

# Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects

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Reproductive disorders of newborn (cryptorchidism, hypospadias) and young adult males (low sperm counts, testicular germ cell cancer) are common and/or increasing in incidence. It has been hypothesized that these disorders may comprise a testicular dysgenesis syndrome (TDS) with a common origin in fetal life. This has been supported by findings in an animal model of TDS involving fetal exposure to *n*(dibutyl) phthalate, as well as by new clinical studies. Recent advances in understanding from such studies have led to refinement of the TDS hypothesis, highlighting the central role that deficient androgen production/action during fetal testis development, may play in the origin of downstream disorders. (Fertil Steril® 2008;89:e33–8. ©2008 by American Society for Reproductive Medicine.)

**Key Words:** Testosterone, Sertoli cell, Leydig cell, fetal germ cells, cryptorchidism, hypospadias, low sperm counts, anogenital distance, compensated Leydig cell failure

Considering the high incidence of disorders of male reproductive health that manifests at birth (cryptorchidism, hypospadias) or in young adulthood (testicular germ cell cancer [TGCC] and infertility) (1, 2), it is surprising that they have attracted so little public health attention. This is all the more remarkable when considering that there is strong evidence for increasing prevalence of these disorders in the West, especially among Caucasians; this increase must have (unidentified) environmental/lifestyle cause(s) (1). This implies that many of the cases of these disorders are intrinsically preventable, provided that the cause(s) can be identified, with health benefits for individuals and reductions in health care costs. Therefore, better understanding of the origins of these reproductive problems is likely to lead to health care benefits without the need for the long and costly development of therapeutic drugs. In the past 5 or so years, there have been major conceptual changes in thinking about the likely origins of male reproductive health disorders, discussed below. The primary purpose of this article is to develop this thinking, based on new understanding that has emerged.

## ORIGINS OF THE HYPOTHESIS OF A TESTICULAR DYSGENESIS SYNDROME (TDS)

Each of the four male reproductive disorders mentioned in the Introduction are risk factors for each other, and they share several, pregnancy-related risk factors (1, 2). Moreover, studies have shown that each of the disorders exhibits a markedly

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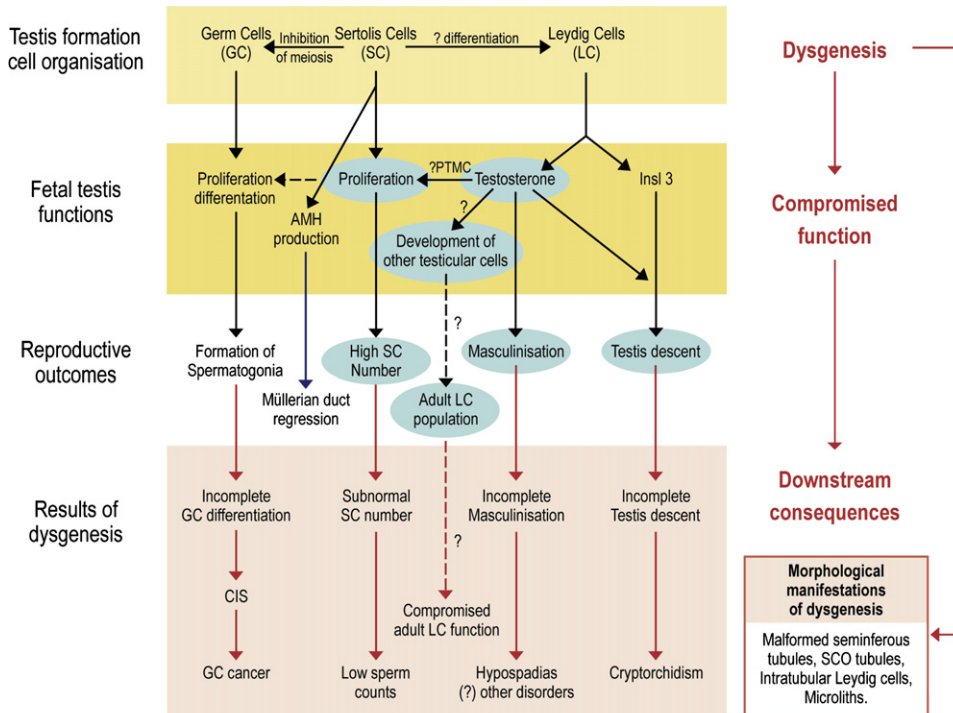
higher incidence in Denmark compared with Finland (3–6). This contrast could reflect environmental/lifestyle differences between the two countries and/or ethnic differences in susceptibility. For example, there is evidence for a faster rate of perinatal testis development in normal Finnish than in Danish boys (7). Based on these observations, it has been hypothesized that TGCC, cryptorchidism, and some cases of hypospadias and low sperm counts, comprise a testicular dysgenesis syndrome (TDS) with a common origin in fetal life (1). The hypothesis proposes that abnormal testis development (dysgenesis), which could have numerous primary causes, leads secondarily to hormonal or other malfunctions of the Leydig and/or Sertoli cells during male sexual differentiation, leading in turn to increased risk of the reproductive disorders (1, 2) (Fig. 1). There are immense difficulties with testing this hypothesis, notably the inaccessibility of the fetal testis at the stage of testis formation and organization (8–12 weeks' gestation) when dysgenesis will occur, plus the lengthy time period (20–45 years) between induction of dysgenesis and the potential (adult) outcomes. Epidemiologic studies in adults with TDS disorders and more recent studies of babies born with cryptorchidism or hypospadias have been the main approaches used to test and refine this hypothesis, but these all have limitations, not least their inability to relate the disorders in a direct way to earlier events within the fetal testis when dysgenesis is presumed to occur. In such situations, an animal model often represents the most satisfactory way forward, but at least for TGCC, proven animal models have not yet been reported.

