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# The correlation between clinical features and ultrastructure of testis of non-mosaic Klinefelter's syndrome patients with hypogonadism and androgen deficiency: A case report

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ABSTRACT

*Background:* Klinefelter Syndrome (KS) is a sex chromosomal syndrome usually with an extra X chromosome (47, XXY) in males, which has various phenotype (mosaicism 47, XXY/46, XY, or more chromosomes 48, XXXY, 49, XXXXY) and clinical features, including eunuchoid body proportions, abnormally long legs and arm span, gynecomastia, ynecomastia, absent or decreased facial and pubic hair, small hyalinized testes, small penis, below-normal verbal intelligence quotient, and learning difficulties. At present, there are no studies on the correlation between the clinical characteristics of patients with KS and the ultrastructural changes of intracellular organelles in testicular tissue in China.

*Case presentation:* Here we report the ultrastructure manifestation of the testis tissues in a KS patient with hypogonadism and androgen deficiency, to find a relationship between ultrastructural changes of organelles and spermatogenic dysfunction, clinical features, timing of surgery and metabolic abnormalities. It has been shown that the spermatocytes are absent and the ultrastructure of Sertoli cells and Leydig cells is obviously abnormal, which may lead to spermatogenic dysfunction, androgen deficiency, impaired glucose tolerance (IGT), and abdominal fat accumulation.

*Conclusions:* Based on the European Academy of Andrology (EAA) Gudilines on Klinefelter Syndrome, this study conducted a retrospective study on the diagnosis and treatment of one adult patient with KS, aiming to provide a standardized diagnosis and treatment for patients with KS. This study is also highly concerned with the correlation between the ultrastructural changes of target organs and clinical symptoms.

## 1. Introduction

Klinefelter's Syndrome (KS) is the most common sex chromosome abnormality in men, with an incidence of approximately 1/600 ~

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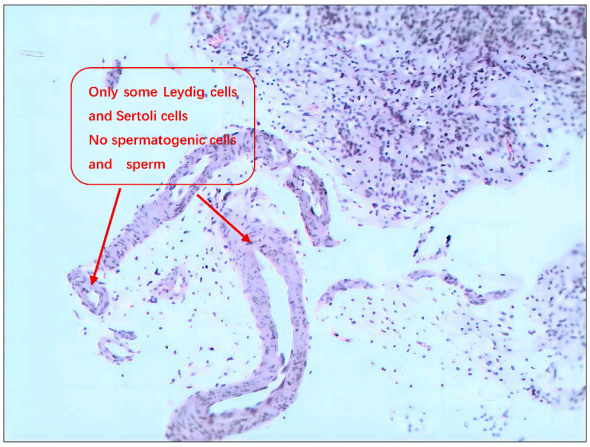
1/660 individuals [1]. The usually karyotype presenting is non-mosaic 47 XXY (80%–85%), and other karyotypes include 46, XY/47, XXY chimeric type, 48, XXXY, 49, XXXXY, etc. [2]. Here, we report a case of KS patients with hypogonadism and androgen deficiency, whose testicular ultrastructure showed hyaline convoluted tubules, vacuolated Sertoli cells and Leydig cells. The changes in the testicular ultrastructure may be the result of gene phenotype and may be related to various clinical characteristics of this patient.

Based on these results, we should pay more attention to the long-term health management of this patient in addition to performing assisted reproductive technology (ART). According to this patient's current impaired glucose tolerance (IGT), fatty liver and other conditions, we should predict the risk of metabolic syndrome (MetS) and cardiovascular, respiratory and gastrointestinal diseases and provide health guidance and corresponding medical interventions.

