Assessing Male Reproductive Toxicity during Drug Development

Mario Sousa1,2, Carolina Ferreira1, Ana Rabaca1 and Rosália Sa*1

1Department of Microscopy, Laboratory of Cell Biology, Multidisciplinary Unit for Biomedical Research (UMIB), Abel Salazar Institute for the Biomedical Sciences (ICBAS), University of Porto (UP), Porto, Portugal
2Centre for Reproductive Genetics Professor Alberto Barros (CGR-ABarros), Porto, Portugal

Abstract

Medical drug development is a crucial research field that attends to population healthcare needs, comprising several preclinical and clinical steps before the approval of a new compound. One major cause of pharmaceutical drug development failure is toxicity, especially in respect to kidney and liver. Nonetheless, the male reproductive system may also be a target for drug toxicity. Although the reproductive health is an important component of the person's life, reproductive toxicology testing is rarely performed, both in preclinical and clinical trial phases. As reproductive and testicular toxicity is found at a low incidence during the early stages of drug development, companies devote the majority of research investments to more frequent areas of toxic occurrence. It is here suggested the inclusion of comprehensive studies and more precise methods to uncover the toxic reproductive effects and causes when developing new pharmacological drugs.

Keywords: Testicular toxicity, Pharmaceutical development; Male infertility

Introduction

Male infertility accounts for about 50% of infertility cases [1]. Anatomic, genetic, endocrinological, environmental, behavioral and nutritional imbalances are critical causes of male infertility [2-5]. Therapeutic drugs can also adversely affect male fertility by injuring testicular cells or instigate hormonal changes that lead to decreased semen quality (Figure 1) thereby compromising the production of competent spermatozoa [6,7]. The male reproductive system is a complex and sensitive regulated process that can be disturbed following exposure to toxic compounds. Besides genetic defects, it is believed that most of male reproductive anomalies depend on exogenous toxic exposures, the so called Testicular Dysgenesis Syndrome [8].

Underlying mechanisms of toxicity depend on the stage of exposure, either in-utero, puberty or adulthood. Frequent presentations comprise cryptorchidism, hypospadias, and anomalies of the excretory channels and accessory glands. Later in life, the main disturbances are reflected in testicular tumors and altered spermatogenesis (Table 1).

Development of medicines is a long and expensive process, with about 30% of failures due to toxic events [9,10]. Toxicity screening aims to identify cell toxicity and the underlying causes, in order to establish the better dose range under which a medicine can be devoid of adverse side-effects, with studies mainly concentrated on kidney, liver and neuronal cells and tissues [9,11,12].

However, the male reproductive system may also be a target for pharmacological drug toxicity and their impact on the reproductive function becomes nowadays a crucial aspect of research especially in cancer patients because their survival has increased, being patients more and more free of the disease still on reproductive age [13,14].

Testicular toxicity testing represents a challenging issue during preclinical trial stages, due to the lack of simple and robust screening methods [15,16]. Histopathological, hormonal and semen parameters evaluations are the most commonly employed methods to assess testicular and pharmacological drug genotoxicity [17,18]. While animal histopathological procedures are an accepted method to evaluate genotoxicity, these are mainly descriptive, being unable to measure the toxicity degree and to discriminate between genotoxicity and nontoxic testicular changes (related to immaturity or to spontaneous conditions) [19,20]. Over the last years, alternative methods, such as evaluation of testicular cell proliferation [21], changes in gene and protein expression [22-24] or epigenetic regulation [25,26] have been developed.

The present study reviews the current methods and advances in the study of the effects and mechanisms involved in pharmaceutical drug toxic effects on male reproductive function.

Method

Drug development a step-by-step process

Development of medicines involves several procedures to attest its safety and efficacy in order to be approved and legalized as a new chemical entity to be population used [9].

The preclinical trial process begins within the laboratory, where a new compound is tested in-vitro and subsequently in-vivo, using at least two animal models [27]. Firstly, exploratory toxicology experiments are of dose-ranging nature, typically acute or of short-term. At this phase, which may exceed 4 years, research is dedicated to identify the major target organs and physiological systems affected by the drug, and screen for specific drug's toxicity and evaluation of pharmacological effects. During the same period genotoxicity is also investigated [9,28]. As a substantial number of compounds do not surpass the preclinical investigation stage due to the lack of evaluation tools that can accurately monitor toxicity, efforts have been made to improve the newly available organ-specific toxicity detection tools through the identification and characterization of toxicity biomarkers [28-31].