## AN ANIMAL MODEL FOR TDS

It has been shown that exposure of rats in utero to the ubiquitous environmental chemical di(*n*-butyl) phthalate (DBP) can induce a TDS-like syndrome in the male offspring (8–10);

**FIGURE 1**

Schematic diagram to illustrate how dysgenesis of the early fetal testis is thought to lead to abnormalities of somatic cell function, resulting in hormonal changes and the downstream disorders that comprise testicular dysgenesis syndrome (TDS). The central role of testosterone is highlighted by the blue boxes. Dashed lines show pathways that are hypothesized but unproven. PTMC = peritubular myoid cell; InsI3 = insulin-like factor 3; AMH = antimüllerian hormone; CIS = carcinoma in situ; SCO = Sertoli cell only.



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this is manifest as dose-dependent induction of cryptorchidism, hypospadias, and impaired spermatogenesis/infertility. Importantly, there is evidence of focal dysgenesis (10, 11), subnormal fetal Leydig cell function (8–10), subnormal Sertoli cell proliferation (12), and possibly function (10), consistent with changes predicted in the TDS hypothesis (1). The focal dysgenesis induced by fetal DBP exposure in rats (10, 11), comprising malformed seminiferous cords, Sertoli cell-only tubules with immature-appearing Sertoli cells, and the abnormal occurrence of intratubular Leydig cells, are all features also reported in the testes of men with TGCC (13–15). The one TDS feature notably lacking in DBP-exposed rats is the occurrence of TGCC or its precursor, carcinoma in situ (CIS) cells. However, recent studies have shown that DBP exposure results in a transient delay of fetal germ cell differentiation in rats (16), which may have some analogy to the formation of CIS cells, which are thought to result from failure of normal germ cell differentiation in fetal life (17). Overall, the studies in rats exposed to DBP have provided strong support at every level for the TDS hypothesis in human males. Therefore, this potentially provides a model system in which to identify and dissect the mechanisms via which TDS and its disorders may arise in fetal life, which can then be

applied in humans; in turn, this may lead to identification of points of vulnerability and thus to potential environmental/lifestyle factors capable of affecting these. However, it should be kept in mind that DBP exposure of the fetal rat only provides a model for human TDS and, like all models, will have limitations.