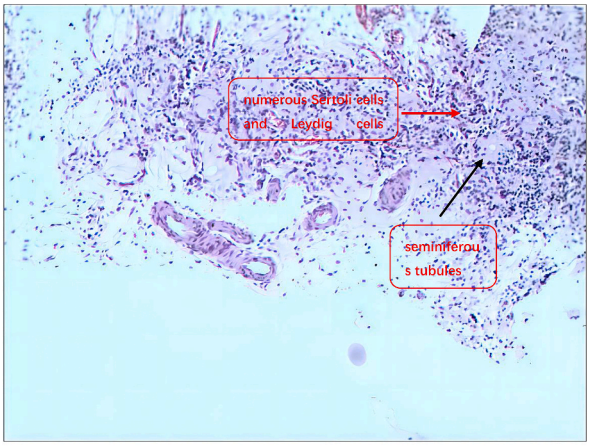
## 2. Case presentation

This couple, who had been married for three years, had been living together, had regular sex without contraception; however they had no child up to now. This patient was a 32-year-old male (his spouse was a 29-year-old woman) consulting Microdissection testicular sperm extraction (mTESE) and seeking long-term androgen treatment for his infertility and androgen deficiency condition at the Urology Center of Joint Logistic Support Force 940th Hospital. The patient had undergone reproductive examination one year before marriage as follows: The results of multiple computer-aided semen analysis revealed azoospermia (two semen analyses after centrifugation), autosomal karyotype analysis (non-mosaic, 47, XXY), microdeletion of the Y chromosome (normal), and serum testosterone (T) (1.18 nmol/L) in the outpatient department of the 940th hospital. He was treated with testosterone replacement therapy (TRT) twice daily (T undecanoate soft capsules 80 mg orally/morning, 40 mg orally/night) for 6 months. The TRT was terminated after marriage. He had no history of surgery, blood transfusion, infectious diseases, mumps, sexually transmitted diseases, gonadal toxin exposure, or family genetic history. He had a history of left upper limb fracture and accepted only plaster fixation.

Upon admission to the hospital, the patient accepted a careful physical examination (PE), which revealed overweight, muscles,




A: testicular tissue (Left) light microscope ×40




B: testicular tissue (Right) light microscope ×40

**Fig. 1.** The results of pathological examination × 40.

obvious secondary sex characteristics, normal facial and body hair, abdominal fat accumulation, slender upper limbs, normal development of penis and with small testes (4 ml in both testes). Some other positive signs were also observed. He also underwent serological detection and imaging examination, including an oral glucose tolerance test (OGTT), hormone test, abdominal color ultrasonography, and male reproductive system color ultrasonography.

After thorough preoperative preparation and multiple conversations, the patinet underwent mTESE. During the operation, it was found that the parenchyma of the bilateral testes was soft in texture. When the white testicular membrane was cut along the central axis of the testicle to expose the testicular parenchyma, we have found that most of the seminiferous tubules were opaque, of which the pipe diameter was relatively small under the microscope (eyepiece 20×). We selected some testicle parenchyma samples, of which the seminiferous tubules were relatively transparent and coarse, sent it to the embryo laboratory of the Reproductive Center, which revealed no any morphological sperm. Some testicular tissues were examined by pathology and transmission electron microscopy (TEM) after the operation.

Material and Methods: Testicular biopsy specimens, measuring 3 × 3 × 5 mm were fixed in 10% formalin and embedded in paraffin, which were cut into sections of 4 μm thickness, and were stained with hematoxylin and eosin (H.E). After prefixing with 3% glutaraldehyde and refixing with 1% osmium tetroxide, light microscopic sections measuring 3 × 3 × 5 mm were progressively dehydrated with acetone and embedded with Epon812, which were cut into sections of 60 nm thickness. These ultrathin sections were flattened and double-stained with uranyl acetate and lead citrate and then studied under a Hitachi-HT7700 electron microscope.

The results of the pathological examination as follows: the parenchyma of testicular tissue morphology was abnormal, the spermatogenic tubule was atrophied, only some Leydig cells and Sertoli cells were seen, and spermatogenic cells were missing. The arrangement of internal cells was disordered, permatogonial cells were absent, and spermatogenesis was not observed in the seminiferous tubules. The cavity area was enlarged, and spermatozoa were absent. Numerous Sertoli cells and Leydig cells nodules (red arrow labelled) were observed to surrounded around small groups of seminiferous tubules (black arrow labelled) in the right testicular tissue (shown in Fig. 1). According to the criteria of the Johnsen score (Table 1), the score was 2.

