Model (2pts, 1 pt)

      ii. Study design

         1. Laboratory, Other (2pts, 1pt)

      iii. Statistical significance of results

         1. Statistically Significant Result (yes/no: 1pt, 0pt)

2. Articles Separated by Pathway Step (Function)

   a. For each pathway step, all individual article grades were summed for a total score for each step (“Step Score”)

3. Tiers and Arrows Created for each "Step Score"

    a. Each tier was constructed based on the relative scores from each "Step Score"

    b. Each tier was assigned a basic arrow symbol

    c. Each arrow was altered according to the magnitude of it's respective "Step Score"

Specifically, each pathway step is connected to the next using arrows to indicate relationship strength. The arrows connecting different segments of the pathological pathway vary based on the strength of evidence found in the literature. The evaluation system and corresponding arrow types are detailed in Tables 1 and 2. For each connection made in the pathological pathway (Figure 2), an arrow was used to connect the two steps. There were three types of arrows used to connect steps of the pathway. Each of the three arrow types was based on a cumulative "Step Score" of each relationship.

Table 1: Strength by Arrow Type

Strength	Arrow
Weak	
Moderate	
Strong	

Table 2: Pathological Pathway - Strength of Relationship

Pathway Step	Representation
B (PDGF)	
A (DHH)	
C (INSL3)	
D (Androgen-Estrogen Ratio, Aromatase)	

RESULTS

Results of the evaluation of articles included in the pathological pathway (Figure 2) are presented here (Table 3). Included with each pathway step is a brief description of the results for each article evaluated and the step score for each step evaluated in the pathway.

Desert Hedgehog Effects on the Leydig Cell

Each of the five studies analyzed received a score of 3, as per the evaluation criteria. In Park, Tong & Jameson (2007), a genetic laboratory study compared homozygous deficient male $DHH^{-/-}$ against XX female. The study found that in these rodents, fetal Leydig cells (FLCs) had failed to develop properly and demonstrated impaired steroidogenesis. Cyclopamine administration at 11.5 days past conception (dpc) in rodents demonstrated near complete reduction in SF-1 expression within the Leydig cell (Yao, Whoriskey & Capel, 2002) and cyclopamine administration at 11.5dpc resulted in greatly depressed Leydig cell differentiation (Yao & Capel, 2002). In Barsoum et al. (2009), activation of the hedgehog signaling pathway in SF-1 positive cells produced steroidogenic fetal Leydig cells. A step score of 15 was assigned for this step in the pathological pathway (Figure 2-A), indicating a “moderate” strength of relationship.

Platelet-Derived Growth Factor Effects on the Leydig Cell

Two of the three studies analyzed were given a score of 3. In one such study, PDGF Receptor α (PDGFR-α) $^{-/-}$ mice exhibited impaired FLC differentiation versus PDGFR- $\alpha^{+/-}$ (Brennan, Tillman & Capel, 2003). In Schmahl, Rizzolo and Soriano (2008), deletion of

PDGFR-α gene (PDGFR- $\alpha^{-/-}$) in mice resulted in a decreased number of steroidogenic Leydig cells in both males and females when compared to PDGFR- $\alpha^{+/-}$. Weisser, Landreh, Soder & Svechnikov (2011) was given a score of 4 due to the statistical significance of supporting data. In this experiment, PDGFα was demonstrated to have increased postnatal FLCs 1.8 fold ($p < 0.05$). This step in the pathological pathway (Figure 2-B) was given a step score of 10, indicating a "weak" strength of relationship.

Insulin-Like Hormone 3 Effects on Gubernacular Development

Four of the seven studies examined each received a score of 3. Among these, Nef and Parada (1999) demonstrated that testis of mice with INSL3 gene disruption do not descend properly (bilateral CO) due to disruption of gubernaculum development. Shortly thereafter, Nef, Shipman & Parada (2000) found mice exposed to exogenous estrogens during fetal life suffered undescended fetal testis and undetectable INSL3 transcripts. Mice exposed to estradiol prenatally exhibited normal upstream factors such as DHH expression and altered INSL3 transcription. In another study, INSL3 gene knockout versus control ($INSL3^{-/-}$; $INSL3^{+/-}$) in male mice resulted in undetectable INSL3 mRNA and bilateral CO (Zimmerman et al., 1999). Huang, Rivas and Agoulnik (2012) demonstrated that conditional knockout of Rxfp2 (receptor for INSL3) versus control ($Rxfp2^{fl}/Rxfp2^{-}/Cre^{-}$) on gubernacular mesenchymal cells resulted in undescended testis, found intraabdominal.

The three other studies evaluated each received a score of 4 due to statistically significant data. One such study demonstrated that INSL3 and dihydrotestosterone (DHT) together stimulated gubernacular cells of the mesenchyme ($p < .001$) more than other factors measured

(Kubota et al., 2002). Another experiment, Zhang et al. (2009) illustrated that fetal offspring of pregnant rats injected with DES experienced decreased INSL3 expression and inhibited migration of testis from the abdominal region. In Emmen et al. (2000), fetal mice exposed to DES exhibited a threefold decrease in INSL3 mRNA and the testis of these mice remained in the abdominal region. A step score of 24 was assigned to this step in the pathological pathway (Figure 2-C), indicating a "strong" strength of relationship.