*Corresponding author: Rosália S, Department of Microscopy, Laboratory of Cell Biology, Multidisciplinary Unit for Biomedical Research (UMIB), Abel Salazar Institute for the Biomedical Sciences (ICBAS), University of Porto (UP), Porto, Portugal, Tel: +351935621131; E-mail: rmsa@icbas.up.pt

Received January 17, 2017; Accepted January 23, 2017; Published July 21, 2017

Citation: Sousa M, Ferreira C, Rabaca A, Sa R (2017) Assessing Male Reproductive Toxicity during Drug Development. Andrology (Los Angel) 6: 185. doi: 10.4172/2167-0250.1000185

Copyright: © 2017 Sousa M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
When the chemical passes the preclinical trial stage, the firm creates an Investigational New Drug Application with the Food and Drug Administration (FDA), presenting the pharmacological profile and the preclinical results of short-term toxicity [9,32]. If the application is approved, clinical trials can be started after a 30 day period.

Clinical trials are divided into three different phases:

Phase-I are studies conducted in a small number of healthy volunteers designed to determine the safe dose range and toxicity [33].

Phase-II begins when the drug reveals safe to be tested in a larger sample of volunteers who have the medical condition to whom the product is intended to treat [34].

Phase-III starts if the compound remains promising, and is tested in a larger sample of subjects with the disease of interest, now using distinct doses and schedules [35].

The principal aim of this final phase is to clinically demonstrate the safety and efficacy of the new product. With this large number of

Table 1: Male reproductive tract toxicity during man’s developmental stage.
Male reproductive function concerns during the drug development phase

The male reproductive system is highly sensitive to toxicant-induced damages and the available procedures for detecting genotoxicity are fairly limited [37]. Medicines may cause endocrinological evident body changes, libido loss, anejaculation, oligozoospermia and azoospermia, which pose a problem during pharmaceutical drug development [31]. The majority of testicular toxicants show an early cell-specific and spermatogenesis stage-specific pattern of damage, and both morphological and molecular evaluations of the testis, epididymis and sperm may give important information [38].

Apart evident body changes, the reproductive function is currently evaluated by measuring hormonal levels and semen parameters before and after treatment [18]. According to World Health Organization guidelines men with normal semen analysis and proven fertility should be included in the analysis under placebo treatment [39]. Regarding hormones, follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin, testosterone, inhibin B (Sertoli cell product), anti-Mullerian hormone (Sertoli cell product) and insulin-like peptide 3 (Leydig cell product) can be easily performed [40,41]. However, changes may be undetectable in cases of mild testicular injury [31].

Consequently, other tools are also used to predict testicular dysfunction, such as histopathology and molecular biomarkers of testis and sperm function. Histopathology studies can only be used in experimental animals [31]. Of the sperm biomarkers, the human sperm membrane protein SP22 was shown to decline after exposure to both epidydimal and testicular toxicants in rat and ram models, changes in human sperm gene expression or mRNA transcript content [24,42-44]. DNA methylation profile during human spermatogenesis were correlated with impair of sperm quality (such as concentration, morphology and motility); and another specific group of sperm mRNA transcripts was shown to predict low level exposures to Sertoli cell toxicants in the rat [45-47].

In the last 5 years, early testicular toxicity was reported in a few drug development programs. Other treatment programs employed patients with distinct life profiles, rendering the effect of the drug or other concomitant environmental factors impossible to discern as the causative testicular toxicity. Additionally, some treatment regimens contain a mixture of different drugs, which precludes individual drug effects [17,18]. These observations suggest that the reproductive welfare should be of major concern during drug development not only on the recognition of clinical sexual function but also on the nonclinical signs of testicular and sperm toxicity in humans, with a research effort in developing new biomarkers or a panel of biomarkers to assess earliest testicular damage.

Male reproductive function evaluation during pharmacological drug development

Human reproductive function is dependent on complex interactions between numerous cells and organs. In-vitro testing appears critical to evaluate pharmaceutical drug toxicity in addition to in-vivo hormonal assays and semen analysis. Even though cooperative efforts have been made to establish strict guidelines for reproductive toxicity assessment during drug development, standard protocols have not yet been established and, as a consequence, discrepancies between research centres and countries still exist [48]. Drug safety relies on studies for genetic, carcinogenic, reproductive and development toxicity [49]. In order to identify the best approach for genotoxicity assessment, researchers have conducted numerous validation studies to calculate the effectiveness of current screening tools. For instance, it was reported that a 2-week drug treatment was sufficient to analyse drug toxicity-induced damages to male rat reproductive organs [48]. In humans, since each spermatogenic cycle takes about 76 days, testicular toxicity analysis should be performed at the beginning and at each two months [50,51].

