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Original Article

Histological analysis of testes in patients with 5 alpha-reductase deficiency type 2: Comparison with cryptorchid testes in patients without endocrinological abnormalities and a review of the literature

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Highlights

- Germ cell number did not decrease during the infantile period in patients with 5αRD.
- Testicular histology in 5αRD during adolescence was similar to that of cryptorchid testes without endocrinological abnormalities.
- Early orchiopexy is recommended to prevent decrease in germ cell number in 5αRD.

Abstract. As evidenced by the intact histology of the testes during infancy, testicular differentiation during the prenatal period occurs normally in individuals with 5 alpha-reductase type 2 deficiency (5αRD); however, a majority of these individuals suffer from azoospermia or oligospermia during adulthood, indicating that impaired spermatogenesis occurs postnatally. Although the accompanying cryptorchidism may be partly responsible for this process, the underlying mechanisms remain largely unknown. To address this issue, we retrospectively compared the histological findings of descended testes in a 3-mo-old patient and undescended testes in an 18-yr-old patient with 5αRD. In the latter, testicular histology was compared to that of cryptorchid testes obtained from five adolescent patients without endocrinological abnormalities. Histological findings of a 3-mo-old patient revealed normal number of germ cells with intact seminiferous tubules. In contrast, an 18-yr-old patient showed marked reduction in germ cell number and atrophic seminiferous tubules. The findings were very similar to those observed in cryptorchid testes without endocrinological abnormalities. These findings suggest that the decrease in germ cells in 5αRD patients may be at least partly caused by accompanying cryptorchidism. As the number of germ cells did not decrease during the infantile period, early orchiopexy is recommended to prevent a decrease in germ cell number and preserve fertility.

Key words: 5 alpha-reductase deficiency type 2, spermatogenesis, cryptorchidism

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Introduction

5 alpha-reductase type 2 deficiency (5αRD, OMIM 264600) is a 46,XY difference/disorder of sex development (DSD) with an autosomal recessive inheritance pattern that is caused by pathogenic variants of the *SRD5A2* gene encoding for 5 alpha-reductase type 2 (1–3), which is highly expressed in specific cells of the seminal vesicles, as well as the prostate and external genitalia (4, 5), catalyzing the conversion of testosterone to dihydrotestosterone (DHT), which has a more potent androgenic effect (6). Because DHT plays an important role in the virilization of the external genitalia (5, 7), loss-of-function variants of the *SRD5A2* gene in patients with the 46,XY karyotype typically result in a characteristic phenotype at birth with comparatively feminine external genitalia with testes and Wolffian duct derivatives.

When reared as a female with retained testes, patients masculinize and may develop a male gender identity during puberty (8), suggesting that prepubertal gonadectomy can be recommended to these patients; however, the decision to perform gonadectomy without their consent is currently challenging. Therefore, male assignment is usually recommended in patients with 5αRD (9). In addition, despite oligospermia and azoospermia being prevalent, there is case-based evidence to show the acquisition of paternity in 5αRD males (10–12), which also supports male assignment for 5αRD patients.

Theoretically, the testes of the affected 46,XY individuals are expected to differentiate normally and indeed, several reports have revealed intact histology of the testes in 5αRD patients during infantile and toddler periods (13, 14); however, almost all patients suffer from azoospermia or oligospermia during adulthood, indicating that deterioration of spermatogenesis likely occurs after infantile and toddler periods (13–16). Although there is evidence to show that the lack of 5 alpha-reductase type 2 activity itself affects germ cell numbers in 5αRD patients (14, 16), accompanying cryptorchidism has also been observed to adversely affect spermatogenesis, suggesting that the underlying mechanisms of defective spermatogenesis are complex. Therefore, to understand the underlying mechanisms, histological evaluation of the testes of 5αRD patients within a wide range of ages is important. However, an extremely limited number of studies have investigated this issue owing to the low incidence of 5αRD, highlighting the importance of case-based accumulation of histological findings in 5αRD patients.