Androgen, Estrogen, Aromatase Effects on Gubernacular Development

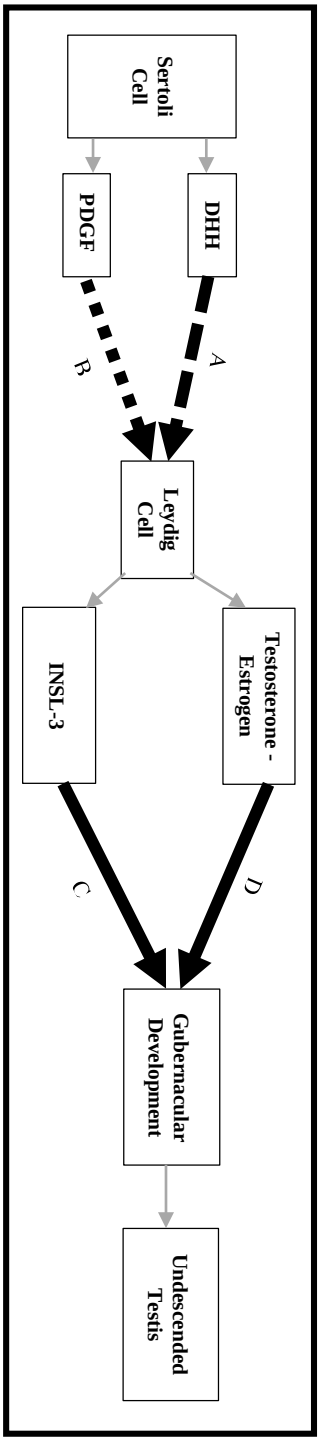
One study included in this section of the pathological pathway was given an individual score of 3. In this experiment, mice that were altered to express human aromatase ($AROM^+$; WT) exhibited intraabdominal CO, decreased testosterone and increased serum estrogen levels (Li et al., 2001). Five articles in this section were given individual scores of 4. Among these, Kotula-Balak et al. (2005) demonstrated that cryptorchid mice produced less testosterone, more estrogen and contained greater amounts of aromatase than control mice.

Additionally, testosterone has been shown to increase INSL3 mRNA levels (Lague & Tremblay, 2013). In one study of pregnant mice with altered sleep cycles, male offspring exhibited decreased testosterone and 19% were cryptorchid at 30 post-natal days (Shono & Suita, 2003). DHT itself significantly promoted gubernacular mesenchymal cell growth ($p < 0.001$) (Kubota et al., 2002). One retrospective cohort study was given an individual score 4. In Palmer et al. (2009), mothers who were exposed to DES during pregnancy increased the risk of CO in their boys by 90%. This step in the pathological pathway (Figure 2-D) was given a step score of 23, indicating a "strong" strength of relationship.

Table 3: Pathological Pathway - Articles and Corresponding Step Scores

Pathway Step	**Study** Lead Author, (year)	**Study Subject Score**	**Study Design Score**	**Statistical Significance Score**	**Score** (Step Score Total)
Desert Hedge Hog Effects on the Leydig Cell	Park, (2007)	1	2	0	3
	Yao, W., C., (2002)	1	2	0	3
	Yao, (2002)	1	2	0	3
	Barsoum, (2009)	1	2	0	3
	Weisser, (2011)	1	2	0	3
Step Score					**(15)**
Platelet-Derived Growth Factor and the Leydig Cell	Brennan, (2003)	1	2	0	3
	Weisser, (2011)	1	2	1	4
	Schmahl, (2008)	1	2	0	3
Step Score					**(10)**
Insulin-like Hormone 3 and Gubernacular Development	Kubota, (2002)	1	2	1	4
	Nef, (1999)	1	2	0	3
	Nef, (2000)	1	2	0	3
	Zimmerman, (1999)	1	2	0	3
	Huang, (2012)	1	2	0	3
	Zhang, (2009)	1	2	1	4
	Emmen, (2000)	1	2	1	4
Step Score					**(24)**
Androgen-Estrogen Ratio and Aromatase Effects on Gubernacular Development	Kubota, (2002)	1	2	1	4
	Lague, (2013)	1	2	1	4
	Kotula-Balak, (2005)	1	2	1	4
	Shono, (2003)	1	2	1	4
	Palmer, (2009)	2	1	1	4
	Li, (2001)	1	2	0	3
Step Score					**(23)**

Figure 2: Evaluation of Pathological Pathway



DISCUSSION

In humans, testicular descent can be separated into two distinct phases: transabdominal and inguinoscrotal. The transabdominal phase (8-15 weeks) is generally accomplished via INSL3, which is released by FLCs, while the inguinoscrotal phase (25-35 weeks) is mediated by testosterone. This process of testicular descent in humans is quite similar to that of rodents (Hutson, Li, Southwell, Newgreen, & Cousinery, 2015). The study of testicular descent in the rodent model may prove to be helpful in understanding CO in humans.

Research has shown that disruption of critical functions during either phase of testicular descent may lead to CO. The factors evaluated in this review appear to be crucial to testicular descent through extensive cooperation and integration. Exposure to EDCs, especially during pregnancy, may disrupt the physiological development of the male fetus. Such exposure may lead to dysregulation of fetal hormones and impair testicular descent. The pathological pathway factors A, C and D (Figure 2) may be especially susceptible to such hormonal dysregulation.