Several animal characteristics must be taken into account when selecting a species for toxicological studies in order to achieve the most similar toxic reactions to those of humans: should have a good reproductive capacity; number of animals required and associated costs; sexually mature, with the presence of semen checked prior to drug exposure; spermatogenesis of immature animals may be erroneously misinterpreted as impaired spermatogenesis; immature animals, rich in spermatogonia stem cells, could be excellent animal models for child medicines [52]; qualitative and quantitative measurements, such as hormonal levels [53], animal weight and overall health status, must be carefully examined; determine drug cell and tissue metabolism, and the pharmacological curve; define length of treatment and dose used; document late onset events such as chronic toxicity [54-56]. Despite the relevant importance for human diseases, experiments should also minimize animal suffering [57].

Nevertheless, the ultimate conclusions will be given from human observations [58]. Several techniques have been developed to study genotoxicity in-vitro, such as testicle organ slice evaluation and sperm suspensions [59-61]. These methods not only can provide more real determinations regarding drug toxic effects but can also aid to develop biomarkers to be latter used for toxicity monitoring [62]. Furthermore, researchers must acknowledge that in-vitro findings do not represent the real physiological conditions as lack tissue interactions [48].

Some of the most common sexual organ drug-induced lesions found on males exposed to therapeutic drugs include the epididymis, seminiferous tubules, testicular dysfunction, altered semen parameters and azoospermia [17]. Even though these issues are a main concern for patients who might possibly be treated with these drugs, most genotoxicity testing focus its attention on pregnancy outcomes and embryo development aspects. Thus, human in-vitro studies for reproductive toxicity are suggested to be fully introduced when developing new pharmacological drugs (Table 2) and before widespread exposure to patients.

Discussion and Conclusion

During drug toxicity assessment it is important to evaluate male’s hormonal profile and reproductive tract function in order to identify any alterations that might result in spermatogenesis and sperm defects. The current methods for evaluating genotoxicity and semen quality in-vitro in humans are well defined and of low invasiveness and thus should be implemented as common practice.
**Diagnostic tools** | **Possible lesions associated with drug toxicity**
---|---
**General physical andrological examination**
- Testicular size, volume and palpation  
  - Hiper-atrophy and atrophy  
  - Testicular tumor  
  - Orchitis  
- Epididymus and Vas deferens palpation  
  - Atrophy, obstruction or inflammation  
- Prostate retroperitoneal palpation  
  - Hiperplasia  
  - Carcinoma  
- Penis  
  - Size changes  
  - Erectile dysfunction  
- Secondary sexual characteristics  
  - Alterations of body proportion, fat distribution and musculature  
  - Voice and hair mutations  
  - Gynecomastia (indicative of endocrinologically active testicular tumor)

**Molecular and Cytogenetics**
- Karyotyping  
- Fluorescence in situ hybridization  
- Molecular genetic diagnostics  
  - Structural chromosome abnormalities (such as Y chromosome microdeletions)  
  - Genetic mutations

**Endocrine Laboratory Diagnosis**
- Gonadotropins [Follicular Stimulating Hormone (FSH) and Luteinizing hormone (LH)]  
  - ↑ levels + ↓ T levels  
  - Primary hypogonadism  
  - ↓ levels  
  - Secondary hypogonadism
- Testosterone (T)  
  - Alterations on reproductive performance  
  - Alterations on social behavior  
  - Alterations of the secondary sexual characteristics
- Estradiol (E2)  
  - Alterations on reproductive performance
- Prolactin  
  - Alterations on reproductive performance
- Inhibin B  
  - Sertoli cell dysfunction  
  - Impaired spermatogenesis
- Anti-Mullerian hormone (AMH)  
  - Alterations of Sertoli cell number, function and maturation

