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Andrology

# Testicular Sonography in Men with Klinefelter Syndrome Shows Irregular Echogenicity and Blood Flow of High Resistance

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***Purpose*:** Klinefelter syndrome is the most common chromosomal aberration among azoospermic men. We wanted to compare testicular echogenicity and intratesticular arterial blood in men with this syndrome versus men with normal sperm parameters.
***Methods*:** Testicular sonography including Doppler imaging, was performed as part of the infertility workup in 26 men with Klinefelter syndrome as well as in 26 men with normal sperm parameters.
***Results*:** In men with Klinefelter syndrome, sonography of the testicular parenchyma revealed a heterogenous irregular pattern with spread hyper- and hypoechoic foci. Doppler sonography resulted in waveforms of high impedance patterns, reflecting intratesticular blood flow of a high resistance. In men with normal sperm parameters testicular echogenicity was of an almost homogenous regular pattern. In these men, intratesticular blood flow typically exhibited a pattern of low vascular resistance.
***Conclusions*:** The study demonstrates that testicular echogenicity as well as intratesticular blood flow are different in men with Klinefelter syndrome versus men with normal sperm parameters.

**KEY WORDS:** Blood flow; echogenicity; Klinefelter syndrome; sonography; testis.

## INTRODUCTION

Men with Klinefelter syndrome have a somatic 47,XXY or less commonly, a 47,XXY/46,XY mosaic karyotype. The incidence of 47,XXY Klinefelter syndrome has been found to be about 0.2% in the general population and 3.1% in the infertile male population (1,2). The clinical features of the classic form are gynecomastia, absence of facial hair, small firm testes, and testicular azoospermia. Klinefelter syndrome is the most common chromosomal aberration among azoospermic men.

Klinefelter men usually have small testes (testicular volume of 2–6 mL) which are intrinsically abnormal. However, focal spermatogenesis is present in some of these men. In 1996 the first successful surgical sperm recovery in Klinefelter patients was reported (3). Since then, several children have been born following intracytoplasmic sperm injection (ICSI) using testicular spermatozoa from men with 47,XXY Klinefelter syndrome.

Traditionally, the infertility workup of the male partner has included a medical history and genital examination, as well as seminal and serological laboratory tests. Since the introduction of surgical sperm retrieval techniques for ICSI, scrotal sonography of men with azoospermia has become a natural part of the infertility investigation in many clinics. A high diagnostic accuracy has been obtained due to training and education of sonologists combined with equipment of still higher resolution. The introduction of color Doppler sonography has made it possible to produce

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a simultaneous display of tissue morphology in gray scale and blood flow in color. Recent reports have demonstrated that color Doppler imaging facilitates the detection of small intratesticular blood vessels and allows the measurement of impedance of blood flow in these vessels (4,5). It has even been suggested that the presence of testicular blood vessels demonstrated by sonography may be of prognostic value for successful sperm recovery (5).

The ultrasonographic findings of testicular screening in men with Klinefelter syndrome have so far only been scantily described. Knowledge of the typical testicular texture and intratesticular blood flow in this group of patients should aid in diagnosing pathology. In the present study we wanted to evaluate testicular texture and intratesticular blood flow, utilizing sonography in combination with color Doppler imaging, in men with nonmosaic Klinefelter syndrome. For comparison, the findings were compared with the results of testicular sonography in a group of men with normal sperm parameters.

## MATERIAL AND METHODS

### Study Population

The study protocol was approved by the human ethics committee of Göteborg University. Twenty-six men with a mean age of $33.5 \pm 3.7$ years (mean $\pm$ SD, ranging from 26 to 44 years) in whom cytogenetic karyotyping as part of the infertility workup had diagnosed a 47,XXY karyotype agreed to participate in the study. Prior to cytogenetic karyotyping azoospermia had been confirmed after analysis of at least two samples from each man. None of the men had ever received any kind of hormone replacement therapy. A control group of 26 men with a mean age of $33.8 \pm 3.4$ years (mean $\pm$ SD, ranging from 25 to 42 years) from infertile couples agreed to take part in the study. These couples had undergone a standard infertility workup and in each case, a female factor had been determined to be the cause of infertility. These men had normal sperm parameters assessed according to strict Tygerberg criteria and WHO (6,7). Ejaculates were collected after 3–5 days' sexual abstinence. All men in the control group were healthy and none of them had a medical history of testicular disease or surgery. All participants underwent semen analysis, clinical examination, testicular sonography, including measurement of testicular volume and color Doppler scanning. Informed consent was obtained from each man before taking part in the study.