Desert Hedgehog and Endocrine Disrupting Chemicals

The Sertoli cell is located in the testis and plays a key role in testicular development, as well as spermatogenesis (Griswold, 1998). A crucial function of Sertoli cells is the production of the signaling molecule DHH. In mice, this signaling molecule has been shown to stimulate FLC differentiation by binding to its receptor Patched 1 (PTC1) on the Leydig cell (Yao & Capel, 2002). This DHH-PTC1 signaling mechanism (Figure 2-A) is partially responsible for the differentiation of Leydig cells (Yao, Whoriskey, & Capel, 2002). It was also found that the DHH signaling pathway is necessary in the testis of mice for adult Leydig cell formation (Clark,

Garland & Russell, 2000). Peritubular myoid cells are also critical for testis cord formation and are located in the testicular interstitium, along with Leydig cells and the DHH signaling pathway. In Clark, Garland, and Russell (2000), DHH gene knockout resulted in abnormal development of peritubular tissue and seminiferous tubules in mice.

In theory, any disruption of the DHH-PTC1 signaling mechanism (Figure 2-A) may disrupt downstream mechanisms and lead to genital disorders. According to Katayama et al. (2006), the DHH signaling system is susceptible to direct estrogenic regulation in the uterus of the mouse model. Additionally, comprehensive laboratory analysis demonstrated that human male fetal mRNA DHH levels were altered significantly by prenatal maternal smoke (Fowler et al., 2008).

According to Fowler et al. (2008), prenatal maternal smoking reduced testicular DHH expression in the second-trimester, suggesting a mechanism by which prenatal maternal smoking may lead to CO. The aforementioned studies indicate DHH signaling is important for testicular descent and that the DHH pathway may be susceptible to EDCs. The present study concluded that DHH signaling (Figure 2-A) was of moderate strength in the pathological pathway of CO. More research is needed regarding the effects of EDC exposure on DHH signaling as it relates to the development of CO.

Insulin-like Hormone 3 and Endocrine Disrupting Chemicals

INSL3 is a hormone secreted by Leydig cells of the testis and is considered to be a key factor in gubernacular development (Hutson, Li, Southwell, Newgreen, & Cousinery, 2015). INSL3 (Figure 2-C) stimulates gubernacular cells of the mesenchyme upon binding to its relaxin

family G-protein coupled receptor (Kubota et al., 2002; Bay, Main, Toppari & Skakkebaek, 2011). It has been shown that the testis of mice with INSL3 gene disruption do not descend properly (bilateral CO) due to disruption of gubernaculum development (Nef & Parada, 1999; Zimmerman et al., 1999).

Several studies have focused particularly on the transabdominal stage of testicular descent, where INSL3 plays a critical role. For example, in DES exposed mice, fetal testis were found to be undescended, but exhibited normal upstream factors such as DHH expression. However, INSL3 expression was greatly modified (Nef, Shipman & Parada, 2000). Critically, a dose dependent relationship between DES exposure and INSL3 expression has been discovered. The fetal offspring of pregnant rats injected with DES showed decreased INSL3 expression and inhibited migration of testis from the abdominal region (Zhang et al., 2009). When fetal mice were exposed to DES, a threefold decrease in INSL3 mRNA was observed and the testis of these mice remained in the abdominal region (Emmen et al., 2000). These findings suggest that the process of INSL3 signaling (Figure 2-C) may be vulnerable to EDC exposure.

Interestingly, DHT in combination with INSL3 are needed in the fetal mouse in order to promote gubernacular mesenchymal cell growth. When an INSL3 antagonist was employed, testosterone dependent inguinoscrotal descent of mouse testis was impaired (Yuan et al., 2010). This finding highlights the complex relationships between factors that facilitate testicular descent and stresses that the testis are very sensitive to exogenous chemicals during embryonic life.

If a disturbance in testis development occurs, it can lead to the malfunction of Sertoli cell or fetal Leydig cell function. The dysfunction of these cells may then alter testicular descent and

other genital development processes (Sharpe & Skakkebaek, 2008). As pointed out by Bay and Andersson (2010), the rodent model has been used to show that certain EDCs such as DBP reduce the expression of INSL3 (Wilson et al., 2004; McKinnell et al., 2005; Lague & Tremblay, 2008). Additionally, overexpression of the P450 aromatase gene in mice resulted in decreased INSL3 expression (Strauss et al., 2009). In a study involving postnatal boys with CO, an increased LH/INSL3 ratio was found, which suggests reduced prenatal INSL3 levels and resulting undescended testis (Bay & Andersson, 2010). Such findings highlight the importance of INSL3 function in testicular descent and its susceptibility to EDCs.

After evaluation, this review concluded that INSL3 (Figure 2-C) is a strong factor in CO etiology. This review finds that testicular development and descent are delicate processes that are vulnerable to EDCs such as DBP and DES. It is possible that the EDCs found in cigarette smoke may act in a similar way to disrupt testicular descent, although research has not greatly supported such exposure as a risk factor for CO. The degree to which INSL3 function is vulnerable to exogenous chemicals is unknown and could vary based on many factors.

Androgen, Estrogen, Aromatase and Endocrine Disrupting Chemicals

The presence of testosterone near the developing gubernaculum is critical to testicular descent. Without testosterone (Figure 2-D), the testis of mice remain in the inguinal canal (Hutson, Li, Southwell, Newgreen & Cousinery, 2015). Among the many functions of testosterone in the male fetus is increased expression of INSL3 in an Androgen Receptor (AR) dependent fashion (Lague & Tremblay, 2013). Sex hormone regulation of testosterone and estrogen appear crucial to testicular descent. Therefore, it is possible that a fetal sex hormone

imbalance may contribute to CO. A possible cause of sex hormone imbalance is prenatal maternal cigarette smoking.