Herein, we present the testicular histology of two patients with 5αRD, one at the age of 3 mo and the other at the age of 18 yr, and compare the number of germ cells with those of cryptorchid testes obtained from adolescent patients without endocrinological abnormalities and previously reported reference values to understand the characteristics of spermatogenesis in patients with 5αRD. We also discuss the characteristics of spermatogenesis in 5αRD, with a brief review of the literature.

Methods

Ethical considerations

This study was approved by the Ethical Review Board of Osaka Women's and Children's Hospital (No. 1345). The opt-out recruitment method for participation in the study was approved by the ethical review board.

Patients

We retrospectively evaluated the medical records of patients diagnosed with DSD who visited Osaka Women's and Children's Hospital prior to 2020 and identified two patients with 5αRD who had undergone histological examination of the testes. The patients at the time of histological evaluation were 3 mo (Patient A) and 18 yr (Patient B) old. Both patients had a 46,XY karyotype. A diagnosis of 5αRD was confirmed by genetic analysis of the *SRD5A2* gene, and compound heterozygous variants were identified in both patients [NM_000348.4:c.16C>T (p.Q6X) and NM_000348.4:c.679C>T (p.R227X) variants in patient A and NM_000348.4:c.78C>G (p.Y26X) and NM_000348.4:c.505C>T (p.Q169X) variants in patient B]. All variants except NM_000348.4:c.505C>T (p.Q169X) have been previously reported as pathogenic variants. The NM_000348.4:c.505C>T (p.Q169X) variant was not listed in the variant database [gnomAD (https://gnomad.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)]. According to the ACMG/AMP standards and guidelines for the interpretation of sequence variants (17), all identified variants were categorized as likely pathogenic (PVS1, PM2, and PM6).

To evaluate whether the histological findings of the testes in patient B were affected by the associated cryptorchidism, we compared the histological characteristics to those of the cryptorchid testes obtained from adolescent patients without endocrinological abnormalities. Based on the medical records, we identified four adolescent patients with isolated cryptorchidism (Nos. 1–4 in **Table 1**). Isolated cryptorchidism was defined as a condition in which any disorder known to cause cryptorchidism was not identified based on the medical records in this study. Among the four patients, three (Nos. 2–4 in **Table 1**) showed elevations in LH, FSH, and T, indicating that the hypothalamic-pituitary-gonadal (HPG) axis is likely intact in these patients. Although serum levels of LH, FSH, and T during the pubertal period were not available in one patient (No.1 in **Table 1**), this patient was included in the study because no findings based on medical records suggested the presence of an abnormal HPG axis. Additionally, one adolescent patient (No. 5 in **Table 1**) had bilateral undescended testes associated with Prune-belly syndrome. Because the pathogenesis of cryptorchidism in patients with Prune-belly syndrome is unlikely to have an endocrinological origin, as shown by the normal levels of LH, FSH, and T during puberty (**Table 1**), this patient was included in the study. The

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histological findings of cryptorchid testes were obtained from the medical records of these patients. The clinical characteristics and histological findings of the patients are summarized in **Table 1**.

Histological analysis

Orchidectomized or biopsied samples were paraffin-embedded and thin-sliced sections were prepared. After deparaffinization, hematoxylin and eosin (H&E) staining was performed. The number of germ cells per seminiferous tubule was examined in 50 seminiferous tubules per sample, and the average was calculated.

Result

Histological characteristics of the testes of 5αRD patients

Patient A had descended testes of diameter 12 mm. Histological analysis of the orchidectomized testes revealed intact seminiferous tubules and germ cells (**Fig. 1A**). Edematous findings were not observed in the interstitium. Patient B displayed bilateral undescended testes, both of which located in the inguinal canal. LH-RH analog treatment was administered for a period of 5 years before orchidectomy. Each testis was 12 mm

Table 1. Histological characteristics of cryptorchid testes in patients without endocrinological abnormalities

