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## DESARROLLO SEXUAL DIFERENTE

# Gonadectomy in DSD

## Gonadectomía en DSD

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### Introduction

In several forms of Differences of Sex Development (DSD), there is an increased risk for the development of gonadal germ cell cancer (GCC). Based on their biological characteristics, (developmental stage, pattern of genomic imprinting, ploidy,…), the resulting malignancies are classified as Type II germ cell cancers of the testis, ovary and dysgenetic gonad. The cell of origin is a pluripotent primordial germ cell (PGC), that is delayed or blocked in its maturation. These GCC, mostly seminomas / dysgerminomas, but also non-seminomas are sometimes seen, do not arise from somatic driver mutations, but from a defective micro-environment (Sertoli, Leydig cells) that is incapable of driving the germ cells through their physiological maturation process[1, 2]. The invasive GCC, seminomas / dysgerminomas and non-seminomas, in general, do not occur prior to (induced) puberty, but they are preceded for many years by two distinct neoplastic lesions: germ cell neoplasia in situ (GCNIS) and gonadoblastoma[3,4].

### Risk factor for GCC development

Several risk factors for GCC development have been identified. GCC in the context of DSD occur in individuals who have Y chromosomal material in their (gonadal) karyotype, more specifically the gonadoblastoma region on Y (GBY); the *testis-specific protein* Y-encoded (TSPY) within this region is the most likely candidate gene[3, 5]. Thus, 46,XX individuals who have a DSD, including men who have 46,XX testicular DSD due to translocation of SRY to one of the X chromosomes, are not at increased risk. GCC arise from pluripotent PGC that fail to accomplish their developmental program in a timely manner. These PGC aberrantly express the pluripotency marker OCT3/4, often in combination with TSPY[6]. Thus, the second risk factor for GCC development in DSD is the presence of (OCT3/4 expressing) PGC. The number of germ cells is most often strongly reduced in DSD, and their maturational stage can vary, along with the functionality of the micro-environment. In a testicular context, most surviving germ will ultimately reach the spermatogonial stage, i.e. they will reside in contact with the basal membrane of the testis tubule, lose their pluripotency and express germ cell-specific genes like *Dead-Box Helicase 4* (DDX4), showing their commitment to the germ line[7]. However, before becoming spermatogonia, many PGC will be delayed in their maturation and some will remain visible as OCT3/4 positive PGC in the center of the tubule well beyond birth, which in typical testicular development is rarely encountered beyond intra-uterine life[8, 9]. In gonadal dysgenesis, the testicularisation process is often incomplete or absent, and no testis tubules can be found. Instead, the gonad impresses as undifferentiated gonadal tissue (UGT), consisting of gonadal stromal cells with or without sex-cord like structures. In UGT, scarce surviving PGC can be found, and most of them are blocked in their maturation, whereas more mature germ cell types are commonly absent. Alternatively, these aberrant germ cells are removed by apopotosis, and as an end stage, the gonad can impress as a streak[10]. UGT is the typical gonadal context where gonadoblastomas develop, whereas GCNIS is invariably encountered in a testicular environment[3].

Another risk factor that has been related to GCC development is aberrant expression of stem cell factor (SCF) in Sertoli (-like) cells and in germ cells. During embryonic life, SCF, in co-operation with c-Kit,

orchestrates the correct migration of PGC from the yolk sac to the developing gonadal ridges. In typical development, SCF expression fades out around birth, but it has been found to be expressed in high-risk DSD gonads, as well as in seminomas / dysgerminomas and in non-seminomas.

Until around 2000, the overall risk for GCC development in DSD (at that time called "intersex"), was estimated at around 30%, and prophylactic gonadectomy was routinely performed following a diagnosis of DSD. With stratification of cases according to etiology (problems of gonadal development versus problems of androgen synthesis or action), it became clear that large differences in GCC risk exist, with the former group being far more at risk than the latter[3, 8, 10- 12].

Subsequent studies have tried to estimate GCC more precisely in specific subgroups of patients. Although no large series exist, individual case reports point at a particularly high risk in DSD associated with WT1 mutations, such as Frasier syndrome or Denys-Drash syndrome[3, 12].