### Testicular Sonography

Testicular assessments were performed with the men in a horizontal position, following clinical examination. An ATL HDI 5000 machine (ATL Ultrasound, Bothell, WA) equipped with a linear broadband transducer operating at a range of 5–12 MHz in both 2D and color was used. The spectral Doppler center frequency was 6 MHz. A high-pass filter with a cutoff level of 125 Hz was used. The output energy of the Doppler instrument did not exceed 500 mW/cm$^2$. Each testis was measured in three projections using frozen B-mode sonography, starting with the largest longitudinal diameter followed by the largest testicular depth. Finally, the width was measured by turning the transducer 90 degrees into a transverse projection of the testis. Testicular volume was calculated automatically by the ultrasound computer. All the testicular parenchyma of each testis was scanned. The testicular texture was evaluated according to a semiquantitative score from 1 to 5 as described earlier (8). Score 1 was given to a very uniform pattern, and score 2 to a slightly irregular echogenicity. Score 3 was to be used if a moderately irregular echogenicity or if many small echogenic points were seen throughout any sectional view. Score 4 was given to a very irregular pattern or if bright echogenic spots were seen. Score 5 was given if tumor was suspected because of demarcated areas inside the testis.

Color Doppler imaging was used to identify vessels within the testicular parenchyma. Flow velocity waveforms were generated by a pulsed Doppler range gate to enable quantitative analysis. Care was taken to obtain the highest possible Doppler shift by varying the angle of the transducer.

Impedance of blood flow was expressed as pulsatility index (PI) and resistance index (RI). The sample gate was placed over the vessel and the probe angled in order to obtain a waveform with the maximum peak systolic velocity. The PI was calculated electronically by smooth curves fitted to waveforms over three cardiac cycles according to the formula $PI = (S - D)/TAMXV$, where $S$ is the peak Doppler shifted frequency, $D$ is the minimum Doppler shifted frequency, and TAMXV is the time-averaged maximum velocity (cm/s) over the cardiac cycle. The RI was calculated according to the formula $RI = (S - D)/S$. One to three intratesticular vessels were monitored on each side and mean values (PI and RI)

were calculated. Images of the testicular parenchyma and Doppler measurements were saved on a magneto optical disk and later evaluated by two independent investigators.

### Statistical Analysis

All statistical analyses were done at the level of the individual patient. The two-tailed Mann–Whitney *U*-test was used for between-group comparison. All data were expressed as mean ± SD. A $P$ value of $<0.05$ was considered significant.

## RESULTS

No significant difference in semen volume and abstinence period before ejaculate collection was found between the Klinefelter group and the control group (Table I).

As expected, the men with Klinefelter syndrome had small firm testes compared to the men with normal sperm parameters (Table II). Sonographic measurements showed that the mean testicular volume in the Klinefelter group was $(3.2 \pm 1.0)$ mL versus $(18.4 \pm 2.4)$ mL in the control group.

Testicular echogenicity was found to be completely different in men with Klinefelter syndrome versus men with normal sperm parameters (Fig. 1). In the Klinefelter group a heterogenous irregular testicular pattern with spread foci of hyper- and hypoechogenic parenchyma was found in all men examined. Thus, the testes of this group of patients were given score 4. In contrast, testicular sonography in men with normal sperm parameter, showed an almost regular homogenous texture of medium echogenicity. The testes of these men were given score 1 or score 2. This almost homogenous pattern was seen in all men with normal sperm parameters, but was not observed in any of the men with Klinefelter syndrome.