In a study involving pregnant women who smoked, testosterone levels were increased by 11% (Toriola et al., 2011). A retrospective cohort study, Palmer et al. (2009), demonstrated a 140% increased risk of CO in boys whose mothers had begun taking DES *before* their 11th week of pregnancy. This time period coincides with the masculinization programming window, wherein testosterone regulation is crucial to masculinization (Scott, Mason & Sharpe, 2009). It is hypothesized that DES exposure during this time contributes to CO development by creating an unbalanced androgen-estrogen ratio and impairing testicular descent.

It is well known that testosterone is needed for testicular descent, particularly during the inguinoscrotal phase (Tremblay, 2015). However, estrogen and aromatase levels are also of importance to hormone regulation and genital development. The effects of androgen, estrogen and aromatase have been investigated as potential factors in CO development. Several studies have discussed how an imbalanced androgen-estrogen ratio may contribute to the pathological development of CO (Figure 2-D). While much research has focused on testosterone levels later in fetal development, testosterone during the masculinization programming window may be a crucial upstream factor of CO as this window exhibits a high testosterone-estrogen ratio and is sensitive to alteration (Scott, Mason & Sharpe, 2009).

A technique used to measure testosterone during the masculinization programming window is known as the Anogenital Distance (AGD) (Scott, Mason & Sharpe, 2009). AGD has been shown to be reduced in boys with CO (Jain & Singal, 2013; Thankamony et al., 2014),

which suggests that alteration of androgen levels may play a role in pathogenesis (Barthold et al., 2015). Exposure to EDCs during the masculinization programming window (estimated to be 8-12 weeks in humans) may disrupt the delicate testosterone-estrogen ratio and disrupt testicular descent (Scott, Mason & Sharpe, 2009b). Studies such as these illustrate the need for additional research regarding the effect EDCs have on the testosterone-estrogen ratio in utero as it relates to the development of CO.

Here, one possible cause of CO is hypothesized: prenatal maternal cigarette smoke may disrupt testicular descent by altering the androgen-estrogen levels *in utero*, perhaps during the masculinization programming window. General hormonal dysregulation caused by cigarette smoking has been established in the literature. In one study, women in the middle of their menstrual cycle were reported to experience an antiestrogenic effect due to cigarette smoke exposure (Westhoff, Gentile, Lee, Zacur & Helbig, 1996). Estrogen levels have also been shown to be decreased and androgen levels increased in pregnant smokers (Bernstein et al., 1989; Toriola et al., 2011). The mechanism of such alteration is unclear.

It is possible that altered testosterone-estrogen ratio is due to modification of hormone receptor function, or antagonism by EDC ligands such as XEs. Importantly, many XEs do not bind to estrogen receptors with high affinity. It has been estimated that some exogenous estrogens bind to ER at 0.0001% to 1% of E2 affinity. However, these compounds are capable of functioning as either agonists of ERα and ERβ or as antagonists to the binding of endogenous ligands (Akingbemi, 2005). The antagonistic capability of XEs poses an additional threat to physiologic hormonal regulation.

In one study, when human aromatase was expressed postnatally in male mice, the down-regulation of over two dozen genes was observed. Among the genes under-expressed were the genes for INSL3 and *Star* (an intracellular cholesterol transporting protein involved in testosterone synthesis). Additionally, the expression of P450 aromatase also increased estradiol levels within the testicle (Strauss et al., 2009). A separate study demonstrated that cigarette smoking elevated levels of aromatase in male fetuses. The end result was that male fetal aromatase levels were on par with those found in female fetuses (O'Shaughnessy, Monteiro, Bhattacharya, Fraser & Fowler, 2013). These results suggest that aromatase expression and hormone dysregulation may eventually lead to CO.

It has also been demonstrated that chemicals present in cigarette smoke, such as DDT, alter sex hormone levels or contribute to genital disorders (Virtanen & Adamsson, 2011). In rabbits, fetal exposure and exposure via lactation to DDT resulted in undescended testis and altered testosterone regulation (Veeramachaneni, Palmer, Amann & Pau, 2007). However, it is important to note that not all gestational exposure to EDCs has led to CO or severe genital disorders (McGlynn et al., 2009).

Further evidence that EDC exposure alters hormone function is found in the cohort study of men whose mothers had been treated with the synthetic estrogen DES during pregnancy. The exposed boys had a 90% increased risk of developing CO (Palmer et al., 2009). These findings introduce greater complexity regarding the effects that EDCs have on proper genital development and suggest that one measurement technique alone may not accurately reflect hormonal dysregulation due to EDC exposure. As indicated by the studies discussed here, there

is growing evidence that EDC exposure contributes to CO. Androgen: estrogen ratio and aromatase expression (Figure 2-D) were evaluated and determined to be a strong factor in CO development. Together, these findings support a pathological mechanism for the development of CO involving EDC exposure and the disruption of physiological factors in testicular descent (Figure 2).

The present study is limited by the selection and evaluation criteria utilized. These criteria were subjectively, yet rationally selected based on the aim of this review. This subjectivity and the limited generalizability of studies evaluated, especially those involving animal experiments, limit the conclusions that may be drawn from this review. The analysis of certain factors such as PDGF signaling in particular, is limited by the experimental methods utilized. In such cases, genetic experimentation resulted in the harvesting of fetal tissue, which prohibited the development of the fetus and consequent development of CO. Therefore, the strength of relationship between genetic insufficiency and CO in these cases is limited.

Conclusion

CO etiology remains poorly understood, as does the relationship between prenatal maternal smoking and CO. The complex and interconnected nature of testicular descent has resulted in a body of research that is scattered and difficult to interpret. This is in large part due to the ethical difficulties encountered in research studies that investigate how CO develops in humans. As a result, the rodent model has been used extensively. This model has allowed greater exploration of the role that EDCs play in the development of genital disorders.

