Doi: 10.1111/j.1742-7843.2007.00197.x
Journal compilation © 2008 Nordic Pharmacological Society. *Basic & Clinical Pharmacology & Toxicology*, **102**, 155–161

MiniReview

Testicular Dysgenesis Syndrome and Leydig Cell Function

Ulla Nordström Joensen, Niels Jørgensen, Ewa Rajpert-De Meyts and Niels Erik Skakkebæk

University Department of Growth and Reproduction, Rigshospitalet, Blegdamsvej, Copenhagen, Denmark

(Received July 3, 2007; Accepted October 20, 2007)

Abstract: Fertility among human beings appear to be on the decline in many Western countries, and part of the explanation may be decreasing male fecundity. A hypothesis has been put forward that decreasing semen quality may be associated with a testicular dysgenesis syndrome (TDS), a spectrum of disorders originating in early foetal life. TDS comprises various aspects of impaired gonadal development and function, including testicular cancer. A growing body of evidence, including animal models and research in human beings, points to lifestyle factors and endocrine disrupters as risk factors for TDS. We present our view of the emerging role of Leydig cell dysfunction with subsequent decreased testosterone levels in the pathogenesis of TDS.

Human fertility rates are on the decline in countries all over the world. In many Western countries, the fertility rate is well below the replacement level of 2.1 children per woman [1]. Most often this is attributed to socio-economic factors and increasing control of fertility, for example, increased use of contraceptives or the choice of career rather than a large family. However, decreasing fecundity (the ability to conceive) is emerging as a possible contributing factor to the declining fertility rates. In Denmark, the fertility rate in teenagers is on the decline with no apparent simultaneous increase in contraceptive use or abortion rates [2]. The use of assisted reproduction is increasing, and in Denmark more than 6% of children are now born as a result of infertility treatments [3]. It has also been shown that a large part of the young male Danish population has low sperm counts. More than 10% may have sperm counts in the infertile range, and up to 30% are in the subfertile range [4].

We do not yet know the long-term health effects of increased use of assisted reproduction on the generations to come. One smaller study of reproductive health among young Danish men conceived after fertility treatment suggested that these men had considerably lower sperm concentrations, lower total sperm counts, fewer morphologically normal spermatozoa and fewer motile sperm than their naturally conceived controls [5]. In addition, there was a tendency towards smaller testis size, lower concentrations of serum testosterone, and lower free androgen index. A tendency towards lower testosterone levels already in infancy was also noted in boys conceived by means of assisted reproductive techniques [6]. The cause of these findings is not known, and larger studies are needed to confirm the results.

Male infertility is closely linked to other aspects of impaired gonadal development and function. The term *testicular dysgenesis syndrome* (TDS) was first coined in 2001 [7]. It was hypothesized that many cases of abnormal spermatogenesis, cryptorchidism (undescended testes), penile malformations (e.g. hypospadias, a congenital malformation with abnormal placement of the external urethral orifice) and testicular cancer may have a common aetiology, and that all these clinical problems may result from an irreversible developmental disorder originating in early foetal life (fig. 1). Patients with TDS-related symptoms present within a range of severity: at the mild end with slight impairment of spermatogenesis and at the severe end of the range with all of the above-mentioned symptoms. The risk of testicular cancer increases with the severity of symptoms, and an association between decreased male fertility and testicular cancer, as well as its pre-invasive precursor, carcinoma *in situ* (CIS), is now well documented [8–11]. We do not yet know the prevalence of TDS symptoms worldwide, but recent estimates indicate that at least 5–10% of the Danish male population may suffer from undescended testes, hypospadias or testicular cancer [12,13]. In addition, some mildly affected men may be free of clinical symptoms, as even severely decreased spermatogenesis is sometimes compatible with fertility. Taking into account the high frequency of low sperm counts, TDS-related symptoms seem to be quite common in Denmark. It should be pointed out, however, that not all cases of male infertility are related to TDS; for example, obstructive azoospermia, varicocele, occupational exposures to toxic compounds, or genetic disorders are causes unrelated to TDS.