No.	Age		Testis location	Histological findings		LH (mIU/mL) FSH (mIU/mL) T (ng/dL)	Type of cryptorchidism
				Seminiferous tubule (ST)	Germ cell number per ST		
1	10	Right Left	Outside inguinal canal Scrotum	Age-appropriate ST diameters Basement membrane thickening not observed	4.5	< 0.2 1.9 5	Isolated cryptorchidism
2	11	Right Left	Intraperitoneal Scrotum	Age-appropriate ST diameters Basement membrane thickening (mild) Sertoli cell-only syndrome (100%)	0	1.2 1.9 124.1	Isolated cryptorchidism
3	16	Right Left	Outside inguinal canal Scrotum	Age-appropriate ST diameters Basement membrane thickening (mild) Sertoli cell-only syndrome (96%)	0.07	3.1 6.2 701.9	Isolated cryptorchidism
4	13	Right Left	Scrotum (retractile) Outside inguinal canal	Atrophic ST Basement membrane thickening (mild) Sertoli cell-only syndrome (100%)	0	4 4.7 185.7	Isolated cryptorchidism
5	14	Right Left	Inguinal canal Inguinal canal	Atrophic ST Basement membrane thickening (mild) Sertoli cell-only syndrome (80%)	0.26	6.2 13.5 566.8	Prune-belly syndrome-associated cryptorchidism

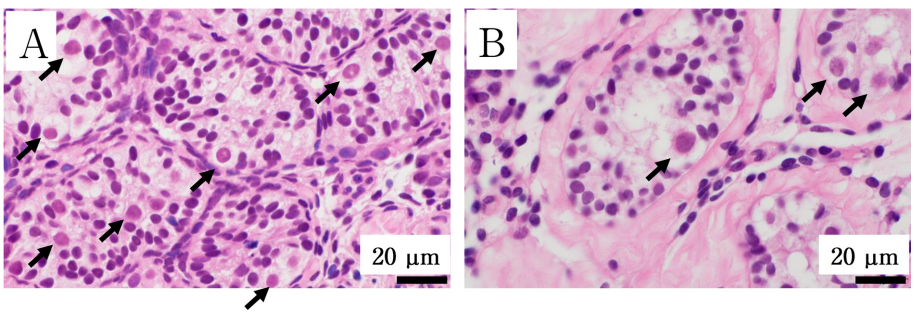

Fig. 1. Hematoxylin and eosin (H&E) staining of testes in patients with 5 alpha-reductase deficiency. A: H&E staining of patient A. Intact seminiferous tubules and spermatogonia are shown. Arrows indicate spermatogonia. B: H&E staining of patient B. Basal membrane was edematous and thick. Although the majority of the seminiferous tubules were histologically compatible with Sertoli cell-only syndrome, some contained spermatogonia, as indicated by arrows.

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in diameter and possessed an epididymis, spermatic cord-like structure, and tunica albuginea of 300–400 μm thickness. The atrophic seminiferous tubules were irregularly embedded in the sparse interstitium (**Fig. 1B**). The basement membranes of seminiferous tubules were edematous and thick (**Fig. 1B**). The majority of seminiferous tubules (95%) were histologically compatible with Sertoli cell-only syndrome, whereas the remaining seminiferous tubules contained germ cells, although their numbers were markedly reduced (**Fig. 1B**).

Germ cell number per seminiferous tubule in 5αRD patients and its comparison with reference values from healthy controls

In patient A, the average number of germ cells per seminiferous tubule of the right and left testes was 2.9 and 2.6, respectively, which was at the lower limit of the age-matched normal range according to a report by Hadziselimovic *et al.* (**Fig. 2**) (18). In patient B, the average number of germ cells per seminiferous tubule was markedly low with 0.28 and 0.08 in the right and left testes, respectively. Germ cells were detected in 4 and 1 of 50 seminiferous tubules in the right and left testes, respectively. Since patient B was treated with an LH-RH analog from the age of 13, the germ cell count in patient B was compared to that from reference values in healthy patients at 13-yr-old (18). According to reference data by Hadziselimovic *et al.*, the germ cell count in patient B was much lower than the lower limit of the normal range (**Fig. 2**) (18).