In 45,X/46,XY (and variants) DSD, a large spectrum of gonadal phenotypes is encountered, ranging from bilateral streak gonads, to asymmetric gonadal development, with a testis on one side and UGT or streak tissue on the other. Immunohistochemical studies reveal a particularly high risk in individuals who have UGT and/or strongly dysgenetic testes, mostly resulting in ambiguous genitalia at birth. In these individuals, over 50% of gonads display gonadoblastoma or OCT3/4+ cells blocked in their maturation. Boys with the 45,X/46,XY karyotype and with bilateral inguinal or scrotal testes have a lower risk, estimated at around 10%; and the lowest risk (5%) is found in non-virilised girls diagnosed with Turner syndrome. Interestingly, the gonadal phenotypes are reflected to a large extent in the patient's external phenotype, as assessed by the external masculinization score[13] or the more widely applicable external genitalia score[14]. Overall, this and other studies show that in 45,X/46,XY individuals with (some degree of) virilization, poorer "testicularisation" of the gonad correlates with a higher GCC risk. Many girls who have Turner syndrome and a 45,X/46,XY (and variants) karyotype have bilateral streak gonads or bilateral gonadal regression, thus eliminating the risk for GCC development due to the absence of germ cells[15-17].

The prevalence of GCC in androgen insensitivity syndrome has been extensively reviewed more recently. In prepubertal cases, GCNIS is extremely rare, and invasive GCC almost inexistent[18]. However, over-diagnosis may occur, due to interpretation of germ cells delayed in their maturation and thus expressing OCT3/4, as pre-GCNIS or even as GCNIS. The position of the OCT3/4 expressing germ cells relative to the basal membrane is of crucial importance in this matter: OCT3/4 positive germ cells in the center of the testis tubule are delayed in their maturation, whereas OCT3/4 expressing germ cells in contact with the basal membrane fail to downregulate their pluripotency while in the spermatogonial niche and are hypothesized to be indicative of (pre-) GCNIS[8]. Post puberty, the incidence of (pre-)GCNIS in androgen insensitivity syndrome rises to around 10%, but invasive cancers remain rare, probably due to the lack of androgen signaling[4,19,20]. A genetic predisposition, due to the presence of high risk alleles for GCC development as identified by genome-wide association studies, may also play a role[19].

**Management of risky gonad**

Management of the risky gonad is determined by several factors combined, such as the risk for malignant transformation, hormonal and reproductive capacity, possibilities for adequate surveillance and patient preferences.

Most boys with dysgenetic testes produce sufficient testosterone to experience puberty and do not need testosterone replacement in (early) adulthood[17, 21-23]. Their testes, when present in a scrotal position, can reliably and easily be investigated by self-palpation and ultrasound, e.g. the latter performed annually, from puberty onwards. GCC risk has been hypothesized to increase from puberty onwards, and to be inversely related to the degree of gonadal differentiation and function[1,4,12,15]. It is important to explain to patients that imaging, whether it is ultrasound or MRI, cannot detect GCNIS, but is aimed at detecting invasive gonadal GCC at an early stage. For scrotal testes, there does not seem to be an added value of MRI as compared to ultrasound[24]. Classic tumor markers perform poorly for the detection of testicular cancer, especially seminoma. Testicular GCC produce specific micro-RNAs, and their value as specific tumor markers in the follow-up of these cancers is currently being investigated. However, micro-RNAs have not been identified in serum or semen of men who had GCNIS without an invasive component[25]. Some men who have dysgenetic testes may produce sperm[17]. Therefore, a gonadal biopsy with the aim to detect GCNIS, if present, at the end of puberty, can efficiently be combined with sperm capture by (micro-) testicular semen extraction (micro-)TESE.

