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# Undetectable anti-Mullerian hormone and inhibin B do not preclude the presence of germ cell tumours in 45,X/46,XY or 46,XY gonadal dysgenesis

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## Abstract

**Objective:** Individuals with 45,X/46,XY or 46,XY gonadal dysgenesis are at increased risk of germ cell malignancies. Therefore, prophylactic bilateral gonadectomy is advised in girls and considered in boys with atypical genitalia for undescended, macroscopically abnormal gonads. However, severely dysgenetic gonads may not contain germ cells rendering gonadectomy unnecessary. Therefore, we investigate if undetectable preoperative serum anti-Müllerian hormone (AMH) and inhibin B can predict the absence of germ cells, (pre)malignant or otherwise.

**Design, Patients and Measurements:** Individuals who had undergone bilateral gonadal biopsy and/or gonadectomy because of suspected gonadal dysgenesis in 1999–2019 were included in this retrospective study if preoperative AMH and/or inhibin B were available. Histological material was reviewed by an experienced pathologist. Haematoxylin and eosin and immunohistochemical stainings for SOX9, OCT4, TSPY and SCF (KITL) were used.

**Results:** Thirteen males and 16 females were included, 20 with 46,XY and 9 with 45,X/46,XY DSD. Three females had dysgerminoma alongside gonadoblastoma; two gonadoblastoma, one germ cell neoplasia in situ (GCNIS) and three males had pre-GCNIS and/or pre-gonadoblastoma. Gonadoblastoma and/or dysgerminoma were present in 3/11 individuals with undetectable AMH and inhibin B, one of whom also had non-(pre)malignant germ cells. Of the other 18, in whom AMH and/or inhibin B were detectable, only one had no germ cells.

**Conclusions:** Undetectable serum AMH and inhibin B cannot reliably predict the absence of germ cells and germ cell tumours in individuals with 45,X/46,XY or 46,XY gonadal dysgenesis. This information should help in counselling about prophylactic gonadectomy, taking into account both the germ cell cancer risk and potential for gonadal function.

KEYWORDS

46,XY DSD, anti-Mullerian hormone, germ cell cancer, germ cells, inhibin, sex chromosome DSD

## 1 | INTRODUCTION

Differences/disorders of sex development (DSD) are congenital conditions characterised by atypical chromosomal, gonadal or anatomical sex. DSD can be subdivided into conditions in which gonadal development is affected, conditions characterised by defects in hormone synthesis or actions, and other conditions. In individuals with 45,X/46,XY or 46,XY gonadal dysgenesis residual function of the gonads varies considerably. As a result, the phenotype can range from typical female external genitalia in cases of complete gonadal dysgenesis, to atypical genitalia, to typical male external genitalia. Characteristics of Turner syndrome, such as short stature, have been reported in 14%–70% of individuals with 45,X/46,XY DSD.[1–3]

The abnormal environment in the dysgenetic gonad leads to disturbed germ cell maturation, with an increased risk of germ cell tumours, estimated to be 15%–40%.[1,4–7] Most commonly found in gonadal dysgenesis is gonadoblastoma, which is an in situ lesion that can progress to dysgerminoma, or more rarely, to non-dysgerminoma. It is estimated based on case reports/case series that dysgerminoma occurs in 3%–10% of individuals.[6,8] Choriocarcinoma, embryonal carcinoma and yolk sac tumours have also been reported.[9–11] Therefore, prophylactic bilateral gonadectomy is advised at diagnosis in girls with 45,X/46,XY or 46,XY gonadal dysgenesis, and should be considered with a low threshold in boys with ambiguous genitalia for undescended, macroscopically abnormal gonads that cannot be surgically moved to a lower position to allow surveillance.[12]

However, severely dysgenetic gonads may not contain differentiated supportive cells and as a consequence may be devoid of any germ cells. Such pathologically confirmed streak gonads cannot develop germ cell cancer so prophylactic gonadectomy would not be necessary. Sertoli cells secrete inhibin B and anti-Müllerian hormone (AMH) and serum levels of these hormones have been shown to correlate with Sertoli cell number and germ cell number in boys with cryptorchidism.[13–15] It has been proposed that in boys with bilateral non-palpable testes, a diagnosis of testicular regression syndrome can be made based on inhibin B being undetectable or close to the lower limit of detection, in combination with clearly elevated gonadotropins, without the need for laparoscopy.[16] The (nearly) undetectable level of inhibin B is thought to indicate an absence of Sertoli cells and thus an absence of germ cells.