Comparison of testicular histology of 5αRD patients with that of cryptorchid testes obtained from adolescent patients without endocrinological abnormalities

To evaluate whether the histological findings of the testes in patient B were affected by the associated cryptorchidism, we compared them with those obtained from four adolescent patients with isolated cryptorchidism and one adolescent patient with Prune-belly syndrome (**Table 1**). In three patients with unilateral cryptorchidism (Nos. 1–3 in **Table 1**), the cryptorchid testes were histologically evaluated, whereas the left testes were investigated in two patients with isolated bilateral cryptorchidism (Nos. 4 and 5 in **Table 1**). The clinical characteristics and histological findings of the patients are presented in **Table 1** and **Fig. 3**. The average number of germ cells per seminiferous tubule markedly decreased in four patients (Nos. 2–5 in **Table 1** and **Fig. 2**). Basement membrane thickening of the seminiferous tubules was observed in four patients (Nos. 2–5 in **Table 1**) but was not observed in the patient with a normal number of germ cells (No. 1 in **Table 1**). Seminiferous tubules were atrophic and reduced in number, particularly in patients Nos. 3, 4, and 5 (**Table 1**). These findings indicate that the histological findings

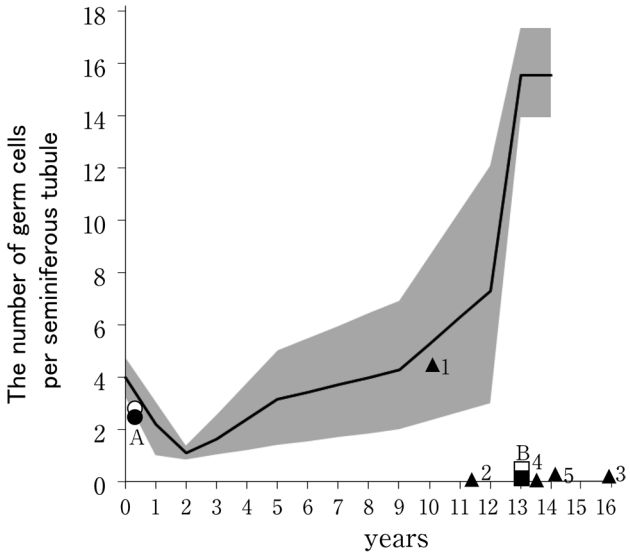

Fig. 2. The number of germ cells per seminiferous tubule in patients with 5 alpha-reductase deficiency. The circles and squares indicate patients A and B with 5 alpha-reductase deficiency, respectively. Open and closed circles indicate the number of germ cells per seminiferous tubule in the right and left testicle, respectively. The letters A and B next to the circles/squares indicate patients A and B, respectively. Triangles show patients with cryptorchidism without endocrinological abnormalities. The numbers next to the triangles represent the number of patients, as described in **Table 2**. The shaded area represents the normal range of the number of germ cells per seminiferous tubule and was adopted from Hadziselimovic *et al.* (18).

of the testes from patients with 5αRD were similar to those of patients with isolated cryptorchidism.

Discussion

Although most previous reports have described impaired spermatogenesis and fertility in patients with 5αRD (10), there is case-based evidence of the acquisition of paternity in adults with 5αRD (12, 19), indicating the diversity in the extent of impaired spermatogenesis in patients with 5αRD. The pathogenesis of infertility has been suggested to include defects in spermatogenesis due to a lack of 5 alpha-reductase activity, undescended testes, or both (10). These assumptions are primarily based on the histological analysis of testes in patients with 5αRD and isolated cryptorchidism; however, owing to the low incidence of 5αRD, few studies have reported the histological evaluation of testes in 5αRD patients. In addition, age-dependent analysis of testicular histology is required to understand the mode of spermatogenesis in 5αRD; therefore, case-based accumulation of histological findings in a wide range of ages is necessary to fully understand the characteristics of spermatogenesis in patients with 5αRD.