GCC risk is much higher in 46,XY or 45,X/46,XY individuals who have UGT and/or gonads that have a very poor level of "testicularisation"[7, 15] (Figure 1). In such gonads, sex steroid production is mostly very limited, and is not likely to sufficiently support start or progression of puberty. Germ cells are either absent or very limited in number and if present, they often aberrantly express pluripotency markers, showing their immaturity and carcinogenic potential. Therefore,

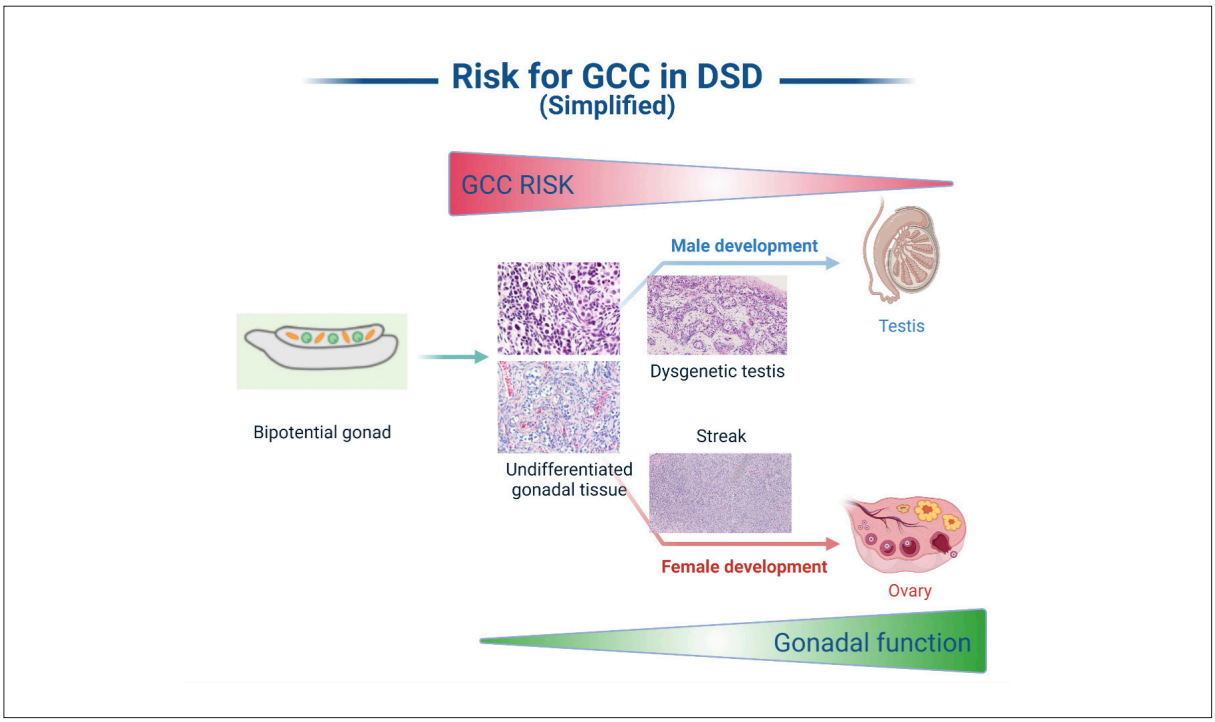


Figure I. Schematic representation of risk estimation for gonadal germ cell cancer in DSD: The risk for developing gonadal germ cell cancer increases with poorer gonadal differentiation ("testicularisation") and decreased gonadal function. Created with BioRender.com
GCC: germ cell cancer; DSD: differences / disorders of sex development.

weighing advantages and risks, the threshold for performing a gonadectomy is much lower. In addition, it Is advisable to combine gonadal biopsies for microscopic investigation with an orchiopexy procedure. Gonadectomy is strongly advised when gonadoblastoma, GNIS or UGT with multiple pluripotent germ cells is found, especially when these gonads are in an abdominal position[1,7,12 15].

Tumor risk has been shown very high in 46,XY women with complete gonadal dysgenesis caused by WT1 mutations, especially Frasier syndrome, and SRY mutations[12, 26, 27], and early gonadectomy is recommended[1] (Figure 2A). It is currently unclear whether this can be extended to other forms of 46,XY complete gonadal dysgenesis, but in the absence of functional gonadal cells, gonadectomy seems to be the most straightforward decision. Girls with Turner syndrome who turn out to have Y chromosomal material almost invariably have bilateral streak gonads, regressed gonads, or sometimes UGT with often extensive gonadoblastoma. Ovarian follicles have almost never been reported in such gonads, and early gonadectomy seems to be the most logic decision given the absence of any gonadal function in most, if not all of these girls[1,21,15].