Despite the usefulness of the rodent model, further human studies regarding EDC exposure and CO are needed in order to better understand CO's etiology. Literature indicates that there are numerous factors and pathways involved in CO development that warrant further study. Currently, there are several hypotheses that may lead to significant discoveries. For example, the synergistic-like effects of DHT and INSL3 on gubernacular mesenchymal cells may elucidate further roles of androgen in testicular descent (Kubota et al., 2002). Promising research is also being conducted regarding the function of calcitonin-gene regulated peptide (CGRP).

Additionally, research suggests that CGRP and the Genitofemoral Nerve (GFN) may contribute to the latter stages of testicular descent. However, the roles of CGRP and GFN in testicular descent are unclear and are the subject of current research (Hutson, Li, Southwell, Newgreen & Cousinery, 2015). Future research regarding DHT, CGRP and GFN function, as well as the effects of EDCs may assist in illuminating CO and TC etiology.

REFERENCES

Akingbemi, B. T. (2005). Estrogen regulation of testicular function. *Reproductive Biology & Endocrinology*, *3*51-13. doi:10.1186/1477-7827-3-51

Annamalai, J., & Namasivayam, V. (2015). Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife. *Environment International*, *76*78-97. doi:10.1016/j.envint.2014.12.006

Barsoum, I. B., Bingham, N. C., Parker, K. L., Jorgensen, J. S., & Yao, H. H. (2009). Activation of the Hedgehog pathway in the mouse fetal ovary leads to ectopic appearance of fetal Leydig cells and female pseudohermaphroditism. *Developmental Biology*, *329*96-103. doi:10.1016/j.ydbio.2009.02.025

Barthold, J. S., Wang, Y., Kolon, T. F., Kollin, C., Nordenskjöld, A., Olivant Fisher, A., & ... Devoto, M. (2015). Pathway analysis supports association of nonsyndromic cryptorchidism with genetic loci linked to cytoskeleton-dependent functions. *Human Reproduction (Oxford, England)*, *30*(10), 2439-2451. doi:10.1093/humrep/dev180

Bay, K., & Andersson, A. (2010). Human testicular insulin-like factor 3: in relation to development, reproductive hormones and andrological disorders. *International Journal Of Andrology*, *34*(2), 97-109.

Bay, K., Main, K. M., Toppari, J., & Skakkebaek, N. E. (2011). Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nature Reviews Urology*, (4), 187.

Beischlag, T. V., Luis Morales, J., Hollingshead, B. D., & Perdew, G. H. (2008). The aryl hydrocarbon receptor complex and the control of gene expression. *Critical Reviews In Eukaryotic Gene Expression*, *18*(3), 207-250.

Bernstein, I. M., Watson, M., Simmons, G. M., Catalano, P. M., Davis, G., & Collins, R. (1989). Maternal brain death and prolonged fetal survival. *Obstetrics And Gynecology*, *74*(3 Pt 2), 434-437.

Brennan, J., Tilmann, C., & Capel, B. (2003). Pdgfr-α mediates testis cord organization and fetal Leydig cell development in the XY gonad. *Genes & Development*, *17*(6), 800.

Buttke, D. E., Sircar, K., & Martin, C. (2012). Exposures to Endocrine-Disrupting Chemicals and Age of Menarche in Adolescent Girls in NHANES (2003-2008). *Environmental Health Perspectives*, *120*(11), 1613-1618 6p. doi:10.1289/ehp.1104748

Calafat, A. M., Xiaoyun, Y., Lee-Yang, W., Reidy, J. A., & Needham, L. L. (2008). Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol: 2003-2004. *Environmental Health Perspectives*, (1). 39

Chevalier, N., Brucker-Davis, F., Lahlou, N., Coquillard, P., Pugeat, M., Pacini, P., & ... Fénichel, P. (2015). A negative correlation between insulin-like peptide 3 and bisphenol A in human cord blood suggests an effect of endocrine disruptors on testicular descent during fetal development. *Human Reproduction (Oxford, England)*, *30*(2), 447-453. doi:10.1093/humrep/deu340

Clark, A. M., Garland, K. K., & Russell, L. D. (2002). Desert hedgehog (Dhh) gene is required in the mouse testis for formation of adult-type Leydig cells and normal development of peritubular cells and seminiferous tubules. *Biology Of Reproduction*, *63*(6), 1825-1838.

Coutts, S., Fulton, N., & Anderson, R. (2007). Environmental toxicant-induced germ cell apoptosis in the human fetal testis. *Human Reproduction*, *22*(11), 2912-2918.

Damgaard, I. N., Skakkebæk, N. E., Toppari, J., Virtanen, H. E., Shen, H., Schramm, K., & ... Nordic Cryptorchidism Study, G. (2006). Persistent Pesticides in Human Breast Milk and Cryptorchidism. *Environmental Health Perspectives*, (7). 1133.

Damgaard, I. N., Jensen, T. K., Petersen, J. H., Skakkebæk, N. E., Toppari, J., & Main, K. M. (2008). Risk Factors for Congenital Cryptorchidism in a Prospective Birth Cohort Study. *Plos ONE*, *3*(8), 1-8. doi:10.1371/journal.pone.0003051

Diamanti-Kandarakis, E., Bourguignon, J., Giudice, L., Hauser, R., Prins, G., Soto, A., & ... Gore, A. (2009). Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocrine Reviews*, *30*(4), 293-342.