**Semen analysis**
- Macroscopic examination  
  - Volume  
  - pH  
  - Appearance  
  - Infection  
  - Obstruction of the efferent system  
  - Semen secretion production dysfunction
- Count  
  - Azoospermia and/or Aspermia
- Concentration  
  - Sertoli cell-only syndrome
- Motility  
  - Asthenozoospermia
- Morphology  
  - Maturation arrest
- Immunological tests  
  - Sperm agglutination  
  - Testicular inflammation (cytotoxicity)  
  - Sperm motility disorders
- Biochemical analysis  
  - Zinc, citric acid and prostatic acid phosphatase measurement  
  - Prostate dysfunction
  - Prostaglandins and fructose measurement  
  - Seminal vesicles dysfunction
  - Neutral α-glucosidase, L-carnitine and glycerophosphocholine measurement  
  - Epididymal dysfunction  
  - Distal obstruction of the efferent system
- Sperm function  
  - Vitality  
  - Metabolic Dysfunction
  - DNA integrity  
  - Sperm DNA fragmentation and / or immature chromatin
  - Reactive oxygen species  
  - Infection  
  - Impaired motility

**Histopathology**
- Target cell type  
  - Sertoli cell  
  - Leydig cell  
  - Germ cell  
  - Testis  
  - Epididymis  
  - Seminal vesicles  
  - Prostate  
- Fertility assessment by fertilization and / or pregnancy rates  
  - Animals  
  - Humans  

To evaluate the drug effect, the above studies should be conducted before and after (at least at the end of a spermatogenic cycle) exposure to determine the reversibility or permanent toxic effects.

**Table 2:** Monitoring of the potential male reproductive toxicity of a drug in development during pre-clinical (*in vitro* and *in vivo*) and clinical phases (*in vivo*).
The current lack of reliable biomarkers for evaluation of testicular toxicity, either in animal models or in in-vitro human studies, is an exciting new field challenge for the pharmaceutical industry. In the future, these will turn prediction more feasible, with decreased time and resources needed to evaluate the safety of a new chemical compound, and may provide a reliable and sensitive monitoring method in the therapeutic setting. Nevertheless, basic research still remains crucial for the determination of the metabolism, pharmacokinetics and mechanisms of action of the drugs.

Acknowledgments

UMIB (Pest-OE/SAU/UI0215/2014) was funded by National Funds through FCT-Foundation for Science and Technology.

Conflict of Interests

Authors disclose any conflict of interests.