GCC risk is manyfold lower in disorder of testosterone biosynthesis (Figure 2B). In some conditions, such as 5 alpha-reductase deficiency, fertility has even been described[28]. Gonadal management in these cases is to a large extent determined by the potential development of (un)desired secondary sex characteristics, rather than by GCC risk. Removal of testes should be avoided in prepubertal children, even when raised as girls, in order to leave all options open with regard to gender identity. In cases where a stable gender identity has not been reached at ages 10-12 years, puberty can be transiently postponed with Gonadotropin Releasing Hormone analogues (GnRHa), while further exploring gender identity. In boys who have a disorder of testosterone biosynthesis, testes need to be placed in a scrotal position, and surveillance with self-palpation and annual ultrasound needs to be organized from puberty onwards[1].

Guidelines for the management of gonads in complete and partial androgen insensitivity syndrome have been published[20]. Briefly, given the very low risk for GCC development in childhood (see above), and testicular testosterone production during puberty, which will subsequently be converted to estradiol, allowing for spontaneous breast-development and bone mass accrual, bilateral gonadectomy should be postponed, at lease until around the age of transition. Young women who have complete androgen insensitivity syndrome can then make an autonomous choice, based on all available information, whether they want

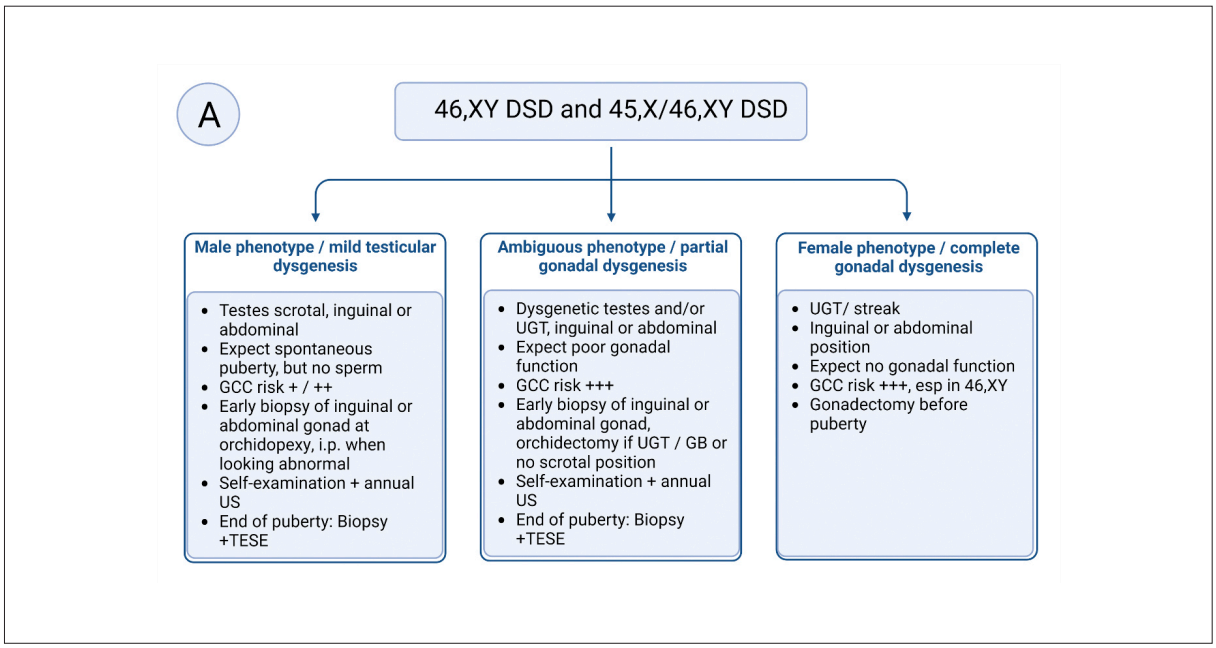


Figure II A. Rationale and summary of recommendations for gonadal management in individuals who have 45,X/46,XY or 46,XY DSD. Created with BioRender.com