Emmen, J. M. (2000). Involvement of Insulin-Like Factor 3 (Insl3) in Diethylstilbestrol-Induced Cryptorchidism. *Endocrinology*, *141*(2), 846-846. doi:10.1210/en.141.2.846

Fowler, P., Cassie, S., Rhind, S., Brewer, M., Collinson, J., Lea, R., & ... O'Shaughnessy, P. (2008). Maternal smoking during pregnancy specifically reduces human fetal desert hedgehog gene expression during testis development. *Journal Of Clinical Endocrinology & Metabolism*, *93*(2), 619-626.

Fowler, P. A., Childs, A. J., Courant, F., MacKenzie, A., Rhind, S. M., Antignac, J., & ... O'Shaughnessy, P. J. (2014). In utero exposure to cigarette smoke dysregulates human fetal ovarian developmental signalling. *Human Reproduction (Oxford), 29*(7), 1471-1489.

Gore, A. C., Chappell, V. A., Fenton, S. E., Flaws, J. A., Nadal, A., Prins, G. S., & ... Zoeller, R. T. (2015). Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews*, *36*(6), 593-602. doi:10.1210/er.2015-1093

Griswold, M. D. (1998). The central role of Sertoli cells in spermatogenesis. *Seminars In Cell & Developmental Biology*, *9*(4), 411-416.

Huang, Z., Rivas, B., & Agoulnik, A. (2012). Insulin-Like 3 Signaling Is Important for Testicular Descent but Dispensable for Spermatogenesis and Germ Cell Survival in Adult Mice. *Biology Of Reproduction*, *87*(6)

Hutson, J., Li, R., Southwell, B., Newgreen, D., & Cousinery, M. (2015). Regulation of testicular descent. *Pediatric Surgery International*, *31*(4), 317. doi:10.1007/s00383-015-3673-4

Jacobsen, P. R., Christiansen, S., Boberg, J., Nellemann, C., & Hass, U. (2010). Combined exposure to endocrine disrupting pesticides impairs parturition, causes pup mortality and affects sexual differentiation in rats. *International Journal Of Andrology*, *33*(2), 434-442. doi:10.1111/j.1365-2605.2009.01046.x

Jain, V., & Singal, A. (2013). Shorter anogenital distance correlates with undescended testis: a detailed genital anthropometric analysis in human newborns. *Human Reproduction*, *28*(9), 2343-2349.

Jensen, M. S., Toft, G., Thulstrup, A. M., Bonde, J. P., & Olsen, J. (2007). Cryptorchidism According to Maternal Gestational Smoking. *Epidemiology*, (2). 220.

Jørgensen, N., Meyts, E. R.-D., Main, K. M. and Skakkebæk, N. E. (2010), Testicular dysgenesis syndrome comprises some but not all cases of hypospadias and impaired spermatogenesis. *International Journal of Andrology*, 33: 298–303. doi: 10.1111/j.1365-2605.2009.01050.x

Katayama, S., Ashizawa, K., Gohma, H., Fukuhara, T., Narumi, K., Tsuzuki, Y., & ... Nagai, K. (2006). The expression of Hedgehog genes (Ihh, Dhh) and Hedgehog target genes (Ptc1, Gli1, Coup-TfII) is affected by estrogenic stimuli in the uterus of immature female rats. *Toxicology And Applied Pharmacology*, *217* 375-383. doi:10.1016/j.taap.2006.10.003

Kitamura, M., & Kasai, A. (2007). Cigarette smoke as a trigger for the dioxin receptor-mediated signaling pathway. *Cancer Letters*, *252*(2), 184-194

Knez, J. (2013). Endocrine-disrupting chemicals and male reproductive health. *Reproductive Biomedicine Online*, *26*(5), 440-448. doi:10.1016/j.rbmo.2013.02.005

Kotula-Balak, M., Gancarczyk, M., Sadowska, J., & Bilinska, B. (2005). The expression of aromatase, estrogen receptor alpha and estrogen receptor beta in mouse Leydig cells in vitro that derived from cryptorchid males. *European Journal Of Histochemistry*, *49*(1), 59-66.

Kubota, Y., Temelcos, C., Bathgate, R., Smith, K., Scott, D., Zhao, C., & Hutson, J. (2002). The role of insulin 3, testosterone, Mullerian inhibiting substance and relaxin in rat gubernacular growth. *Molecular Human Reproduction*, *8*(10), 900-905.

Lague, E., & Tremblay, J. (n.d). Antagonistic effects of testosterone and the endocrine disruptor mono-(2-ethylhexyl) phthalate on INSL3 transcription in Leydig cells. *Endocrinology*, *149*(9), 4688-4694.

Lague, E., & Tremblay, J. (2013). Antagonistic effects of testosterone and the endocrine disruptor mono-(2-ethylhexyl) phthalate on INSL3 transcription in Leydig cells. *Endocrinology*, *149*(9), 4688-4694.

Lee, K. W., Richmond, R., Hu, P., French, L., Shin, J., Bourdon, C., & ... Pausova, Z. (2015). Prenatal exposure to maternal cigarette smoking and DNA methylation: epigenome-wide association in a discovery sample of adolescents and replication in an independent cohort at birth through 17 years of age. *Environmental Health Perspectives*, (2), doi:10.1289/ehp.1408614

Li, X., Nokkala, E., Yan, W., Streng, T., Saarinen, N., Warri, A., & ... Poutanen, M. (2001). Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase. *Endocrinology*, *142*(6), 2435-2442.

Lip, S. L., Murchison, L. D., Cullis, P. S., Govan, L., & Carachi, R. (2013). A meta-analysis of the risk of boys with isolated cryptorchidism developing testicular cancer in later life. *Archives Of Disease In Childhood*, (1), 20.