**Table I.** Semen Characteristics

| | Mean ± SD (range) | | |
|---|---|---|---|
| | Klinefelter men | Fertile controls | *P*-value |
| Abstinence period (days) | 3.5 ± 0.6 (3–5) | 3.4 ± 0.6(3–5) | NS |
| Semen volume (mL) | 3.1 ± 1.0 (2.0–7.4) | 3.3 ± 0.8 (2.4–7.2) | NS |
| Sperm count ($10^6$/mL) | 0 (azoospermia) | 49.0 ± 24.0 (22–156) | |
| Motility (% motile) | | 50.0 ± 4.9 (40–60) | |
| Morphology (% normal) | | 11.7 ± 1.9 (10–16) | |

*Note.* NS: $P > 0.05$.

**Table II.** Comparison Between Testicular Findings in Men with Klinefelter Syndrome and Fertile Controls

| | Klinefelter men | Fertile controls | P-value (Klinefelter vs.controls) |
|---|---|---|---|
| No of men | 26 | 26 | |
| Age (years) | | | |
| Mean (±SD) | 33.5 (3.7) | 33.8 (3.4) | NS |
| Range | 26–44 | 25–42 | |
| Testicular volume (mL) | | | |
| Mean (±SD) | 3.2 (1.0) | 18.4 (2.4) | <0.0001 |
| Range | 1.4–5.7 | 15.5–24.2 | |
| Testicular echogenicity | Heterogenous | Homogenous | |
| Pulsatility index (PI) | | | |
| Mean (±SD) | 1.55 (1.03) | 0.75 (0.12) | <0.0001 |
| Range | 1.32–3.20 | 0.58–1.02 | |
| Resistance index (RI) | | | |
| Mean (±SD) | 0.78 (0.25) | 0.55 (0.06) | <0.0001 |
| Range | 0.52–0.98 | 0.38–0.66 | |

*Note.* Mann–Whitney *U*-test, NS: $P > 0.05$.

Intratesticular blood vessels were identified in all participants. While intratesticular arteries in men with Klinefelter syndrome exhibited high-impedance waveforms with narrow systolic peaks and low diastolic flow, intratesticular blood flow in men with normal sperm parameters was characterized by high levels of diastolic flow, reflecting low vascular resistance (Fig. 2). The mean Doppler flow resistance of these vessels ($PI = 0.75 \pm 0.12$; $RI = 0.51 \pm 0.06$) was significantly lower ($P < 0.0001$) than that in the Klinefelter group ($PI = 1.55 \pm 1.03$; $RI = 0.78 \pm 0.25$).

Unexpectedly, in one of the men with Klinefelter syndrome, sonography revealed a intratesticular tumor (Fig. 3). This testis was given echo-score 5. The man was treated with a unilateral orchidectomy. Histopathological diagnosis showed a Sertoli cell tumor. The tumor was considered benign, but with a potential of becoming malignant.

## DISCUSSION

Scrotal sonography has become an important procedure in the evaluation and treatment of men with infertility. It has proven superior to physical examination to discern between testicular and extratesticular lesions despite the organs' accessibility for clinical

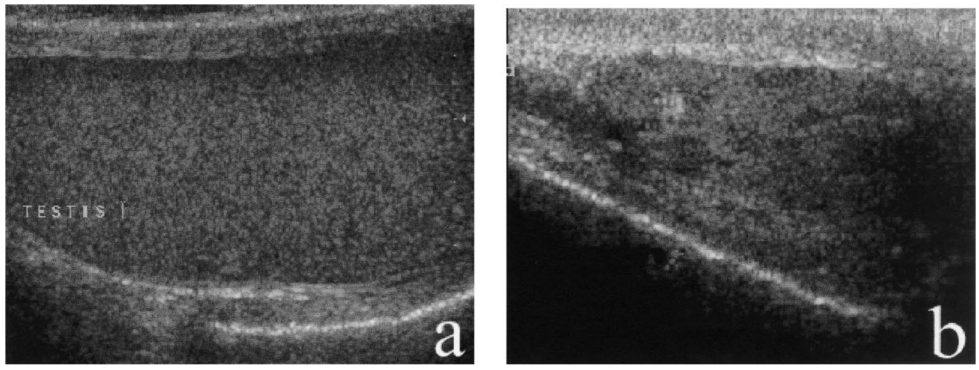


**Fig. 1.** Longitudinal testicular sonography demonstrating characteristic homogenous parenchyma (score 1) of a man with normal sperm parameters (a) and typical heterogenous parenchyma (score 4) with hyper- and hypoechoic foci of a man with nonmosaic Klinefelter syndrome (b).

examination (9–11). Knowledge of the expected testicular characteristics is important in evaluating testicular disease. The testicular texture and color Doppler ultrasound findings of the normal testis have previously been described in detail and are in accordance with our findings (8,12).