In addition to the need for gonadectomy to prevent germ cell cancer, the loss of potential residual gonadal function needs to be considered in a decision about gonadectomy. Spontaneous onset of puberty, spontaneous menarche and pregnancy have been reported in girls and women with 45,X/46,XY DSD.[6,17] However, in prepubertal children, it can be difficult to assess gonadal function. AMH and inhibin B have been shown to predict ovarian function in girls with Turner syndrome, but this has not been investigated specifically in girls with 45,X/46,XY DSD.[18,19]

Thus, on the one hand, it would be valuable if serum inhibin B and AMH could be used to predict the absence of Sertoli cells and thereby germ cells in individuals with gonadal dysgenesis, who as a consequence would not need to undergo a prophylactic gonadectomy. On the other hand, it would be useful if these markers could be used to predict the presence of ovarian follicles, and thus residual gonadal function, in prepubertal girls. Therefore, in this study, we examined the correlation between serum levels of these hormones before prophylactic gonadectomy and the presence of germ cells and germ cell tumours in individuals with 45,X/46,XY or 46,XY gonadal dysgenesis.

## 2 | MATERIALS AND METHODS

### 2.1 | Inclusion criteria

Individuals who fulfilled the following inclusion criteria were eligible for the study: (1) diagnosed with DSD; (2) had undergone bilateral gonadal biopsy and/or gonadectomy because of suspected gonadal dysgenesis at the Erasmus MC between 1999 and 2019; and (3) AMH and/or inhibin B were available from before the biopsy/gonadectomy.

### 2.2 | Laboratory investigations

Until 2006, the ultra-sensitive immuno-enzymometric assay (Immunotech, Beckman-Coulter) was used for measurement of AMH. Within-assay and between-assay coefficients of variation (CVs) were <5% and <8%, respectively. Until 2012, inhibin B was measured using ELISA (Oxford Bio-Innovation). Within-assay and between-assay CVs were <9%, and <15%, respectively. The detection limits for the assays were 10 ng/L for inhibin B, and 0.05 μg/L for AMH. Since 2006, an in-house ELISA has been used to measure AMH.[20] Intra-assay and interassay CVs were <5% and <10%, respectively. The detection limit defined as the absorbance of blank replicates +2 standard deviations of this assay was 0.078 mcg/L; serum AMH < 0.1 mcg/L was reported as undetectable.

Since 2012, both serum AMH and inhibin B levels were measured by the Gen II ELISA (Beckman Coulter, Inc.). Inter-assay CVs were 15.1% for AMH and 11.4% for inhibin B, respectively.[21,22] The detection limits for these assays were 0.08 mcg/L and 2.9 ng/L, respectively.

### 2.3 | Pathology

Histological material was reviewed by a single experienced pathologist (JWO), who had performed the initial assessment of pathology for 49% of the samples. The pathologist was blinded to the results of serum AMH and inhibin B. Haematoxylin and eosin stainings, as well as immunohistochemical stainings for SOX9, OCT4, TSPY and SCF (KITL), and double stainings for OCT4 and TSPY were carried out as previously described.[23] The panel of immunohistochemical stains was chosen to enable the differentiation between dysgerminoma, gonadoblastoma and germ cell

neoplasia in situ (GCNIS), pre-gonadoblastoma and pre-GCNIS, and maturation delay as outlined in the WHO Classification of Tumours of the Urinary System and Male Genital Organs.[24]

The presence of germ cell malignancies or precursor lesions was classified as follows[25]:

a. Dysgerminoma
b. Gonadoblastoma or GCNIS
c. Pre-gonadoblastoma or pre-GCNIS
d. Maturation delay

### 2.4 | Statistical analysis

Data are described as mean ± standard deviation if normally distributed, or median and range if non-normally distributed. No statistical tests were performed.