McGlynn, K., Guo, X., Graubard, B., Brock, J., Klebanoff, M., & Longnecker, M. (2009). Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. *Environmental Health Perspectives*, *117*(9), 1472-1476 5p. doi:10.1289/ehp.0800389

McKinnell, C., Atanassova, N., Williams, K., Fisher, J., Walker, M., Turner, K., & ... Sharpe, R. (2005). Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. *Journal Of Andrology*, *22*(2), 323-338.

National Institute of Environmental Health Sciences. (2015, August 11). Endocrine Disruptors. Retrieved from http://www.niehs.nih.gov/health/topics/agents/endocrine/

Nef, S., & Parada, L. F. (1999). Cryptorchidism in mice mutant for Insl3. *Nature Genetics, 22*(3), 295.

Nef, S., Shipman, T., & Parada, L. F. (2000). Regular Article: A Molecular Basis for Estrogen-Induced Cryptorchidism. *Developmental Biology*, *224*354-361. doi:10.1006/dbio.2000.9785

Ohtake, F., Baba, A., Takada, I., Okada, M., Iwasaki, K., Miki, H., & ... Kato, S. (2007). Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. *Nature, 446*(7135), 562-566

O'Shaughnessy, P., Monteiro, A., Bhattacharya, S., Fraser, M., & Fowler, P. (2013). Steroidogenic enzyme expression in the human fetal liver and potential role in the endocrinology of pregnancy. *Molecular Human Reproduction*, *19*(3), 177-187.

Palmer, J., Herbst, A., Noller, K., Boggs, D., Troisi, R., Titus-Ernstoff, L., & ... Hoover, R. (2009). Urogenital abnormalities in men exposed to diethylstilbestrol in utero: a cohort study. *Environmental Health, 8*

Park, S. Y., Tong, M., & Jameson, J. L. (2007). Distinct roles for Steroidogenic factor 1 and Desert hedgehog pathways in fetal and adult leydig cell development. *Endocrinology, 148*(8), 3704-3710.

Qin, X., Kojima, Y., Mizuno, K., Ueoka, K., Massart, F., Spinelli, C., & ... Sone, H. (2012). Association of variants in genes involved in environmental chemical metabolism and risk of cryptorchidism and hypospadias. *Journal Of Human Genetics, 57*(7), 434-441. doi:10.1038/jhg.2012.48.

Rodgman, A., & Green, C. (2014). Toxic Chemicals in Cigarette Mainstream Smoke - Hazard and Hoopla. *Beiträge Zur Tabakforschung International : Contributions To Tobacco Research, 20*(8), 481.

Sasaki, G., Ogata, T., Ishii, T., Kosaki, K., Sato, S., Homma, K., & ... Matsuo, N. (2003). Micropenis and the 5alpha-reductase-2 (SRD5A2) gene: Mutation and V89L polymorphism analysis in 81 Japanese patients. *Journal Of Clinical Endocrinology & Metabolism, 88*(7), 3431-3436.

Schmahl, J., Rizzolo, K., & Soriano, P. (2008). The PDGF signaling pathway controls multiple steroid-producing lineages. *Genes & Development, 22*(23), 3255-3267. doi:10.1101/gad.1723908

Scott, H., Mason, J., & Sharpe, R. (2009). Steroidogenesis in the Fetal Testis and Its Susceptibility to Disruption by Exogenous Compounds. *Endocrine Reviews, 30*(7), 883-925.

Sharpe, R. M., & Skakkebaek, N. E. (2008). Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. *Fertility And Sterility*, *89*(Supplement), e33-e38. doi:10.1016/j.fertnstert.2007.12.026

Shono, T., & Suita, S. (2003). Disturbed pituitary-testicular axis inhibits testicular descent in the prenatal rat. *BJU International*, *92*(6), 641-643.

Skakkebaek, N. E., Rajpert-De Meyts, E., & Main, K. M. (2001). Testicular dysgenesis syndrome: An increasingly common developmental disorder with environmental aspects. *Human Reproduction (Oxford)*, *16*(5), 972-978.

Smith, C., & Hansch, C. (2000). The relative toxicity of compounds in mainstream cigarette smoke condensate. *Food And Chemical Toxicology*, *38*637-646. doi:10.1016/S0278-6915(00)00051-X

Strauss, L., Kallio, J., Desai, N., Pakarinen, P., Miettinen, T., Gylling, H., & ... Poutanen, M. (2009). Increased Exposure to Estrogens Disturbs Maturation, Steroidogenesis, and Cholesterol Homeostasis via Estrogen Receptor alpha in Adult Mouse Leydig Cells. *Endocrinology*, *150*(6), 2865-2872.

Thankamony, A., Ngee, L., Carroll, D., Williams, M., Dunger, D. B., Acerini, C. L., & ... Hughes, I. A. (2014). Anogenital Distance and Penile Length in Infants with Hypospadias or Cryptorchidism: Comparison with Normative Data. *Environmental Health Perspectives*, *122*(2), 207-211 5p. doi:10.1289/ehp.1307178

Thorup, J., Cortes, D., & Petersen, B. L. (2006). The incidence of bilateral cryptorchidism is increased and the fertility potential is reduced in sons born to mothers who have smoked during pregnancy. *The Journal Of Urology*, *176*(2), 734-737.