The results of the ultrastructure of Leydig cells and Sertoli cells under TEM are shown, The spermatogenic tubules were shown transparent. Being insufficient organelles, the Leydig cells and Sertoli cells exhibited obvious changes and damages of ultrastructure, in which they showed obvious swelling and vacuolation (blue arrow labelled). The structure of the nucleus (N) was irregular, in which the nucleoli (Nu) shrinkage (red arrow labelled) can be seen in individual cells, and the perinuclear space was significantly widened. Most cells lacked the nucleolus (Nu), mitochondria (M), and Golgi apparatus (Go). The appearance of Leydig cells was different from that of normal mature cells, exhibiting an unusually increased cytoplasm, and displaying several morphological abnormalities. The normal nuclei (N) of Leydig cells were almost invisible and not found. The N morphology was disordered and most nucleoli (Nu) was disappeared (red arrow). It was seen that the smooth endoplasmic reticulum (SER) presented concentric multilayer pore-free poolsin, of which the part surrounding the cytoplasm contained microcrystalline inclusion bodies (black arrow labelled), in Leydig cells. Some microcrystalline inclusions of the nuclei were deposited in N (shown in Fig. 2).

The 940th Hospital is not a cadcorpse institution, and ethical approval is the reason why the normal testicular tissue cannot be obtained from autopsies as a control group. Therefore, the above interpretation of the results of ultrastructure under TEM was based on the detailed review and interpretation after referring to the reports issued by Wuhan Servicebio Technology Co., Ltd. and the classical references [3,6,31] for TEM images and descriptions of infertility patients. The obvious limitation of this study was the lack of use of healthy testicular tissue to be compared with the proband.

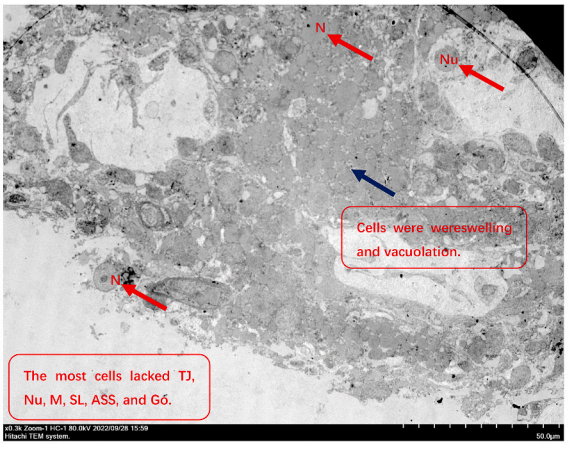
The final diagnosis was as follows: 1. Primary male infertility, nonobstructive asthenospermia (NOA), 2. Klinefelter's syndrome, karyotype: 47, XXY, 3. IGT, 4. Fatty liver, 5. Chronic cholecystitis, 6. Gallbladder stones, multiple, 7. Neurodermatitis, 8. Foot moss, onychomycosis.

We recommended that this couple to receive artificial insemination with donor semen (AID) or *in vitro* fertilization and embryo transfer (IVF-ET) by donor. The couple subsequently entered the AID preparation stage. The total results of this patient are presented in Table 2.

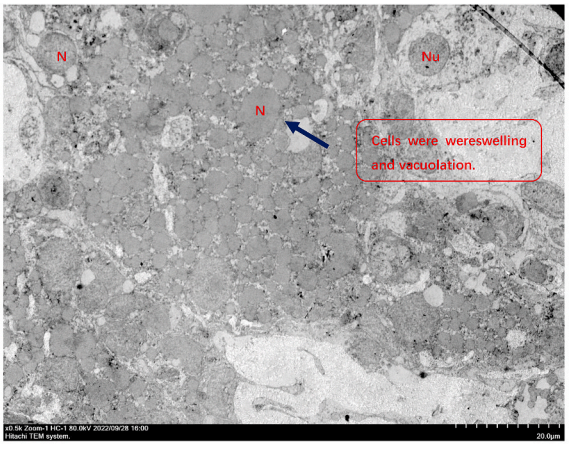
**Table 1**
Johnsen score criteria.