The frequency of patients with TDS symptoms is rapidly increasing. We know from the Danish testicular cancer registry

Author for correspondence: Ulla Nordström Joensen, University Department of Growth and Reproduction, Section GR-5064, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (fax +45 3545 6054, e-mail ulla.nordstroem.joensen@rh.regionh.dk).

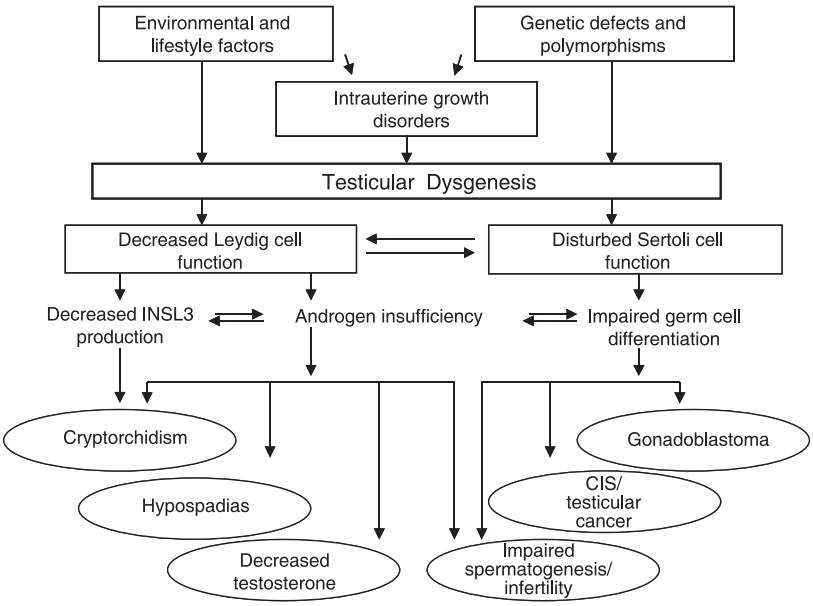

Fig. 1. The aetiology and symptoms of testicular dysgenesis syndrome (TDS). Schematic diagram illustrating the general pathways in which the disorders that comprise TDS may arise, including genetic, environmental and lifestyle factors and intrauterine growth disorders, which may act separately or in concert. Disorders shown in the lower part of the figure vary greatly in frequency, from common (e.g. impaired spermatogenesis), to rare (e.g. CIS, carcinoma *in situ*) and testicular germ cell cancer. INSL3 (insulin-like factor-3) is produced in Leydig cells and may be involved in antenatal descent of the testes. Adapted from Skakkebæk et al. [14]. With permission.

that the rate of testicular cancer is now several times higher than 60 years ago. It has been proposed that increasing rates of this disease may be seen as a 'whistle blower' of fertility problems in the population, as the cancer registers are usually of better quality than registers of congenital abnormalities. Health authorities should thus regard increasing testicular cancer incidence as a forewarning of deteriorating reproductive health in the male population [14].

Such trends of rapidly rising cancer incidence occurring over a few generations are more likely due to environmental and lifestyle influences than to genetic changes. Exposure to endocrine-disrupting compounds has been suggested to play a role in the increase of TDS-like symptoms. A growing body of evidence, including animal models [15] and research in human beings, points to commonly used chemicals ubiquitous in our environment, such as phthalates and persistent pesticides. We know from studies of phthalates and their metabolites in serum and urine that human beings are exposed to considerable amounts of these chemicals [16], which are found in a wide variety of consumer products for daily use [17]. Our point of view is that environmental factors associated with our lifestyle, including probably endocrine disrupters, are the most important determinants of TDS.

Histological aspects of TDS

Structural changes can be seen in the testicular tissue of men with different TDS symptoms (fig. 2). This applies to both the spermatogenic tubules and the androgen-producing cells of the testicle, the Leydig cells. Microscopically, changes may consist of immature tubules containing undifferentiated Sertoli cells, impaired spermatogenesis or complete absence of spermatogenic cells (Sertoli-cell-only pattern) or CIS [18]. There may be signs of abnormal development and dysfunction of the Leydig cells, which will be discussed in detail below.