The present study demonstrates that the testicular echogenicity observed in men with Klinefelter syndrome is completely different from that in men with normal sperm parameters. A heterogenous irregular pattern with spread hyper- and hypoechoic echogenicity was found to be typical in men with Klinefelter syndrome. Testicular histopathology from Klinefelter patients typically reveals atrophy with fibrotic, hyalinized seminiferous tubules and Leydig cell hyperplasia. Clearly, these characteristics of the testicular parenchyma are reflected in the very irregular hyper- and hypoechoic echogenicity observed at sonographic scanning. In men with normal karyotype it has previously been shown that if testicular carcinoma in situ is present, the testis will be given an echo score of 4 (13). However, in most cases where an echo score of 4 is visualized, carcinoma in situ is not present.

In men with normal sperm parameters, suprat-esticular, capsular, and intratesticular arteries were easily visualized bilaterally in all participants. Only

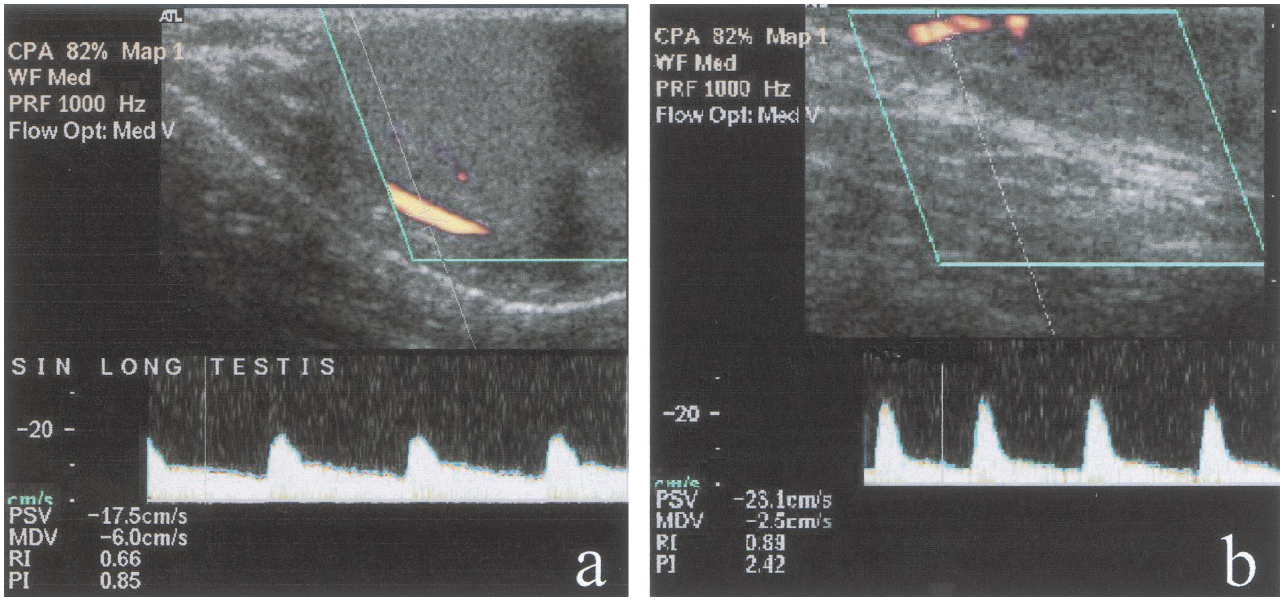


**Fig. 2.** Representative intratesticular blood flow velocity waveforms of a man with normal sperm parameters, showing blood flow with low vascular resistance (a) and of a man with nonmosaic Klinefelter syndrome, demonstrating blood flow with high vascular resistance (b).