### 2.5 | Ethics

This study was part of a larger observational study on DSD, which was assessed by the local medical ethical committee who determined that the Medical Research Involving Human Subject Act (WMO) did not apply.

## 3 | RESULTS

Twenty-nine individuals were included, 13 males and 16 females. Twenty had a 46,XY karyotype. Nine had sex chromosomal DSD including 45,X/46,XY ($n = 3$), 45,X/46,X,idic(Yq) ($n = 4$), 45,X/46,X,der(Y) ($n = 1$) and 45,X/46,XY/47,XYY ($n = 1$). In three females, the 45,X cell line was only found in a buccal smear; their karyotype in peripheral blood lymphocytes was 46,XY or 46,X,idic(Yq).

One individual had undergone unilateral gonadectomy in the past, before AMH or inhibin B was first measured. Histology from this first gonadectomy was not available. From this individual, only histology from the second gonadectomy, performed 14 years later and laboratory data from before this second procedure were included in the study.

### 3.1 | Endocrine evaluation

Ten individuals, all females, had undetectable AMH and inhibin B levels; in one only inhibin B was available from before gonadectomy, which was undetectable. In two boys, AMH was not available and in one inhibin B had not been measured before gonadal biopsy/gonadectomy. In one female, AMH was detected but at a level below the limit of quantitation for this assay (1.0 µg/L; LOQ 1.2 µg/L); in this individual, inhibin B was undetectable.

Seven individuals (four boys and three girls) were prepubertal at the time of their last visit/at the time of gonadectomy. Three out of nine females with undetectable AMH and inhibin B in the pubertal age range had had spontaneous onset of breast development; they had hypergonadotropic hypogonadism (FSH > 35 U/L). One had a 45,X/46,X,der(Y) karyotype and the other two 46,XY DSD of unknown cause. In contrast, all individuals (13/13) in whom AMH and/or inhibin B were detectable and who were in the pubertal age range had spontaneous onset of puberty.

### 3.2 | Gonadal surgery

All gonadectomies were prophylactic without clinical evidence of a germ cell cancer before the surgery. In males, gonadectomy was performed for abdominal gonads with abnormal appearance at surgery. Biopsies were taken from gonads that remained in situ at the time of orchidopexy.

### 3.3 | Germ cells and germ cell tumours

In three females aged 14.6–23.1 years, germ cell malignancies were identified, all dysgerminomas alongside gonadoblastoma. One had 45,X/46,XY DSD and two 46,XY DSD; two had typical female external genitalia (EGS 0), whereas the third had some virilisation (EGS 3.5). Two additional females with EGS 0, aged 11.2 and 14.4, had unilateral gonadoblastoma. They had 45,X/46,XY/47,XYY and 46,XY DSD of unknown cause. One female aged 13.6 years, with EGS 4.5, with 46,XY DSD had bilateral GCNIS.

Three males aged 0.1–1.9 years had pre-GCNIS and/or pre-gonadoblastoma. One had 45,X/46,XY karyotype; the others had 46,XY DSD.

### 3.4 | Relation between Sertoli cell markers and the presence of germ cells and germ cell tumours

The median time between the last measurement of AMH and/or inhibin B and the last gonadectomy/gonadal biopsy was 1.4 years (range 0–7.6 years). Germ cells were present in 3/11 (27%) of those with undetectable AMH and inhibin B (Table 1). In one female, sporadic gonocytes were observed scattered in ovarian-type stroma in one gonad, whereas a microscopic gonadoblastoma was present in the other gonad; in the other female, only one gonad contained germ cells, all within nests of gonadoblastoma (Figure 1); in the third individual, germ cells were also present in nests of gonadoblastoma and dysgerminoma. Out of the other 18 individuals, in whom either AMH or inhibin B or both were detectable, there was only one in whom no germ cells were identified. This was a male with karyotype 45,X/46,X,idic(Yq) who had undergone gonadectomy of a streak gonad and a biopsy at age 7.5 years of the other gonad, which showed dysgenetic testicular tissue without germ cells.