Another histological feature seen in association with testicular dysgenesis is the presence of microcalcifications in the testicular tissue, termed microliths. Microscopically, microliths have been strongly associated with CIS [18,19]. Ultrasonic examination of testes sometimes reveals a pattern of small hyperechogenic points, termed testicular microlithiasis. This finding does not necessarily correspond to the testicular microliths found microscopically, but is associated with an increased risk of CIS and development of germ cell tumour if found either in the contralateral testis of a patient with unilateral testicular germ cell cancer [20] or in bilateral biopsies of an infertile man [21]. A normal ultrasound pattern, however, does not exclude CIS, and we do not yet know if ultrasonographic microlithiasis found in men from the general population implies an increased risk of CIS [22].

Leydig cell clusters (micronodules) are more frequently found in the testes of infertile men and men with contralateral testicular cancers, than in control samples from necropsy specimens of young men with no visible signs of disease [18,23]. The frequently used term of 'Leydig cell hyperplasia' may be incorrect, because even if the proportion of Leydig cells may appear increased in histological samples from dysgenetic testes, there is often no significant increase in total Leydig cell volume when testis size is taken into account [23].

Histological findings of Leydig cell micronodules have been correlated with abnormal serum concentrations of sex

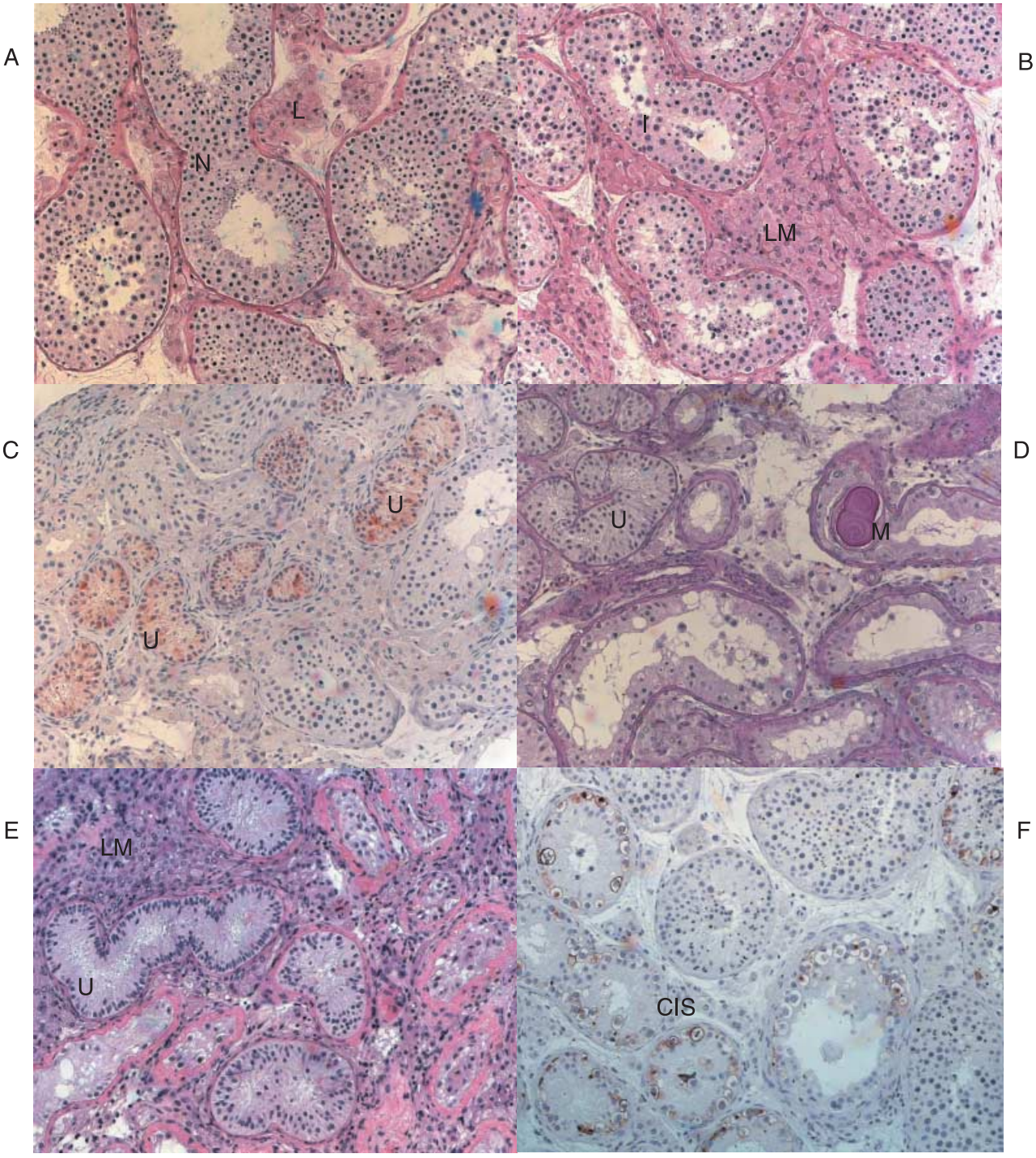

Fig. 2. Micrographs of testicular biopsies from adult patients from an infertility clinic. (A) Normal spermatogenesis in tubules (N). Slight Leydig cell hyperplasia (L). (B) Slightly impaired spermatogenesis (I). Large Leydig cell micronodules (LM). (C) Severe dysgenesis. Undifferentiated tubules (U) with undifferentiated Sertoli cells, stained positive for anti-Müllerian hormone, a hormone produced by immature Sertoli cells and normally not seen after puberty and the onset of meiosis. (D) Undifferentiated tubules (U) and microliths (M) in the same patient as in image (C). (E) Undifferentiated tubules (U) and Leydig cell micronodule (LM). (F) CIS (carcinoma *in situ*) cells visible by PLAP staining.

hormones. They are found more frequently in the testes of patients with elevated follicle-stimulating hormone and luteinizing hormone and are rare in testes with apparently normal spermatogenesis and normal gonadotrophins (follicle-stimulating hormone and luteinizing hormone). In a systematic study of testicular biopsies of men with oligozoospermia, the number of Leydig cell micronodules was increased in testes with histological signs of impaired spermatogenesis. Micronodules were also associated with hyperstimulated testes with increased luteinizing hormone/