### REFINEMENT AND ELABORATION OF THE TDS CONCEPT: THE CENTRAL ROLE OF ANDROGENS

As a result of the clinical and animal model findings outlined above, there has been widespread acceptance of the concept of a testicular dysgenesis syndrome, and more details are being added gradually to the hypothesised model (Fig. 1). A particularly important recent development has been the demonstration that inhibition of androgen production/action in rodents by transgenesis (18), DBP exposure (12), or flutamide treatment (19) reduces Sertoli cell number substantially in the perinatal period. This implies that androgens play a determining role during the most important periods (fetal and early postnatal life) of Sertoli cell proliferation (20) (Fig. 1). This would fit with data for the human showing that Sertoli cell number increases during fetal life (when testosterone

levels are high) and during the period of the neonatal testosterone rise (20, 21). As Sertoli cell number in adulthood is the primary determinant of sperm production/counts in men (20), it can be envisaged that reduction in testosterone levels in the fetal testis, as a secondary consequence of dysgenesis, could lead to low sperm counts in adulthood because of reduced Sertoli cell numbers, as proposed in the hypothesis (Fig. 1). An important reason why this detail did not form part of the original TDS hypothesis is because Sertoli cells in the fetal testis in all species so far examined, do not express androgen receptors; therefore, the effects of androgens are indirect, probably mediated via the peritubular myoid cells (12). The demonstration that androgens regulate Sertoli cell numbers perinatally adds to the view that disturbance of testosterone production/action in fetal life is of central importance in leading to the downstream TDS disorders (Fig. 1). Moreover, recent data suggesting that androgens may also positively regulate the expression of insulin-like factor 3 (Insl3) postnatally in mice (22) could imply that reduction in intratesticular testosterone in fetal life could lead to reduced Insl3 production, thus increasing the risk of failure of testis descent, in which both hormones play a role (Fig. 1). Indeed, in the DBP-exposed rat model of TDS there is a parallel reduction in intratesticular levels of testosterone and expression of Insl3 mRNA and protein (23, 24).

Further evidence for the important role of reduced androgen action in TDS comes from the study of individuals with androgen insensitivity syndromes (AIS). In these patients there is increased risk of TGCC (17, 25), but it remains unknown why the germ cells are affected as, like the fetal Sertoli cells, they are not direct androgen targets. It is presumed that this effect is mediated via the Sertoli cells, and if so, this would be a tertiary effect. If this is the case, it implies that androgen action on fetal Sertoli cells plays a role in regulating interactions with the fetal germ cells that are important for their normal differentiation; the factors involved remain to be identified (Fig. 1). It has also been reported that focal dysgenetic areas may be found in testes of AIS patients (26), so dysfunction of the Sertoli cells could occur secondary to this

(Fig. 1). The latter observation is puzzling because testosterone is not thought to play any role in initial differentiation of the testis (15, 27). Indeed, mice with defective androgen receptors form their testes normally with no reported dysgenesis; such findings fit with the evidence that seminiferous cord formation in rodents is completed before testosterone production begins (15, 27). The occurrence of dysgenetic areas in the testes of some AIS patients suggests a difference from rodents, and could mean that, for example, the final stages of seminiferous cord formation occur more slowly in the human and overlap with the early period of androgen production and action.

### POTENTIAL NEW ENDPOINTS OF TDS

An important question is whether there are other potential adult health consequences of impaired androgen production/action in fetal life in TDS that have not yet been looked for. Some possibilities are listed in Table 1. Perhaps the most important of these is compromised Leydig cell function in adulthood, as there is already evidence pointing to this in patients with CIS in their testes (28). Furthermore, adult men with low sperm counts/infertility exhibit compensated Leydig cell failure/function, such that they need a higher than normal LH drive to maintain their testosterone levels within the normal range (29); as a consequence, they exhibit an increase in the ratio of LH/testosterone levels in peripheral blood (29). As some of these are likely to be men with TDS, it raises the question of whether events within the fetal and/or early postnatal testis might affect the numbers and/or function of adult Leydig cells. As fetal and adult generations of Leydig cells are completely separate (30), and the adult generation does not develop until puberty in the human, this presupposes that any effect would have to be on the stem/progenitor cell of the adult Leydig cell population that was present in the perinatal testis. It is unknown if these cells are present or not, as they cannot yet be identified, but it would seem logical to expect their presence as every other cell type in the adult testis is present also in the fetal testis. At present, it is not known what

**TABLE 1**

**Potential new endpoints of TDS in adult men.**

| Endpoint <sup>a</sup>                       | Evidence <sup>a</sup>                                     |
|---|---|
| Compensated Leydig cell function/failure    | Men with low sperm counts/infertility.                    |
| Reduced AGD                                 | Secular decline in adult male testosterone levels.        |
| Reduced prostate volume <sup>b</sup>        | Evidence from rodent TDS/antiandrogen effects.            |
| Reduced seminal vesicle volume <sup>b</sup> | Evidence from rodent TDS/antiandrogen effects.            |
| Altered “masculine behaviors”               | TGCC and prostate cancer incidence are inversely related. |
|   | Evidence from rodent TDS/antiandrogen effects.            |
|   | Known role of fetal androgens in brain masculinization.   |

<sup>a</sup> See text for references.