## 4 | DISCUSSION

Care for individuals with DSD is constantly evolving and in recent years, there has been more emphasis on patient autonomy and, if safe from a medical viewpoint, postponing surgeries until an age when the individual can be involved in the decision.[4] Clinicians may be more inclined to postpone gonadectomy as demonstrated by a recent report of two girls with 46,XY gonadal dysgenesis due to an *NR5A1* variant in whom gonads were left in situ, one of them being treated with a GnRH analogue to prevent virilization.[26] However, the safety of such an approach has not been established. Predictors of the presence or absence of germ cells would be highly valuable in clinical decision-making around gonadectomy.

Therefore, we set out to investigate if undetectable serum levels of inhibin B and AMH could predict the absence of germ cells, meaning gonadectomy would not be necessary. In the current study, all nine individuals in the pubertal age range in whom AMH and inhibin B were undetectable had hypergonadotropic hypogonadism indicating severely affected endocrine function, although three had experienced some spontaneous breast development. However, three individuals with undetectable AMH and inhibin B had a gonadoblastoma and/or dysgerminoma; the youngest was 11 years old. Thus, we conclude that undetectable serum levels of inhibin B and AMH, even in combination with hypergonadotropic hypogonadism, cannot be relied upon to diagnose the absence of germ cells. This in contrast to what has previously been found in boys with suspected testicular regression syndrome in whom it was suggested that laparoscopy to look for testicular remnants is not necessary in case of (nearly) undetectable inhibin B in combination with very high gonadotropins.[16]

**TABLE 1** The presence of germ cells and abnormal germ cells in individuals with and without detectable serum levels of AMH and inhibin B. Individuals are listed according to the most severe germ cell abnormality present (i.e., an individual with both dysgerminoma and gonadoblastoma is listed under dysgerminoma).

| Presence of: | Undetectable AMH and inhibin B (*n* = 11) | Detectable AMH and/or inhibin B (*n* = 18) |
|---|---|---|
| Any germ cells | 3 | 17 |
| Abnormal germ cells | | |
| Dysgerminoma | 1 | 2 |
| Gonadoblastoma/ GCNIS | 2 | 1 |
| Pre-gonadoblastoma/ pre-GCNIS | 0 | 3 |
| Maturation delay | 0 | 0 |

Abbreviations: AMH, anti-Müllerian hormone; GCNIS, germ cell neoplasia in situ.