testosterone ratios, compared to necropsy specimens as controls with normal spermatogenesis [23].

The reason for the formation of Leydig cell micronodules in the hypogonadal men is unknown, but it is speculated that it may be caused by primary Leydig cell failure as a result of testicular dysgenesis. This could, in turn, lead to rising luteinizing hormone levels to compensate for the decreased testosterone levels, causing hyperstimulation of the Leydig cells and formation of Leydig cell clusters that are dysfunctional in spite of their large size.

Serum testosterone levels

The pathogenic role of Leydig cell dysfunction in patients with TDS-associated symptoms has been poorly investigated. Until recently, the genital malformations, testicular cancer and impaired semen quality have been the main concern in reports dealing with TDS. It is known that a patient diagnosed with unilateral CIS, a precursor of testicular cancer, often has impaired spermatogenesis in the contralateral testis. These patients also have higher levels of luteinizing hormone and follicle-stimulating hormone, as well as a tendency towards lower levels of testosterone [24]. This could indicate that Leydig cells may be dysfunctional in TDS, or else a defect in Sertoli cells, presence of CIS cells or a relatively lower number of haploid germ cells may theoretically have a negative effect on testosterone production in the nearby Leydig cells.

A large study comparing reproductive hormone levels in infertile men (with severe abnormalities of sperm production) to those of fertile controls revealed signs of impaired Leydig cell function in the infertile men. There was a shift towards lower testosterone levels, as well as lower testosterone/luteinizing hormone ratios and higher oestradiol/testosterone ratios. These results clearly suggested impaired Leydig cell function, although partially compensated by increased luteinizing hormone levels [25]. It cannot yet be pinpointed whether the Leydig cell dysfunction originated during development; however, there is some indirect evidence to suggest that this may be the case. Not only adults with TDS-associated conditions, such as testicular cancer and infertility, seem to have signs of decreased Leydig cell function. Some boys with hypospadias have Leydig cell dysfunction [26], and a subtle impairment of Leydig cell function was recently noted in newborn boys with cryptorchidism [27].