<sup>b</sup> Such reductions might be associated with a reduction in ejaculate volume.

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effects, if any, fetal androgens might exert on these “adult Leydig stem cells,” but if this action is reduced because of dysgenesis-induced reduction in intratesticular testosterone levels, there could be consequences that then emerged in adulthood (Fig. 1). This is speculation, but two pieces of data lend indirect support. First, in marmosets, suppression of the neonatal testosterone rise results in adulthood in changes consistent with compensated Leydig cell failure (31). Second, a recent study from the United States has shown evidence for a decline in adult testosterone levels according to later year of birth (32), matching the similar increase in incidence of TGCC with later year of birth (33) and the more controversial, reported decline in sperm counts (34).

Based on studies in rats involving administration of antiandrogens or the suppression of intratesticular testosterone by DBP exposure in the rat model of TDS, a number of other changes can be predicted to occur as a consequence of TDS that could be searched for in humans. These include reduced size of the prostate and seminal vesicles (8), which could be evaluated by ultrasound. Moreover, as it is the products of these organs that form the ejaculate, it might be predicted that ejaculate volume is reduced in TDS patients. Androgen programming of aspects of masculine behavior (Table 1) is a complex area but certain traits are established as endpoints of fetal androgen exposure (35, 36). However, the most testable and interesting of the proposed new endpoints is reduced anogenital distance (AGD) (Table 1). Anogenital distance in males is normally 1.5- to twofold larger in males than in females, both in rodents (37) and humans (38), a difference that is known in rodents to be programmed by androgen exposure in fetal life (37). No measurements of AGD have yet been reported in humans with TDS disorders, but one study has produced evidence linking increased phthalate exposure in pregnancy and reduced anogenital index (AGD corrected for bodyweight) in male babies (39), data consistent with that found in the rat DBP model (8). New data on AGD is likely to emerge in the coming years, and this could provide fresh insights into TDS. In particular, it may provide an accurate measure of fetal androgen exposure, at least for the period when AGD is being determined, a measurement that is presently only possible by indirect measurement of testosterone levels in amniotic fluid or in cord blood at birth, both of which are probably imprecise (40). Probably the weakest aspect of the TDS hypothesis from a data point of view is the lack of direct measures of fetal testosterone exposure, a major limitation when considering the central importance assigned to androgen action in TDS (Fig. 1). AGD measurements will hopefully provide new information on this aspect, which should enable further refinement or rethinking of the TDS hypothesis.

## NEW INSIGHTS INTO THE MECHANISTIC BASIS FOR TDS

At present, understanding of the mechanisms leading to TDS disorders are descriptive, and are based mainly on reasonable interpretation. In turn, this is based on what we currently know about the processes of development of the testis and reproductive system in fetal life, an area in which new informa-

tion is accruing (27). The challenge is to identify the various molecular/biochemical pathways that first result in dysgenesis and how this then radiates out into the downstream endpoint disorders of TDS. It is hoped that the availability of the rat model for TDS may provide much of this information, but it is unclear how accurate a model this is for human TDS, so other avenues need to be explored. Two lines of investigation appear particularly worthwhile: first, to exploit further the marked difference between Denmark and Finland in prevalence of TDS disorders so as to gain better understanding of what underlies this difference. It is already clear that the rate of perinatal testis development differs in babies from these two countries (7), but it is unknown what genetic and/or environmental differences explain this. Based on the prevalence of TGCC, a similar difference may also exist between Caucasian and Afro-Americans within the United States (41), and this could be exploited in a similar way to the Danish-Finnish difference by prospective studies of newborn boys (7). Such studies should at the least establish if genetic “predisposition” factors are at the root of these ethnic differences in TDS, which will then provide an important focus for identification of the environmental factors that must interact with these pathways to explain, for example, the progressive increase in incidence of TGCC.