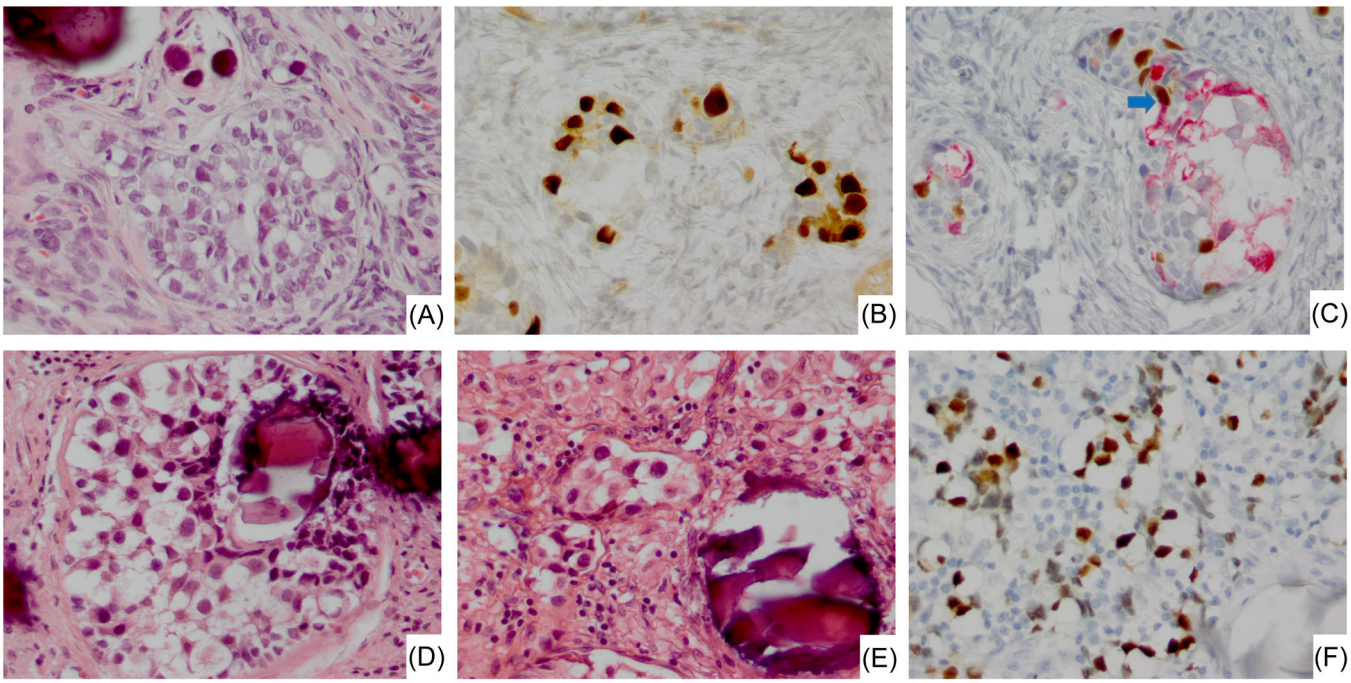


**FIGURE 1** Lesions identified in individuals with undetectable serum levels of AMH and inhibin B. Microphotographs showing two gonadoblastomas respectively in (A–D), both characterised by round nests composed of granulosa cells, gonocytes and gonadoblastoma cells in close proximity with microliths. The gonocytes with small nuclei and the gonadoblastoma cells with large, atypical nuclei both showed nuclear staining for OCT4 (B). Occasional cells with double staining for OCT4 and TSPY (C, arrow) are at risk for malignant transformation. Gonadoblastoma shown in (D) was associated with dysgerminoma composed of dysgerminoma cells (E, F), which show nuclear expression of OCT4 (F), and are intermingled with lymphocytes (E). (A, D and E, haematoxylin and eosin staining; B and F, OCT4 staining; C, double staining for OCT4 and TSPY; all at 200× magnification). AMH, anti-Müllerian hormone.

The advantage of preventing germ cell malignancies by prophylactic gonadectomy needs to be carefully weighed against the loss of endocrine function/need for hormone replacement and loss of any fertility potential. Most girls with 45,X/46,XY or 46,XY gonadal dysgenesis develop hypergonadotropic hypogonadism, although up to half may initially undergo spontaneous onset of puberty, as found in the current study as well as a previous study.[6] In the current study, none of the girls had follicles on pathological examination. However, normal ovaries on pathology, spontaneous menarche and, very rarely, pregnancy have been described in girls and women with 45,X/46,XY DSD.[6,17] Unfortunately, levels of AMH and inhibin B were not reported in those studies. In girls with partial gonadal dysgenesis, undesired virilization may occur during puberty, which may be another reason for gonadectomy in addition to the increased risk of germ cell cancer.

The current study shows that germ cell tumours can be present even if levels of inhibin B or AMH are undetectable, and that germ cell tumours occur at a young age. Monitoring using ultrasound or MRI has been shown to be unreliable to detect germ cell tumours.[6,27] This supports current recommendations for girls with 45,X/46,XY or 46,XY gonadal dysgenesis to undergo prophylactic gonadectomy at diagnosis.[4,12] However, the potential for ovarian function and possibly fertility needs to be taken into account in this decision. In the current study, only five females had detectable AMH or inhibin B, one of whom had a 45,X/46,XY karyotype. Although none had follicles, the number is too small to draw definite conclusions about the chance of functional ovarian tissue being present in those with detectable AMH or inhibin B. Preservation of gonadal tissue for fertility at gonadectomy is complex due to the considerable risk of germ cell cancer.