Serum testosterone levels decrease with age in the individual male [28], but low serum testosterone levels are also associated with various health conditions in younger men, including abdominal obesity, diabetes and dyslipidaemia. US and Danish observational studies [29,30] of population-level serum testosterone and sex hormone-binding globulin (SHBG) concentrations suggested an age-independent decrease in testosterone and SHBG levels over the last 20 years that cannot entirely be explained by health and lifestyle factors. The reason behind the fact that testosterone levels appear to be decreasing more than could be expected from ageing is unknown, but it may indicate an environmental factor.

Possible role of environmental factors

The clinical symptoms of TDS are thought to originate in early foetal life and share risk factors. Formation of the testis and its early development are both hormone-independent, but later testosterone insufficiency may contribute to dysgenetic tubule formation. Various animal models, as well as patients with complete androgen insensitivity syndrome, provide some evidence that androgens are important for early proliferation of Sertoli cells. There is no doubt that the most severe forms of TDS observed in patients with disorders of sex differentiation have a genetic cause (e.g. mosaicism for sex chromosome aneuploidy). What causes the bulk of moderate and mild forms of TDS is yet unknown, but it is our belief that environmental causes must play a significant role in the rapidly increasing frequency of these disorders.

Several environmental exposures have been proposed to play a role as endocrine disrupters. Perhaps the best documented is the group of anti-androgenic compounds, in particular phthalates, for which we now have an animal model and proposed mechanisms of action (see below). There are also several epidemiological studies suggesting an association between early phthalate exposure and male reproductive health symptoms, including maternal phthalate exposure during pregnancy and decreased ano-genital length in male infants [31], and phthalates in breast milk and changes in the hypothalamic–pituitary–gonadal axis in male offspring [32].

Apart from the endocrine disrupting compounds in our environment already under suspicion, other factors of modern lifestyle may play a role in the increase of male fertility problems. Maternal smoking during pregnancy has been shown to adversely affect the sons' testicular function, including spermatogenesis [33,34]. There is also a study suggesting that increased body mass index in young men is associated with lower levels of testosterone, SHBG and inhibin B in serum, as well as reduced sperm output, compared to men within the normal range of body mass index (20–25) [35].

In addition to endocrine-disrupting chemicals, some chemical substances may influence male fertility by exposure in adulthood. Lead and cadmium, for instance, accumulate in the male reproductive organs, and some studies have associated reduced sperm quality with a high exposure to these substances [36]. Another example of late exposure with deleterious effects on male fertility is the pesticide 1,2-dibromo-3-chloropropane, which permanently impaired spermatogenesis in male workers exposed to this chemical [37]. The occupational damage to testis function evidently is not related to the gonadal development, and are thus not considered as a part of TDS.

Leads from animal models

In order to provide information on the possible mechanisms of environmental endocrine disrupters that may induce TDS we need animal models, as intervention studies evidently cannot be performed on human beings. In existing animal models, effects of endocrine disrupters have proved similar to most aspects of TDS in human beings. This is with the

exception of testicular cancer derived from CIS, for which there is no existing animal model, unless it is confirmed that the observed CIS-like changes in rabbits are true tumour precursors [38].

Different models have been proposed as to how exogenous endocrine disruption could lead to the male reproductive disorders associated with TDS [39–41]. These mechanisms include increased exposure to oestrogens, decreased androgen production by suppression of Leydig cell function (directly or as a secondary feature of Sertoli cell dysfunction), suppression of the expression of androgen receptors or distortion of the androgen–oestrogen balance that may itself be important for the normal development of the reproductive tract. Originally, a hypothesis was put forward in 1993 [42] that increasing incidence of reproductive abnormalities in the human male may be related to oestrogen exposure *in utero*, as similar abnormalities occurred in sons of women exposed to diethylstilobestrol during pregnancy and were inducible in animals by exposure to diethylstilboestrol during pregnancy. The proposed mechanisms were suppression of gonadotrophin secretion via enhanced negative feedback by oestrogens, or impairment of Leydig cell development, leading to inadequate testosterone production. More direct anti-androgenic mechanisms have since emerged, namely suppression of androgen production, suppression of androgen receptor expression or suppression of secretion of insulin-like factor-3 by foetal Leydig cells [43].