The second approach is to focus on a key risk factor for all of the TDS disorders, fetal growth restriction (1, 2). It is unknown why this should lead to dysgenesis and the TDS disorders, but identification of the pathways that underlie this should provide important insight into processes vulnerable to disruption. For example, does the fact that the testis grows rapidly when it first differentiates (27) make it especially vulnerable to fetal growth restriction? The latter can occur at various gestational stages, but if the TDS hypothesis is correct it must be very early growth restriction that is important (i.e., weeks 8–12). Or do factors that cause dysgenesis also cause or lead to fetal growth restriction? Testosterone has been implicated in male–female differences in fetal growth in humans (42). It is also noteworthy that male fetuses appear more susceptible than females to maternal smoking-induced growth restriction (43), and maternal smoking has been linked with major deficits in adulthood in testis size and sperm counts (44, 45). However, a recent study has shown that maternal testosterone levels in pregnancy in humans are *positively* related to fetal growth restriction (46), and there are supporting data from sheep (47). These various findings suggest that our present understanding is insufficient to provide a clear interpretation of cause and effect, although the take-home message appears to be that testosterone is related in one or more ways to fetal growth (Fig. 1). Some of these possibilities can probably be evaluated in animal models of fetal growth restriction that are of proven relevance to the human (48).

## ENVIRONMENTAL CHEMICAL EXPOSURES AND TDS

It is unfortunate that many people wrongly consider that the TDS hypothesis is centered on the idea that it is caused by exposure to environmental chemicals, in particular, endocrine

disruptors. The original hypothesis stated that there are likely to be multiple causes of TDS, one of which is exposure to environmental chemicals. However, there is still only limited evidence to support this possibility (39, 49, 50). Perhaps confusion has arisen because the TDS hypothesis states that “endocrine disruption,” as encapsulated by altered testosterone production/action by the fetal testis, is at the center of the hypothesis (Fig. 1), but this alteration could result from any genetic, lifestyle, or environmental factor that causes dysgenesis. Nevertheless, as studies with DBP and certain other phthalates have demonstrated, environmental chemicals that can cause dysgenesis and/or inhibit testosterone production or action, obviously have the right credentials for causing TDS. Whether the human fetus is exposed to sufficient levels of such chemicals to result in any adverse effect remains a point for debate, and is unlikely to be resolved easily because of the inherent difficulties in both obtaining accurate chemical exposure data for the early human fetus and then relating this to clinical outcomes months or decades later. One important recent development that is highly relevant is the demonstration that exposure of the developing rat fetus to mixtures of antiandrogenic chemicals at concentrations at which none of the chemicals individually has a significant effect, results in major perturbation of androgen-dependent masculinisation, for example, reduced AGD (51, 52). Based on these findings and the demonstration of high numbers of environmental chemicals with antiandrogenic activity, it is a distinct possibility that human fetal exposure to such chemicals could contribute causally to the high/rising incidence of TDS disorders. Direct proof or refutation of this possibility will, however, be a Herculean task.

## FUTURE PROSPECTS

The past 10–15 years has witnessed enormous strides forward in our understanding about the prevalence of human male reproductive disorders, culminating in the TDS hypothesis. Much of the accrued data is alarming, in that it suggests that young men born today (at least in Europe) have remarkably low average sperm counts (53), a high prevalence (~1 in 6) of abnormally low sperm counts likely to cause fertility problems (53), and a lifetime risk of TGCC approaching 1% in some countries (54). If, as the new data suggests (32), declining testosterone levels is to be added to this list, then the reproductive health outlook for the present and next generation of men looks bleak. If many of these disorders are a result of TDS, then it is likely that their origin is attributable to lifestyle and/or environmental factors that have changed over the past 50–100 years. This being the case, then such cases are preventable. If this conclusion is to be realised, it first requires that we identify the causes of TDS and, in turn, this requires that we first gain a fuller understanding of the pathways that lead to disordered development of the fetal testis. As with all of science, progress toward objectives is aided by proposing hypotheses that can then be tested. Our hope is that the present elaboration of the original TDS hypothesis will help in this task.

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