Boys with 45,X/46,XY and 46,XY gonadal dysgenesis were found to undergo spontaneous pubertal development in line with previous studies that found the majority had spontaneous puberty.[2,11,28] Preserved testosterone synthesis may be a reason to leave gonads in situ, although men may nonetheless develop testosterone deficiency over time.[2,11,28] For boys, it is currently recommended to perform orchidopexy for any gonad that is not in a scrotal position, and to obtain a gonadal biopsy at the time of surgery if the gonad is macroscopically abnormal, to assess the presence of germ cell (pre)malignancy. The gonads can be monitored by periodic self-examination and by annual ultrasound from puberty onwards, and a biopsy can be repeated post-puberty if indicated.[4,12] However, patients need to be informed that self-examination and imaging with ultrasound, or MRI, is not informative to detect premalignant changes.[29] Gonadectomy is advised for gonads that are macroscopically abnormal or that cannot be brought into a position where monitoring is possible.[12]

Future studies should establish if the risk of germ cell cancer varies between the different genetic etiologies of testicular dysgenesis. Individuals with *WT1* mutations seem at particularly high risk of germ cell cancer[5] but for gonadal dysgenesis due to mutations in other genes, such as *NR5A1*, the exact risk is not clear. In addition, as the current study showed that undetectable serum AMH and inhibin B cannot reliably predict the absence of germ cells, there is a need to find better prognostic markers.

Although this was not the focus of this study, it is notable that in three females with 45,X/46,XY DSD the 45,X cell line was only detected upon investigation of a second tissue, that is, a buccal smear, whereas a 46,XY or 46,X,idic(Yq) karyotype had been found in blood. Because of the significant clinical consequences of mosaicism with a 45,X cell line, that is, increased risk of cardiovascular complications such as aortic dilatation and dissection but also hypertension and ischaemic heart disease, as seen with Turner syndrome,[30] we recommend to perform a buccal smear for FISH analysis of the X and Y chromosome in girls with 46,XY gonadal dysgenesis if there is any feature suggestive of Turner syndrome. Such features include short stature, webbed neck, cubitus valgus or short fourth metacarpal/metatarsal. Of the three females in our study, two had short stature and one had normal stature but a history of aortic coarctation. However, clinical features of Turner syndrome are not present in all individuals with 45,X/46,XY mosaicism and variants.[1] Therefore, one could even consider screening all girls with 46,XY complete gonadal dysgenesis of unknown cause for sex chromosomal mosaicism, although no studies have investigated the yield of this approach.

Strengths of the current study are the revision of pathology by a single experienced pathologist, making use of a uniform protocol for immunohistochemistry. In addition, the study cohort is of a reasonable size considering 46,XY and 45,X/46,XY gonadal dysgenesis are rare conditions. Limitations are the fact that in some individuals, considerable time has elapsed between laboratory evaluation and gonadal biopsy/gonadectomy; the median time was 1.4 years, but in one individual, this was as long as 7.5 years, meaning serum levels of inhibin B and AMH might have changed in the meantime. In addition, for some individuals, only gonadal biopsies were available which might not have been representative of the entire gonad. However, the paediatric urologist who took the biopsies specifically sampled areas of the gonad that appeared abnormal, if such areas were identifiable at surgery, to increase the chance of identifying dysgenetic gonadal tissue if present, which would have the highest risk of germ cell tumours. Finally, the AMH assays used in the current study have now largely been replaced by automated assays with lower detection limits. It might be possible that AMH values reported as undetectable in the current study would have been detectable with these newer assays.

In conclusion, undetectable levels of AMH and inhibin B cannot reliably predict the absence of germ cells and germ cell tumours in individuals with 45,X/46,XY or 46,XY DSD. Although nearly half of the females had undergone spontaneous breast development, we did not find ovarian follicles at gonadectomy in any of the females, with or without detectable AMH and inhibin B. The results of this study can be used when counselling individuals about prophylactic gonadectomy, taking into account both the risk of germ cell cancer and the potential for gonadal function.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflicts of interest.

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