| scores | Spermatogenesis level |
|---|---|
| 10 | The whole process of spermatogenesis is observed. |
| 9 | Spermatogenesis was slightly impaired. |
| 8 | Each seminiferous tubule has less than 5 sperm. |
| 7 | Mature spermatogenic cells are absent. Most early spermatogenic cells can be observed. |
| 6 | Early spermatogenic cells are reduced or missing and the spermatogenesis process is halted at the stage of spermatogenic cell formation. |
| 5 | Many spermatocytes. |
| 4 | Few spermatocytes, and the spermatogenesis process is halted at the stage of primary spermatocytes. |
| 3 | Only spermatogonia. |
| 2 | No spermatogenic cells, only supporting cells. |
| 1 | No vas deferens epithelial cells. Tubular sclerosis is noted. |

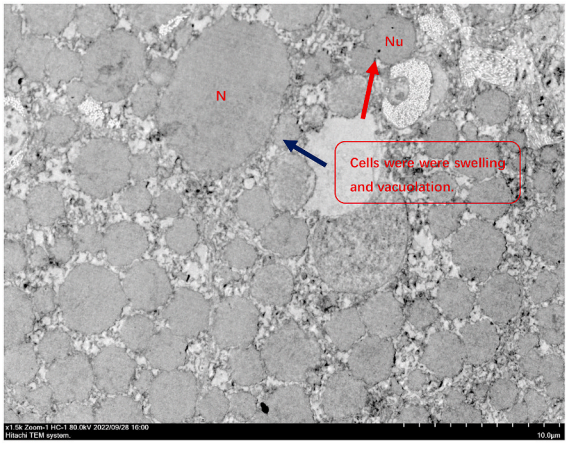
**Note:** The morphological changes of seminiferous tubules, Sertoli cells, and Leydig cells in the patient's testis tissue are observed under a light microscope. The higher the scores, the better the spermatogenesis. The lower the scores, the worse the quality.