An anti-androgenic action of phthalates was proposed in 1998 [44], when rodent studies suggested specific impairment of the androgen-dependent development of the male reproductive tract. It emerged that the effect of phthalates differed from flutamide, an androgen receptor antagonist, in that the active metabolite of di(n-butyl)phthalate (DBP) does not interact with the androgen receptor *in vitro*, and different patterns of anti-androgenic effect were observed for the two substances [45,46]. There is a substantial number of studies of *in utero* exposure of rats to phthalates such as DBP with a consequential range of dysgenetic features in the male offspring that has many similarities with TDS in human beings. This spectrum of disorders in rats has been termed the 'phthalate syndrome', first coined in 2003 [47], and consists of dysgenetic seminiferous tissue, multinucleated gonocytes, as well as reduced androgen production and abnormal Leydig cell aggregation seen in rats when exposed to phthalates in foetal life. These Leydig cell abnormalities are proliferative lesions of developmental origin, as they are seen antenatally in phthalate-exposed male rats. Lesions resembling human Leydig cell adenomas were seen in male rats exposed before birth to DBP, but were dissimilar to traditional Leydig cell adenomas as the Leydig cells were poorly differentiated and the lesions contained aberrant seminiferous tubules [48]. Interestingly, aside from the effects associated with the 'phthalate syndrome', no other adverse effects of DBP exposure were observed in the pregnant rats or their offspring [49].

Further studies were able to link the reproductive malformations with impaired foetal testosterone production, suggesting a causal contribution of decreased hormone production in disruption of sexual differentiation during a critical stage of reproductive tract development [45,50,51]. The mechanism by which phthalates exert their effects on the foetal testis has been further elucidated by a recent study where pregnant rats were exposed to DBP, DMBA [7,12-dimethyl-benz(α)anthracene, a PAH (polycyclic aromatic hydrocarbon)], flutamide and testosterone in various combinations. This study was designed to shed some light on whether decreased testosterone/hormone levels contribute causally to the rest of the phthalate syndrome. The results were a clear association between DBP treatment and major reductions in intratesticular testosterone, as well as a reduction in Sertoli cell number [52]. Although animal data cannot be directly extrapolated to human beings, studies of this kind will undoubtedly contribute to the identification of target pathways and the mechanisms involved in the pathogenesis of human TDS.

Conclusions

In summary, more and more evidence is emerging that Leydig cell dysfunction may be a central part of the testicular dysgenesis syndrome, and that androgen insufficiency may be important either causally or as a consequence of developmental disruption of both the androgen producing and spermatogenic compartment of the human testicle. We do not yet know what causes TDS, but there is increasing suspicion that endocrine-disrupting compounds in our environment may be a central risk factor. Animal models of the TDS-like phthalate syndrome are contributing to our understanding of the mechanisms involved. However, we must keep in mind that human beings are exposed to multiple categories of environmental agents that may act in concert to produce the endocrine disrupting effect thought to be contributing to the rise in TDS and consequently declining fertility and increasing rates of testicular cancer.

Acknowledgements

The authors greatly appreciate the support of Kirsten and Freddy Johansen's foundation.

References

1 Lutz W, O'Neill BC, Scherbov S. Demographics. Europe's population at a turning point. Science 2003;**299**:1991–2.
2 Jensen TK, Carlsen E, Jørgensen N et al. Poor semen quality may contribute to recent decline in fertility rates. Hum Reprod 2002;**17**:1437–40.
3 Andersen AN, Erb K. Register data on assisted reproductive technology (ART) in Europe. Including a detailed description of ART in Denmark. Int J Androl 2006;**29**:12–6.
4 Jørgensen N, Asklund C, Carlsen E, Skakkebæk NE. Coordinated European investigations of semen quality: results from studies of Scandinavian young men is a matter of concern. Int J Androl 2006;**29**:54–61.
5 Jensen TK, Jørgensen N, Asklund C, Carlsen E, Holm M, Skakkebæk NE. Fertility treatment and reproductive health of male offspring: a study of 1925 young men from the general population. Am J Epidemiol 2007;**165**:583–90.