Testicular histology during infancy has previously

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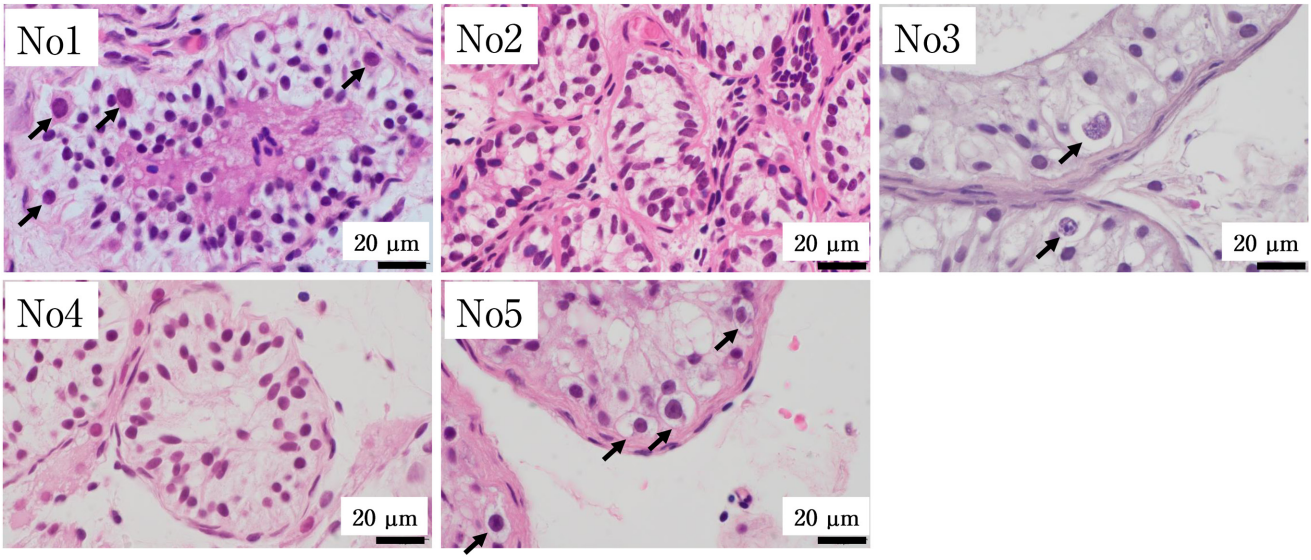

Fig. 3. Hematoxylin and eosin staining of testes in patients with isolated cryptorchidism. The numbers in each figure correspond to the number of patients in **Table 1**. Mild thickening of the basement membrane and a decreased number of germ cells were observed in all patients except for No1. Arrows indicate germ cells.

been reported in one patient (14). Hadziselimovic *et al*. described the testicular histology of an 8-mo-old boy with undescended testes and found that the number of spermatogonia did not decrease (No. 2 in **Table 2**) (14). Consistent with this, we herein report the histology of a 3-mo-old patient and show that the number of germ cells did not decrease with the normal structure of the seminiferous tubules (No. 1 in **Table 2**). In addition, there are four reported cases of testicular histology during the toddler period (Nos. 3–6 in **Table 2**). Hadziselimovic *et al*. investigated the number of germ cells in 2- and 4-yr-old patients with undescended testes and found it to be unchanged from that of age-and testicular location-matched patients with isolated cryptorchidism (Nos. 5 and 6 in **Table 2**) (14). Steger *et al*. also reported the presence of spermatogonia in 1- and 2-yr-old patients; however, quantitative analysis of germ cell numbers was not performed (Nos. 3 and 4 in **Table 2**) (13). The location of the testes is not described in this study. Taken together, although limited evidence is available, the number of germ cells may be maintained during the infantile and toddler periods in patients with 5αRD, even when accompanied by undescended testes, suggesting that the lack of 5 alpha-reductase activity is unlikely to cause decreased germ cell numbers during these periods.