References

1. World Health Organization (2017) WHO manual for the standardized investigation and diagnosis of the infertile couple.
2. Sharpe RM, Franks S (2002) Environment, lifestyle and infertility-an inter-generational issue. Nat Cell Biol 4: 33-40.
3. Pizzolo D, Ferlin A, Garolla A, Lenzi A, Bertoldo A , et al. (2014) Genetic and molecular diagnostics of male infertility in the clinical practice. Front Biosci (Landmark Ed) 19: 291-303.
4. Ferlin A, Foresta C (2014) New genetic markers for male infertility. Curr Opin Obstet Gynecol 26: 193-198.
5. Fullston T (2015) Paternal obesity induces metabolic and sperm disturbances in male offspring that are exacerbated by their exposure to an "obesogenic" diet. Physiol Reprod 3: e12338.
6. Khaki A (2015) Assessment on the adverse effects of Aminoglycosides and Fluoroquinolone on sperm parameters and male reproductive tissue: A systematic review. Iran J Reprod Med 13: 125-134.
7. Pompe SV, Strobach D, G. Stief C, J. Becker A, Trottmann M (2016) Drug use and investigation of toxic metabolites during drug development. Toxicol Appl Pharmacol 207:425-434.
8. Shakkebaek NE, Rajpert-De Meyts E, Main KM (2001) Testicular dysgenesis syndrome: An increasingly common developmental disorder with environmental aspects. Hum Reprod 16: 972-978.
9. Kelly J (2009) Principles of CNS Drug Development: From Test Tube to Patient, UK.
10. Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 3: 711-715.
11. Nolte T, Harleman JH, Jahn W (1995) Histopathology of chemically induced testicular atrophy in rats. Exp Toxicol Pathol 47: 267-286.
12. Park K, Williams DP, Naisbitt DJ, Kitteringham NR, Pirmohamed M (2005) Effect of tamoxifen treatment on global and insulin-like growth factor 2-H19 locus-specific DNA methylation in rat spermatozoa and its association with embryo loss. Fertil Steril 91: 2253-2263.
13. Stokes WS (2004) Selecting appropriate animal models and experimental designs for endocrine disruptor research and testing studies. ILAR J 45: 387-393.
14. Adams CP, Brantner VV (2010) Spending on new drug development.1. Health Econ 19: 130-141.
15. Lipsky M, Sharp L (2001) From idea to market: The drug approval process. J Am Board Fam Pract 14: 362-367.
16. Currie RA (2012) Toxigenomics: The challenges and opportunities to identify biomarkers, signatures and thresholds to support mode-of-action. Mutat Res 746: 97-103.
17. Campion S (2013) The current status of biomarkers for predicting toxicity. Expert Opin Drug Metab Toxicol 9: 1391-1408.
18. Boekelheide K (2011) Commentary on incidence and nature of testicular toxicity findings." Birth Defects Res B Dev Reprod Toxicol 92: 501-503.
19. Creasy DM (2011) Commentary on incidence and nature of testicular toxicity findings in pharmaceutical development survey: A pathologist's perspective. Birth Defects Res B Dev Reprod Toxicol 92: 508-510.
20. Cooper TG (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16: 231-245.
21. Stewart J, Turner KJ (2005) Inhibin B as a potential biomarker of testicular toxicity. Cancer Biomark 1: 75-91.
22. Dere E (2013) SOT symposium highlight: Translatable indicators of testicular toxicity: Inhibin B, microRNAs, and sperm signatures. Toxicol Sci 136: 265-471.
23. Klinefelter GR (2008) Spermatozoal RNA profiles of normal fertile men. Lancet 360: 772-777.
24. Krawetz SA (2005) Paternal contribution: New insights and future challenges. Nat Rev Genet 6: 663-642.
25. Wang H, Zhou Z, Xu M, Li J, Xiao J, et al. (2004) A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. J Mol Med 82: 317-324.
26. Houshdaran S, Cortessis VK, Siegmund K, Yang A, Laird PW, et al. (2007) Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. Plos One 2: e1289.
27. Parkah S, Kedia-Mokashi N, Saxena M, D’Souza R, Maltra A, et al. (2009) Effect of tamoxifen treatment on global and insulin-like growth factor 2-H19 locus-specific DNA methylation in rat spermatozoa and its association with embryo loss. Fertil Steril 91: 2253-2263.
28. Adams CP, Brantner VV (2010) Spending on new drug development.1. Health Econ 19: 130-141.
29. Lipsky M, Sharp L (2001) From idea to market: The drug approval process. J Am Board Fam Pract 14: 362-367.
30. Currie RA (2012) Toxigenomics: The challenges and opportunities to identify biomarkers, signatures and thresholds to support mode-of-action. Mutat Res 746: 97-103.
31. Campion S (2013) The current status of biomarkers for predicting toxicity. Expert Opin Drug Metab Toxicol 9: 1391-1408.
32. Landis SC (2012) A call for transparent reporting to optimize the predictive value of preclinical research. Nature 490: 187-191.
33. Shamoo AE (2006) The myth of equipoise in phase 1 clinical trials. Medscape J Med 10: 254.
34. Simon R (1989) Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10: 1-10.
35. DeMets D (2010) Fundamentals of Clinical Trials (4th edn) Springer 1: 4419-1585.
36. Dickson M, Gagnon JP (2004) Key factors in the rising cost of new drug discovery and development. Nat Rev Drug Discov 3: 417-429.
37. Boekelheide K (2011) Commentary on incidence and nature of testicular toxicity findings." Birth Defects Res B Dev Reprod Toxicol 92: 501-503.
38. Creasy DM (2011) Commentary on incidence and nature of testicular toxicity findings in pharmaceutical development survey: A pathologist’s perspective. Birth Defects Res B Dev Reprod Toxicol 92: 508-510.
39. Cooper TG (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16: 231-245.
40. Stewart J, Turner KJ (2005) Inhibin B as a potential biomarker of testicular toxicity. Cancer Biomark 1: 75-91.
41. Dere E (2013) SOT symposium highlight: Translatable indicators of testicular toxicity: Inhibin B, microRNAs, and sperm signatures. Toxicol Sci 136: 265-471.
42. Klinefelter GR (2008) Spermatozoal RNA profiles of normal fertile men. Lancet 360: 772-777.
43. Krawetz SA (2005) Paternal contribution: New insights and future challenges. Nat Rev Genet 6: 663-642.
44. Wang H, Zhou Z, Xu M, Li J, Xiao J, et al. (2004) A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. J Mol Med 82: 317-324.
45. Houshdaran S, Cortessis VK, Siegmund K, Yang A, Laird PW, et al. (2007) Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. Plos One 2: e1289.
46. Parkah S, Kedia-Mokashi N, Saxena M, D’Souza R, Maltra A, et al. (2009) Effect of tamoxifen treatment on global and insulin-like growth factor 2-H19 locus-specific DNA methylation in rat spermatozoa and its association with embryo loss. Fertil Steril 91: 2253-2263.
47. Stokes WS (2004) Selecting appropriate animal models and experimental designs for endocrine disruptor research and testing studies. ILAR J 45: 387-393.
Identification of the SP22 sperm protein in Santa Ines and Dorper rams. Reprod Domest Anim 45: 323-330.