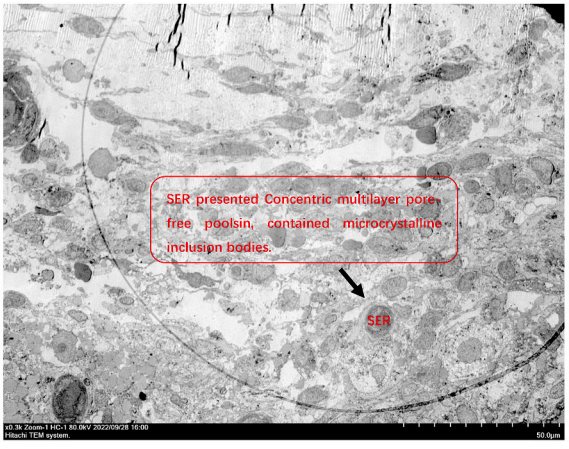

C: Ultrastructure of Sertoli cells TEM×50.0μm



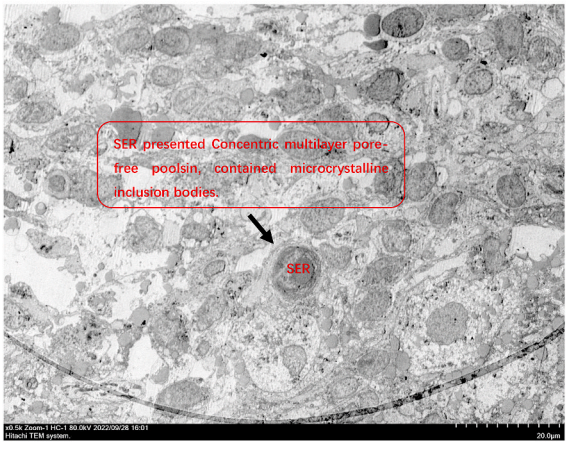

D: Ultrastructure of Sertoli cells TEM×20.0μm



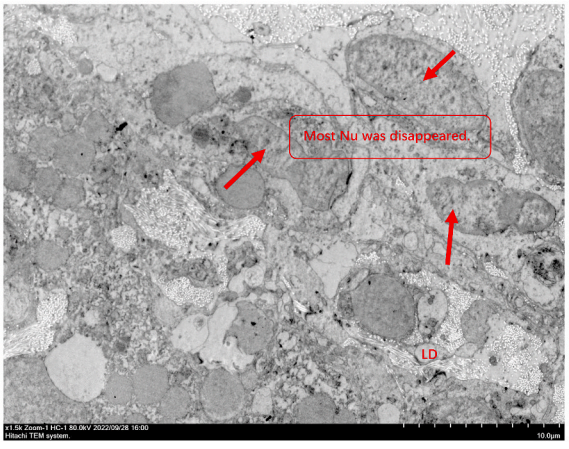

E: Ultrastructure of Sertoli cells TEM×10.0μm




F: Ultrastructure of Leydig cells TEM×50.0μm




G: Ultrastructure of Leydig cells TEM×20.0μm




H: Ultrastructure of most nucleoli (Nu) was disappearedmost nucleoli (Nu) was disappearedLeydig cells TEM×10.0μm

**Fig. 2.** The results of ultrastructure of Leydig cells and Sertoli cells under TEM
**Note**: TJ: tight junctions, N: nuclei, Nu: nucleoli, M: mitochondria, SER: smooth endoplasmic reticulum, LD: lipid droplets, SL: secondary lysosomes, ASS: autophagic lysosomes, Go: Golgi.

**Table 2**
The total clinical results.

| The results of the male spouse | | |
|---|---|---|
| General Clinical Data | Age | 32-year-old |
| | Height/Weight | 175 cm/90 kg |
| | BMI | 29.39 |
| Physical Examination (PE) | General appearance | Obvious secondary sex characteristics, muscles, normal facial and body hair, abdominal fat accumulation, slender upper limbs. |
| | Reproductive specialist PE | Normal development of penis and with small testes (4 ml in both testes). |
| | Other positive signs | The red maculopapular lesion (about 3 × 5cm in size) on the left neck. The thumb of the foot shows a grayish-yellow change, suspected fungal infection. |
| Serum Detection | OGTT: FPG | 6.2 mmol/L |
| | 30min PG | 7.9 mmol/L |
| | 2h PG | 10.3 mmol/L |
| | TG hormone test: T | 2.81 mmol/L |
| | FSH | 1.47 nmol/L |
| | LH | 16.25 mIU/ml |
| | PRL | 8.03 mIU/ml |
| | E2 | 14.9 μg/L<br>63.0 pmol/L |
| Color Ultrasonography | Abdominal color ultrasonography: | 1. Fatty liver, chronic cholecystitis, gallbladder multiple stones. |
| | Male reproductive system color ultrasonography: | 2. There were no obvious abnormalities in the sonograms of spleen, pancreas and bilateral kidney.<br>1.There were no obvious abnormalities in the sonograms of bladder and prostate.<br>2. There are unclear in the sonograms of bilateral seminal vesicles.<br>3. Bilateral testicle volume is small, about 4 ml respectively.<br>4. There were no obvious abnormalities in the sonograms of bilateral epididymal ultrasonography. |
| Computer-aided Semen Analysis | Azoospermia | twice semen analyses after centrifugation |
| Autosomal Karyotype Analysis | Non-mosaic, 47, XXY | |
| Microdeletion of The Y chromosome | Normal | |
| History and Treatment | 1. Marriage for three years, living together, and regular sex without contraception; however they had no child up to now.<br>2. No history of open and endoscopic surgery, blood transfusion, infectious disease, mumps, sexually transmitted diseases, gonadal toxin exposure, or family genetic history.<br>3. The patient was treated with TRT twice daily (T undecanoate soft capsules 80 mg orally/morning, 40 mg orally/night) for 6 months. He stoppedTRT after marriage.<br>4. A left carpal fracture has healed. | |
| Preoperative Diagnosis | Primary Male infertility, Nonobstructive asthenospermia (NOA) and Klinefelter's syndrome | |
| Surgical Condition | 1. Most of seminiferous tubules were opaque, of which pipe diameter was relatively small.<br>2. A small part of seminiferous tubules were relatively transparent and coarse. (by surgical microscope x 20) | |
| Pathological Examination | The parenchyme of testicular tissue morphology was abnormal: spermatogenic tubule was atrophy, only Leydig cells and Sertoli cells were seen, and spermatogenic cells were missing. The Johnsen score was 2. | |
| TEM | The Leydig cells and Sertoli cells exhibited obvious changes and damages of ultrastructure (shown in Fig. 2) | |
| Final Diagnosis | 1. Primary male infertility, NOA<br>2. Klinefelter's syndrome, karyotype: 47, XXY<br>3. IGT<br>4. Fatty liver<br>5. Chronic cholecystitis<br>6. Gallbladder stones, multiple<br>7. Neurodermatitis<br>8. Foot moss, onychomycosis | |
| Subsequent treatment | They have entered the ART preparation stage | |