There have also been limited studies that have analyzed the testicular histology of 5αRD patients during childhood, adolescence, and young adulthood (Nos.7–27 in **Table 2**) (13, 14, 16, 20–24). As shown in **Table 2**, germ cells were present during childhood irrespective of the presence of cryptorchidism, whereas during adolescence, there is evidence of the lack of germ cells in those accompanied by cryptorchidism (Nos. 17 and 18 in **Table 2**). Importantly, germ cells were present in all adolescent patients without cryptorchidism (Nos. 11, 12, and 19 in **Table 2**), indicating that testicular location may be an important factor that compromises spermatogenesis during adolescence. However, in adulthood, there is evidence of the lack of germ cells in the absence of cryptorchidism (No.24 in **Table 2**) (16). Consistent with this, Cai *et al*. performed semen analysis in nine adult patients with 5αRD, which showed that one out of six patients with descended testes showed normospermia, two showed oligospermia, whereas the remaining three patients showed azoospermia (16). These findings indicate that factors other than testicular location are also involved in impaired spermatogenesis in 5αRD.

As spermatocytes usually appear at four years of age (25–27), the lack of spermatocytes during this period is indicative of a defect in spermatogenesis from spermatogonia to spermatocytes. Although there are limited publications available on this issue, Hadziselimovic *et al*. performed a histological analysis of the testes of a 9-yr-old patient (No. 7 in **Table 2**) with descended testes and found a lack of spermatocytes, although the number of germ cells resided at the lower limit of normal germ cell numbers (14), indicating that the lack of 5 alpha-reductase activity may inhibit the development of spermatogonia into spermatocytes independent of testicular location (14). However, there is conflicting evidence as seen in a 16-yr-old patient, where spermatogenesis was arrested at the level of spermatocytes (Nos. 13 and 15 in **Table 2**) (13, 20). These findings indicate that the developmental stage of spermatogenesis varies among patients with 5αRD during childhood and that the effects of genetic (lack of 5α reductase activity) and environmental (testicular location) interactions on spermatogenesis in 5αRD remain largely unknown because of the paucity of investigation. Further studies are required to elucidate this issue.

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Table 2. Summary of histological findings of testes in patients with 5α reductase deficiency aged below 30 yr old

No.	Age	Cryptorchidism	Testis location	Histological analysis			Reference
				Seminiferous tubule	Germ cell number **	Characteristics of Spermatogenesis	
1	3 m	–	Labial-scrotal folds	No specific findings reported	+++	Spermatogonia observed	Present case
2	8 m	+	Pubic tubercle	ND	+++	Spermatogonia observed	14
3	1 y	ND	ND	No specific findings reported	present	Immature spermatogonia observed	13
4	2 y	ND	ND	No specific findings reported	present	Immature spermatogonia observed	13
5	2 y	+	Pubic tubercle	ND	+++	Typical Ad spermatogonia observed	14
6	4 y	+	Pubic tubercle	ND	+++	Typical Ad spermatogonia observed	14
7	9 y	–	Scrotum	ND	++	Decreased number of spermatogonia Lack of spermatocytes	14
8	10 y	ND	ND	No specific findings reported	present	Spermatogonia observed	13
9	11 y	+	Pubic tubercle	ND	+++	Normal number of spermatogonia Lack of spermatocytes	14
10	14 y	+	Inguinal canal	ND	present	Spermatogonia observed Lack of spermatocyte	20
11	15 y	–	Labial-scrotal folds	No interstitial fibrosis observed	present	Germ cell maturation occasionally observed	15
12	16 y	Rt: – Lt: +	Rt: Scrotum Lt: Inguinal canal	ND	present	Spermatids observed	20
13	16 y	+	Inguinal canal	Normal basement membrane Tubules lined by Sertoli cells	+++ / –	No germ cells observed in one testis Spermatocyte observed in the other	20
14	16 y	+	Inguinal canal	ND	present	Spermatogonia observed Lack of spermatocyte	20
15	16 y	ND	ND	Immature seminiferous tubules included (1%) Sertoli cell-only syndrome (60%)	present	Impaired spermatogenesis Spermatocytes observed	13
16	17 y	+	Inguinal canal	Basement membrane thickening Immature seminiferous tubules (8%) Sertoli cell-only syndrome (92%)	+	Few spermatogonia observed	21
17	17 y	+	Inguinal canal	Tubular atrophy Slight thickening of basement membrane Sertoli cell-only syndrome	–	No germ cells observed	22
18	18 y	+	Inguinal canal	Tubular atrophy Slight thickening of basement membrane Sertoli cell-only syndrome	–	No germ cells observed	22
19	18 y	–	Labial-scrotal folds	Normal interstitial and tubular structures	present	Spermatogenesis observed	23
20	18 y	ND	ND	Immature seminiferous tubules included (8-12%) Sertoli cell-only syndrome (88-92%)	ND	ND	13
21	18y	ND	ND	Immature seminiferous tubules included (4%) Sertoli cell-only syndrome (96%)	ND	ND	13
22	18y	+	Inguinal canal	Atrophic seminiferous tubules with basement membrane thickening Relatively prominent interstitium Sertoli cell-only syndrome (95%)	+	Germ cell number markedly decreased Lack of spermatocyte	Present case
23	20 y	–	Labial-scrotal folds	Basement membrane thickening Low lying appearance of germinal epithelium	present	Impaired spermatogenesis with few mature sperm	16
24	21 y	–	Labial-scrotal folds	Atrophy with basement membrane sclerosis Immature Sertoli cells	–	No germ cells observed	16
25	22 y	–	Labial-scrotal folds	Some luminal sloughing Prominent tunica propria	present	Moderately severe hypospermatogenesis	16
26	24 y	+	Inguinal canal	Diffuse mild peritubular fibrosis	+	Markedly decreased number of germ cells Nearly arrested at primary spermatocyte step	16
27	25 y	+ → – *	Inguinal canal →Scrotum*	ND	present	Spermatogenesis limited to the spermatid step	24