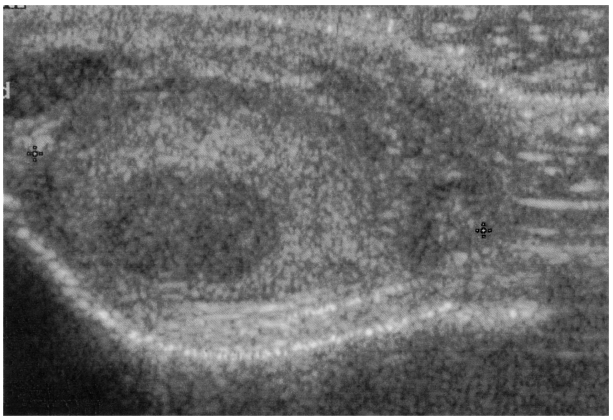

**Fig. 3.** Longitudinal scrotal ultrasonography of a man with nonmosaic Klinefelter syndrome. An intratesticular tumor of less echogenicity than the surrounding testicular parenchyma is seen (score 5). The tumor was not palpable by clinical examination and proved to be a Sertoli cell tumor.

intratesticular arteries were examined by color Doppler imaging. Velocity waveforms from these arteries demonstrated high levels of antegrade diastolic flow, reflecting low vascular resistance in the testis. Similar results were described in a previous publication (12). In that study, Doppler imaging of capsular and supratesticular arteries was also performed. While velocity waveforms from capsular arteries showed high levels of diastolic flow, Doppler imaging of supratesticular arteries was variable.

In the present study, color Doppler imaging of testicular arteries in men with Klinefelter syndrome displayed high impedance waveforms with sharp narrow systolic peaks and low diastolic flow. Although diastolic flow was seen in all the men examined, blood flow was typically of high resistance. These findings indicate that intratesticular arterial blood flow in men with Klinefelter syndrome is different from that of the normal testis. It is unclear whether a vascular pattern of high resistance is present prior to the development of testicular atrophy or if it develops during this process. Another possibility is that the high resistant intratesticular blood flow is secondary to the parenchymal atrophy with fibrotic, hyalinized seminiferous tubules. To our knowledge, blood flow of high resistance has not previously been described in other groups of men with testicular atrophy. It would therefore be of interest to find out if men with testicular atrophy usually exhibit an intratesticular blood flow of high resistance. High impedance waveforms are often an indication of pathology. Intratesticular blood flow of high resistance may have a negative effect on spermatogenesis. In addition, if similar changes occur in other parts of the body it may play a role in the development of cardiovascular disease. It is noteworthy that mortality in men with nonmosaic Klinefelter syndrome is significantly raised from diseases of the cardiovascular system (14).

The clinical value of testicular sonography with color Doppler imaging in predicting successful sperm recovery in azoospermic men has recently been examined. In one study a lower number of intratesticular vessels were observed in men with absence of spermatic activity versus men with successful surgical sperm retrieval (5). Thus, highly vascularized areas may represent foci of spermatogenesis. However, in another study examining men with nonmosaic Klinefelter syndrome, intratesticular blood flow was not found to be predictive for successful surgical sperm recovery (15).

It is well known that there is an association between testicular tumors and infertility (16). In the present study a testicular tumor was found by sonographic scanning in one of the men with Klinefelter syndrome. No palpable irregularity was detected when clinical examination of the testis was performed prior to sonography. This fact clearly demonstrates that sonography in some cases is superior to clinical examination. According to a previous report, testicular sonography was found to have a sensitivity of 100% and a specificity of 99% for testicular tumors (17). It is our opinion that clinical examination as well as scrotal sonography should be performed prior to surgical sperm retrieval.

To conclude, the present study demonstrates a difference in testicular echogenicity and intratesticular blood flow in men with nonmosaic Klinefelter syndrome versus men with normal sperm parameters. A heterogenous pattern with spread hyper- and hypoechoic foci and an intratesticular blood flow of high impedance is typical in Klinefelter patients. The knowledge of these characteristics is important in evaluating testicular disease and may be of interest in enlightening the pathogenesis behind testicular azoospermia in men with Klinefelter syndrome.

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