## 3. Discussion and conclusions

According to the EAA Guidelines on Klinefelter's Syndrome [31], finding focal spermatogenesis is the most important way to extract spermatozoa using mTESE. mTESE combined with ICSI has become the treatment of choice for patients with NOA related to KS who want to have children in their kinship. This patient has sought ART treatment and chose mTESE. Notably, we did not find or obtain mature sperm for ICSI treatment. In the next steps, what problems do patients face besides continuing to the ART treatment?

**Question 1**. What is the relationship between the ultrastructural changes of organelles and spermatogenic dysfunction, clinical features, and timing of surgery?

Normal Leydig cells are distributed in groups between spermatogenic tubules, which can synthesize and secrete male hormones, promoting the development of male reproductive organs and the occurrence of sperm, as well as maintaining the secondary sex characteristics and sexual function. Sertoli cells surround spermatogenic cells with elongated processes and secrete inhibin, androgen binding protein, and testicular fluid to protect and feed spermatogenic cells. According to the results of pathology and TEM after the

operation, spermatogenic tubules were atrophied, only some Leydig cells and Sertoli cells could be seen, and spermatogenic cells could not be found. It has also been found that the ultrastructure of Leydig cells and Sertoli cells have undergone obvious changes and damages. At present, there are a few studies on the ultrastructure of testicular tissue in patients with KS compared with normal testicular tissue were shown: The abundance of SER is a common finding in normal human Leydig cells and Sertoli cells. There have also found the developed Golgi (Go) complexes and abundant mitochondria are also developed in normal Sertoli cells [3]. Ultrastructure and immunostaining were studied in the peritubular myofibroblasts of testes from normal men and KS patients, which showed that the seminiferous tubules were presented a progressive degree of sclerosis measured as thickening of the lamina propria [4]. However, undifferentiated spermatogonia remained positive, which could be harvested and potentially used for infertility therapy in a patients with KS [5]. Ultrastructurally abnormal Leydig cells have little or no function, whereas ultrastructurally normal Leydig cells have been principally responsible for T biosynthesis in these patients, in which the level of T expression was very low. These observations in KS, Castillo's syndromes, and cryptorchid testes, have suggested that disorders of Leydig cells and Sertoli cells reflected a congenital deficiency producing abnormal development [6,7]. However, the Leydig cells and Sertoli cells exhibited obvious swelling and vacuolation, which constitutes a peculiar feature of this KS patient. Since the functional decline and partial loss of the Leydig cells at the source of androgen synthesis have been shown, this patient has presented with androgen deficiency and hypogonadism with NOA. It has been shown that IGT, fatty liver, abdominal fat accumulation, and left carpal fracture were most likely related to the decrease in bone density, which was closely related to androgen deficiency.

Although there are options of mTESE combined with ICSI for KS patients, the use of ICSI may be limited [8]. Due to a certain traditional education culture and possible macho factors, the patient did not receive a reproductive specialist examination until he was an adult before marriage. He accepted mTESE at the age of 32 years, which might have been a little late. There has always been controversy regarding fertility protection and the timing of mTESE for patients with KS [9–15]. Banking testicular tissue from boys with prepubertal KS should be considered in a research framework with the help of diagnosed KS patients owing to the use of noninvasive prenatal testing [9]. Adolescents and their parents should undergo a detailed reproductive consultation process and share decision-making discussions before considering testicular sperm retrieval [10]. Although studies have suggested that age should not be a deterrent for KS patients to undergo mTESE [11] and that age between age 15 and young adulthood is the best stage of Leydig cell function [12], it is still recommended to perform mTESE before the critical age of 35 years in adults [13–16]. Preoperative attention should be paid to TRT and auxiliary examination results, especially color ultrasound of the reproductive system [16].