y, years; m, months; Rt, right; Lt, left. ND: not described. * Spontaneously moved from the inguinal canal to the scrotum during puberty. ** present: germ cells are present, but germ cell number is not evaluated: +++, normal; ++, decreased; +, markedly decreased, –: not observed.

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As described above, there is evidence that the number of germ cells does not decrease during the infantile period or early childhood in patients with 5αRD. As cryptorchidism is known to be a risk factor for a reduction in the number of germ cells, coexisting cryptorchidism is likely to be partly responsible for the reduced germ cell number in 5αRD. Indeed, the present study showed that the histological findings of isolated cryptorchidism, including decreased germ cell number and basal membrane thickening of seminiferous tubules, share similarities with those of 5αRD, supporting the notion that testis location is also an important determinant of germ cell number in 5αRD. Importantly, there is evidence to show that the number of germ cells does not decrease during the first six months after birth, even in cases with intra-abdominal testes in patients with isolated cryptorchidism (18), indicating that early orchiopexy is beneficial for the preservation of germ cell number. This supports the case-based evidence, including the present case, which shows that the number of germ cells does not decrease during infancy in 5αRD.

In summary, we herein report the testicular histology of 3-mo-old and 18-yr-old patients with 5αRD and show histological findings similar to those reported previously. The similarity in testicular histology between 5αRD patients with undescended testes and cryptorchid patients without endocrinological abnormalities may suggest the importance of testicular position in the reduction of germ cells in patients with 5αRD. Importantly, there is evidence of paternity by intrauterine insemination in a 5αRD patient with bilaterally descended testes (19), indicating that a lack of 5α reductase activity does not necessarily mean a lack of fertility in 5αRD. Although there are several limitations in this study, such as the paucity of samples, the intact histology without reductions in germ cell numbers during the infantile period indicates that early orchiopexy is recommended to prevent the additional loss of germ cells by undescended testes. Further accumulation of case-based analyses is required to better understand the pathogenesis of reduced germ cell numbers in patients with 5αRD.

Conflict of interests: The authors have no conflicts of interest to disclose.

Acknowledgments

T.W. and M.K. designed the study and prepared the manuscript. C. I. and M.T. performed the histological evaluations. T.W., C.I., F. Matsui, and F. Matsumoto collected samples and data. All authors analyzed the data. All authors have read and approved the submission of this manuscript. No funding support was received for this study.

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