Tong, V. T., Dietz, P. M., Morrow, B., DÂ'Angelo, D. V., Farr, S. L., Rockhill, K. M., & England, L. J. (2013). Trends in Smoking Before, During, and After Pregnancy - Pregnancy Risk Assessment Monitoring System, United States, 40 Sites, 2000-2010. *MMWR Surveillance Summaries*, *62*(6), 1-19 19p.

Toppari, J., Rodprasert, W., & Virtanen, H. (2014). Cryptorchidism - Disease or symptom?. *Annales D Endocrinologie*, *75*(2), 72-76.

Toriola, A., Vaarasmaki, M., Lehtinen, M., Zeleniuch-Jacquotte, A., Lundin, E., Rodgers, K., & ... Lukanova, A. (2011). Determinants of Maternal Sex Steroids During the First Half of Pregnancy. *Obstetrics And Gynecology*, *118*(5), 1029-1036.

Tremblay, J. J. (2015). Molecular regulation of steroidogenesis in endocrine Leydig cells. *Steroids*, 3. doi:10.1016/j.steroids.2015.08.001

Trsinar, B., & Muravec, U. (2009). Fertility potential after unilateral and bilateral orchidopexy for cryptorchidism. *World Journal Of Urology*, *27*(4), 513-519.

Veeramachaneni, D., Palmer, J., Amann, R., & Pau, K. (2007). Sequelae in male rabbits following developmental exposure to p,p′-DDT or a mixture of p,p′-DDT and vinclozolin: Cryptorchidism, germ cell atypia, and sexual dysfunction. *Reproductive Toxicology*, *23*(Fetal Basis of Adult Disease), 353-365. doi:10.1016/j.reprotox.2007.01.001

Vilahur, N., Bustamante, M., Byun, H., Fernandez, M. F., Santa Marina, L., Basterrechea, M., & ... Baccarelli, A. A. (2014). Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. *Environment International*, *71*81-87. doi:10.1016/j.envint.2014.06.006

Virtanen, H. E., Bjerknes, R., Cortes, D., Jørgensen, N., Rajpert-De Meyts, E., Thorsson, A. V., & ... Main, K. M. (2007). Cryptorchidism: classification, prevalence and long-term consequences. *Acta Paediatrica*, *96*(5), 611-616. doi:10.1111/j.1651-2227.2007.00241.x

Virtanen, H. E., Cortes, D., Rajpert-De Meyts, E., Ritzén, E. M., Nordenskjöld, A., Skakkebaek, N. E., & Toppari, J. (2007). Development and descent of the testis in relation to cryptorchidism. *Acta Paediatrica*, *96*(5), 622-627. doi:10.1111/j.1651-2227.2007.00244.x

Virtanen, H. E., & Adamsson, A. (2012). Cryptorchidism and endocrine disrupting chemicals. *Molecular And Cellular Endocrinology*, *355*(2), 208-220. doi:10.1016/j.mce.2011.11.015

Watanabe, M., Yoshida, R., Ueoka, K., Aoki, K., Sasagawa, I., Hasegawa, T., & ... Ogata, T. (2007). Haplotype analysis of the estrogen receptor 1 gene in male genital and reproductive abnormalities. *Human Reproduction*, *22*(5), 1279-1284.

Weisser, J., Landreh, L., Söder, O., & Svechnikov, K. (2011). Steroidogenesis and steroidogenic gene expression in postnatal fetal rat Leydig cells. *Molecular And Cellular Endocrinology*, *341*18-24. doi:10.1016/j.mce.2011.03.008

Westhoff, C., Gentile, G., Lee, J., Zacur, H., & Helbig, D. (1996). Predictors of ovarian steroid secretion in reproductive-age women. *American Journal Of Epidemiology*, *144*(4), 381-388.

Willingham, E., & Baskin, L. (2007). Candidate genes and their response to environmental agents in the etiology of hypospadias. *Nature Clinical Practice Urology*, *4*(5), 270-279.

Wilson, V. S., Lambright, C., Furr, J., Ostby, J., Wood, C., Held, G., & Gray, J. E. (2004). Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicology Letters*, *146*207-215. doi:10.1016/j.toxlet.2003.09.012

Yao, H. H., Whoriskey, W., & Capel, B. (2002). Desert Hedgehog/Patched 1 signaling specifies fetal Leydig cell fate in testis organogenesis. *Genes & Development*, (11), 1433.

Yao, H. H., & Capel, B. (2002). Regular Article: Disruption of Testis Cords by Cyclopamine or Forskolin Reveals Independent Cellular Pathways in Testis Organogenesis. *Developmental Biology*, *246*356-365. doi:10.1006/dbio.2002.0663

Yuan, F., Li, X., Lin, J., Schwabe, C., Bullesbach, E., Rao, C., & Lei, Z. (n.d). The role of RXFP2 in mediating androgen-induced inguinoscrotal testis descent in LH receptor knockout mice. *Reproduction*, *139*(4), 759-769.

Zhang, L., Zheng, X., Hubert, J., Zheng, H., Yang, Z., & Li, S. (2009). Prenatal exposure to diaethylstilbestrol in the rat inhibits transabdominal testicular descent with involvement of the INSL3/LGR8 system and HOXA10. *Chinese Medical Journal*, *122*(8), 967-971.

Zhang, W., Luo, Y., Zhang, L., Cai, Q., & Pan, X. (2014). Known and emerging factors modulating estrogenic effects of endocrine-disrupting chemicals. *Environmental Reviews*, (1), 87.

Zimmermann, S. (1999). Targeted Disruption of the Insl3 Gene Causes Bilateral Cryptorchidism. *Molecular Endocrinology*, *13*(5), 681-691. doi:10.1210/me.13.5.681