According to the recommendations in the guidelines [31], prenatal diagnosis, karyotype analysis, follow-up and treatment of children and adolescents with KS should be strengthened. The patient in this study clearly missed these critical points.

Question 2: What about long-term TRT, MetS, follow-up, health guidance, etc., for the patient?

KS is the most common hereditary cause of male hypogonadism and androgen deficiency, which are the sex chromosome abnormalities associated with male infertility. Attention must be paid to the following: Long-term male hypogonadism comprises a significant health risk, such as IGT, obesity, loss of muscle and bone mass, and MetS, introducing a vicious circle and further worsening the hypogonadism [17]. These complications can lead to type 2 diabetes, high blood pressure and cardiovascular disease, which can also lead to a markedly decreased quality of life [18,19]. TRT is the foundation stone of the treatment for KS patient with hypogonadisms [32], which takes 12 months and improves the quality of lifeof patients [20–22]. Since long-term clinical benefits, safety, optimal timing and T dosing of TRT in functional hypogonadism remain to be fully documented, we still need to explicitly discuss the uncertainties and benefits of TRT [20,32]. Reports from Italy prove that 25.5% of KS patients had MetS, for which early detection and timely TRT are mandatory [23]. The quality of life of young patients with KS will be affected [24], and early detection and timely treatment are mandatory [23]. The idea that TRT should be administered before mTESE is controversial notwithstanding, the beneficial effects of TRT may stimulate spermatogenesis in patients [25]. We still needed to obtain evidence regarding androgen deficiency in target tissues as soon as possible, which may help to optimize the timing of TRT [26].

Analyzing this patient's condition: No mature sperm was extracted, though this patient had undergone mTESE before the age of 35 years. He underwent TRT for only 6 months and the preoperative level of serum T was less than 5.17 nmol/L (approaching and exceeding this level is helpful to improve the success rate of mTESE) [27], which may lead to the surgical failure of mTESE for this patient. Therefore, he may miss the opportunity, including early gene and reproductive screening and timely TRT [28,29,31,32]. Attention should be paid to enhancing long-term TRT and follow-up to attenuate the consequences of hypogonadism and prevent the frequent comorbidity. Lifelong management of these risk factors, especially increased BMI, during TRT is necessary. Referring to the study in Sweden, lifelong TRT and maintaining a serum T level $>$ 10.41 nmol/L may benefit this patient with hypogonadism, which may be a cost-effective treatment for him [30].

In summary, the typical KS patient has typical clinical symptoms including overweight, small testes, low T, elevated LH and FSH, and MetS, whose ultrastructure of testicular tissue has been significantly changed and damaged, accompanied by spermatogenic dysfunction, hypogonadism and androgen deficiency. According to the recommendation in EAA Guidelines on Klinefelter's Syndrome [31,32], long-term TRT and follow-up management should be given importance to avoid syndromes caused by hypogonadism.

## 4. Limitation of the study

Since the 940th hospital was not a cadcorpse institution, and ethical approval. The lack of use of a healthy testicular tissue to be compared with the proband is an obvious limitation of this study.

## Ethical approval and consent to participate

This study was approved by the Institutional Ethics Committee of Joint Logistic Support Force 940th Hospital of PLA (Lanzhou, Gansu, China; approval No. 2021KYLL199). Informed consent form was obtained from this couple included in the study. Consent for Publication This study obtained this couple consent and permission for publication, but their name and related family data were withheld to protect the patient's privacy.

Availability of supporting data All data generated or analysed during this study are included in this published article and its supplementary information files.

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## Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

## Data availability statement

Data included in article/supp. material/referenced in article.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19940.

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