6 Mau KC, Main KM, Nyboe AA, Loft A, Skakkebæk NE, Juul A. Reduced serum testosterone levels in infant boys conceived by Intra-Cytoplasmic Sperm Injection (ICSI). J Clin Endocrinol Metab 2007;**92**:2598–603.

7 Skakkebæk NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001;**16**:972–8.

8 Berthelsen JG. Testicular cancer and fertility. Int J Androl 1987;**10**:371–80.

9 Møller H, Skakkebæk NE. Risk of testicular cancer in subfertile men: case-control study. Br Med J 1999;**318**:559–62.

10 Baker JA, Buck GM, Vena JE, Moysich KB. Fertility patterns prior to testicular cancer diagnosis. Cancer Causes Control 2005;**16**:295–9.

11 Mancini M, Carmignani L, Gazzano G et al. High prevalence of testicular cancer in azoospermic men without spermatogenesis. Hum Reprod 2007;**22**:1042–6.

12 Boisen KA, Kaleva M, Main KM et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 2004;**363**:1264–9.

13 Boisen K, Chellakooty M, Schmidt I et al. Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at 3 months of age. J Clin Endocrinol Metab 2005;**90**:4041–6.

14 Skakkebæk NE, Rajpert-De Meyts E, Jørgensen N et al. Testicular cancer trends as 'whistle blowers' of testicular developmental problems in populations. Int J Androl 2007;**30**:198–205.

15 Sharpe RM. Pathways of endocrine disruption during male sexual differentiation and masculinization. Best Pract Res Clin Endocrinol Metab 2006;**20**:91–110.

16 Blount BC, Silva MJ, Caudill SP et al. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 2000;**108**:979–82.

17 Frederiksen H, Skakkebæk NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res 2007;**51**:899–911.

18 Høi-Hansen CE, Holm M, Rajpert-De Meyts E, Skakkebæk NE. Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. J Pathol 2003;**200**:370–4.

19 Kang JL, Rajpert-De Meyts E, Giwercman A, Skakkebæk NE. The association of testicular carcinoma in situ with intratubular microcalcifications. J Urol Pathol 1994;**2**:235–42.

20 Holm M, Høi-Hansen CE, Rajpert-De Meyts E, Skakkebæk NE. Increased risk of carcinoma in situ in patients with testicular germ cell cancer with ultrasonic microlithiasis in the contralateral testicle. J Urol 2003;**170**:1163–7.

21 De Gouveria Brazao CA, Pierik FH, Oosterhuis JW, Dohle GR, Looijenga LHJ, Weber RFA. Bilateral testicular microlithiasis predicts the presence of the precursor of testicular germ cell tumors in subfertile men. J Urol 2004;**171**:158–60.

22 Holm M, Lenz S, Rajpert-De Meyts E, Skakkebæk NE. Microcalcifications and carcinoma in situ of the testis. BJU Int 2001;**87**:144–9.

23 Holm M, Rajpert-De Meyts E, Andersson A-M, Skakkebæk NE. Leydig cell micronodules are a common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreases testosterone/LH ratio. J Pathol 2003;**199**:378–86.

24 Petersen PM, Giwercman A, Hansen SW et al. Impaired testicular function in patients with carcinoma in situ of the testis. J Clin Oncol 1999;**17**:173–9.

25 Andersson A-M, Jørgensen N, Larsen LF, Rajpert-De Meyts E, Skakkebæk NE. Impaired leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. J Clin Endocrinol Metab 2004;**89**:3161–7.

26 Rey RA, Codner E, Iniguez G et al. Low risk of impaired testicular Sertoli and Leydig cell functions in boys with isolated hypospadias. J Clin Endocrinol Metab 2005;**90**:6035–40.