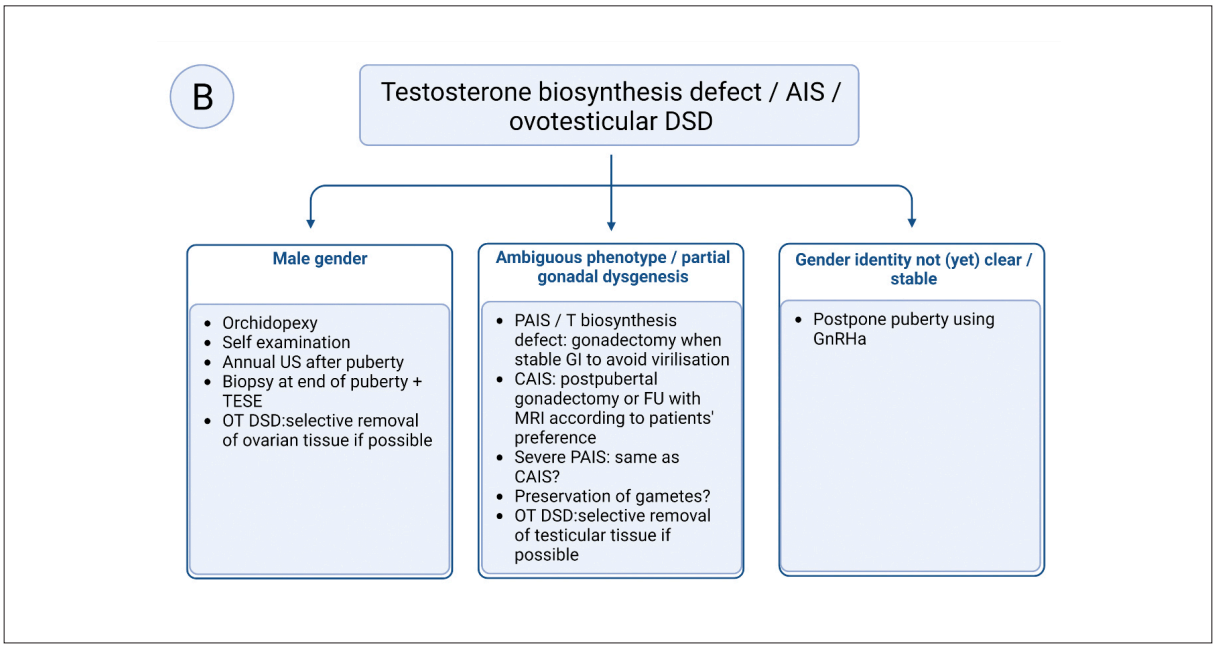


Figure II B. Rationale and summary of recommendations for gonadal management in individuals who have testosterone biosynthesis disorders, androgen insensitivity syndrome or ovotesticular DSD. Created with BioRender.com

GCC: germ cell cancer; DSD: differences / disorders of sex development; US: ultrasound; TESE: testicular semen extraction; GB: gonadoblastoma; AIS: androgen insensitivity syndrome; CAIS: complete androgen insensitivity syndrome; PAIS: partial androgen insensitivity syndrome; OT DSD: ovotesticular DSD; GI: gender identity; GnRHa: gonadotropin releasing hormone analogues.

to keep their gonads or have them removed. Men who have partial androgen insensitivity syndrome can enter a surveillance program, e.g. by regular self-palpation and annual ultrasound, when their gonads are in a stable scrotal position. In youngsters with unclear or developing gender identity, GnRHa can be used temporarily to prevent the development of secondary sex characteristics until a clear gender identity is established[20].

## Conclusion

In conclusion, clinical management of gonads in DSD is complex, and is determined by many variables, such as the degree of gonadal differentiation, the functionality of the gonad, the intrinsic GCC risk associated with the condition under scope, underlying genetic etiology, position of the gonad, and personal preferences. Expert review of gonadal material in the context of DSD is necessary because of the rarity of the individual conditions, as well as their complexity.

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