44. Steger K, Wilhelm J, Konrad L, Staf T, Greb R, et al. (2008) Both protamine-1 to protamine-2 mRNA ratio and Bcl2 mRNA content in testicular spermatids and ejaculated spermatozoa discriminate between fertile and infertile men. Hum Reprod 23:11-16.

45. Navarro-Costa P, Nogueira P, Carvalho M, Leal F, Cordeiro I (2010) Incorrect DNA methylation of the DAZL promoter CpG island associates with defective human sperm. Hum Reprod 25:2647-2654.

46. Sato A (2011) Assessing loss of imprint methylation in sperm from subfertile men using novel methylation polymerase chain reaction Luminescence analysis. Fertil Steril 95: 129-134.

47. Pacheco SE, Anderson LM, Sandrof MA, Vantangoli MM, Hall SJ, et al. (2012) Sperm mRNA transcripts are indicators of sub-chronic low dose testicular injury in the Fischer 344 rat. PLoS One 7: e44280.

48. Sakai T, Takahashi M, Mitsumori K, Yasuhara K, Kawashima K, et al. (2009) Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats—overview of the studies. J Toxicol Sci 25 Spec No: 1-21.

49. Li AP (2004) A comprehensive approach for drug safety assessment. Chem Biol Interact 150: 27-33.

50. Heller CG, Clermont Y (1964) Kinetics of the germinal epithelium in man. Recent Prog Horm Res 20:545-575.

51. Heller CG, Heller GV, Rowley MJ (1969) Human spermatogenesis: an estimate of the duration of each cell association and of each cell type. Excerpta Medica Inter Cong Ser 184:1012-1018.

52. Working PK (1988) Male reproductive toxicology: comparison of the human to animal models. Environ Health Perspect 77:37-44.

53. Narayan P (2015) Genetic models for the study of luteinizing hormone receptor function. Front Endocrinol 6:152.

54. Martin PL, Breslin W, Rocca M, Wright D, Cavagnaro J (2009) Considerations in assessing the developmental and reproductive toxicity potential of biopharmaceuticals. Birth Defects Res B Dev Reprod Toxicol 86:176-203.

55. Dannat K, Tillner J, Winckler T, Weiss M, Eger K, et al. (2003) Effects of diethylstilbestrol (DES) on the rete testis and efferent ductules by ectopic Sertoli and Leydig cells causes dysgenesis and cryptorchidism. Endocrinology 148:5507-5519.

56. Jef至于 Ernstoff L, Troisi R, Hatch EE, Palmer JR, Hyer M, et al. (2010) Birth defects in the sons and daughters of women who were exposed in utero to diethylstilbestrol (DES). Int J Androl 33:377-384.

57. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD et al. (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ Sci Techno 136:1202-1211.

58. Jobling S, Williams R, Johnson A, Taylor A, Sorokin GM, et al. (2006) Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. Environ Health Perspect 114:32-39.

59. Cederoth CR, Schaad Q, Descombes P, Chambon P, Vassalli JD, et al. (2007) Estrogen receptor alpha is a major contributor to estrogen-mediated fetal testis dysgenesis and cryptorchidism. Endocrinology 148:5507-5519.

60. Guyot R, Odet F, Ledique P, Forest MG, Battistoni BLM (2004) Diethylstilbestrol inhibits the expression of the steroidogenic acute regulatory protein in mouse fetal testis. Mol Cell Endocrinol 220:87-95.

61. Haavisto T (2001) Prenatal testosterone and luteinizing hormone levels in male rats exposed during pregnancy to 2,3,7,8- tetrachlorodibenzo-p-dioxin and diethylstilbestrol. Mol Cell Endocrinol 175:169-179.

62. Hoei-Hansen CE, Melte Holm M, De Meyts RE, Skakkebaek NE (2003) Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. J Pathol 200:370-374.

63. Jeffs B, Meeks JJ, Ito M, Martinson FA, Matzuk MM, et al. (2001) Blockage of the rete testis and efferent ducts by ectopic Sertoli and Leydig cells causes infertility in Dax1- deficient male mice. Endocrinology 142:4485-4495.