27 Suomi A-M, Main KM, Kaleva M et al. Hormonal changes in 3-month-old cryptorchid boys. J Clin Endocrinol Metab 2006;**91**:953–8.

28 Travison TG, Araujo AB, Kupelian V, O'donnell AB, McKinlay JB. The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. J Clin Endocrinol Metab 2007;**92**:549–55.

29 Travison TG, Araujo AB, O'donnell AB, Kupelian V, McKinlay JB. A population-level decline in serum testosterone levels in American men. J Clin Endocrinol Metab 2007;**92**:196–202.

30 Andersson AM, Jensen TK, Juul A, Petersen JH, Jørgensen T, Skakkebæk NE. Secular decline in male testosterone and sex hormone binding globulin serum levels: a study of more than 5000 men participating in Danish population surveys. J Clin Endocrinol Metab 2007;**92**:4696–4705.

31 Swan SH, Main KM, Liu F et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005;**113**:1056–1061.

32 Main KM, Mortensen GK, Kaleva M et al. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in three months old infants. Environ Health Perspect 2006;**114**:270–6.

33 Jensen TK, Jørgensen N, Punab M et al. Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1770 young men from the general population in five European countries. Am J Epidemiol 2004;**159**:49–58.

34 Storgaard L, Bonde JP, Ernst E et al. Does smoking during pregnancy affect sons' sperm counts? Epidemiology 2003;**14**:278–86.

35 Jensen TK, Andersson A-M, Jørgensen N et al. Body mass index in relation to semen quality and reproductive hormones among 1558 Danish men. Fertil Steril 2004;**82**:863–70.

36 Abadin HG, Hibbs BF, Pohl HR. Breast-feeding exposure of infants to cadmium, lead, and mercury: a public health viewpoint. Toxicol Ind Health 1997;**13**:495–517.

37 Whorton MD, Meyer CR. Sperm count results from 861 American chemical/agricultural workers from 14 separate studies. Fertil Steril 1984;**42**:82–86.

38 Veeramachaneni DN, Palmer JS, Amann RP, Pau KY. 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. Reprod Toxicol 2007;**23**:353–65.

39 Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 2000;**58**:350–65.

40 Foster PM, Mylchreest E, Gaido KW, Sar M. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 2001;**7**:231–5.

41 Sharpe RM, Atanassova N, McKinnell C et al. Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development. Biol Reprod 1998;**59**:1084–94.

42 Sharpe RM, Skakkebæk NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 1993;**341**:1392–5.

43 Sharpe RM. The 'oestrogen hypothesis' – where do we stand now? Int J Androl 2003;**26**:2–15.

44 Mylchreest E, Cattley RC, Foster PM. Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? Toxicol Sci 1998;**43**:47–60.

45 Mylchreest E, Sar M, Wallace DG, Foster PM. Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. Reprod Toxicol 2002;**16**:19–28.
46 Mylchreest E, Sar M, Cattley RC, Foster PM. Disruption of androgen-regulated male reproductive development by di(n-butyl) phthalate during late gestation in rats is different from flutamide. Toxicol Appl Pharmacol 1999;**156**:81–95.
47 Gray LE Jr, Foster PMD. Significance of experimental studies for assessing adverse effects of endocrine-disrupting chemicals. Pure Appl Chem 2003;**75**:2125–41.
48 Barlow NJ, McIntyre BS, Foster PM. Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. Toxicol Pathol 2004;**32**:79–90.
49 Sharpe RM. Hormones and testis development and the possible adverse effects of environmental chemicals. Toxicol Lett 2001;**120**:221–32.
50 Parks LG, Ostby JS, Lambright CR et al. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 2000;**58**:339–49.
51 Shultz VD, Phillips S, Sar M, Foster PM, Gaido KW. Altered gene profiles in fetal rat testes after *in utero* exposure to di(n-butyl) phthalate. Toxicol Sci 2001;**64**:233–42.
52 Scott HM, Hutchison GR, Mahood IK et al. Role of androgens in fetal testis development and dysgenesis. Endocrinology 2007;**148**:2027–36.

