

# Micro-dissection testicular sperm extraction in Klinefelter's syndrome patients, King Faisal Specialist Hospital and Research Center, Riyadh experience

Abdulmalik H. Almardawi<sup>1</sup>, Rabea Akram<sup>1</sup>, Yara Kattan<sup>1</sup>, Shaheed Saleh Al Suhaibani<sup>1,2</sup>, Hamed AlAli<sup>1</sup>, Said Kattan<sup>1</sup>, Naif Al Hathal<sup>1</sup>

<sup>1</sup>Department of Urology, King Faisal Specialist Hospital and Research Center, Riyadh, <sup>2</sup>Department of Urology, College of Medicine, King Fahad University Hospital, Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia

## Abstract

**Background:** In Klinefelter's syndrome patients with azoospermia, microscopic testicular sperm extraction (m-TESE) can be proposed as a therapeutic option.

**Aim of Study:** The aim of this study is to assess the sperm retrieval rate in patients with Klinefelter syndrome in King Faisal Specialist Hospital, Riyadh.

**Methodology:** Retrospective, Chart review of 32 patients with Klinefelter syndrome who underwent m-TESE were reviewed and analyzed. All patients had two sets of semen analysis after 3 – 5 days abstinence of ejaculation with further study of semen by *in vitro* fertilization (IVF) wash. The hormonal analysis was studied. Ultrasonography of testes was assessed preoperatively. Testicular tubules were sent to the IVF laboratory and were studied under the microscope looking for sperms. Some testicular tissues were sent for the histopathology diagnosis

**Results:** Patients' mean age was  $34.9 \pm 6.0$  years. Mean hormonal levels of E2, FSH, LH, prolactin, and testosterone were  $96.0 \pm 22.0$  pmol/L,  $29.8 \pm 5.4$  IU/L,  $19.0 \pm 2.9$  IU/L,  $15.4 \pm 3.6$  ug/L, and  $10.0 \pm 1.9$  nmol/L, respectively. There were two mosaic Klinefelter syndrome patients (6.25%), whereas 30 patients had a nonmosaic form (93.75%). The overall sperm retrieval rate was 37.5%. All patients had small bilateral testes. Sperm retrieval was successful in three patients with hypospermatogenesis, one patient with maturation arrest, and 8 patients with Sertoli-cell-only pattern. Four patients with complete hyalinization of testicular tissues had complete failure to retrieve sperms. The pregnancy rate after intra-cytoplasmic sperm injection was 50%.

**Conclusions:** The sperm retrieval rate in Klinefelter syndrome patients with m-TESE is in accordance with most of those reported in the literature. Regarding histopathology, hypo-spermatogenesis showed a favorable outcome. The pregnancy rate with intra-cytoplasmic sperm injection was 50%.

**Keywords:** Klinefelter syndrome, microscopic testicular sperm extraction, sperm retrieval rate, spermatogenesis

**Address for correspondence:** Dr. Abdulmalik H. Almardawi, Department of Urology, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia.

E-mail: abadi\_84@yahoo.com

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## INTRODUCTION

Infertility and problems of impaired fecundity have been a continued concern. Globally, these problems affect about 8%–12% of couples. Almost half of the infertility cases are attributed to male factors of infertility, while about 20% of all men exhibit suboptimal sperm parameters<sup>[1]</sup>.

One of the common sex chromosome abnormalities among males is Klinefelter syndrome. It occurs in approximately one in 500–600 phenotypic males.<sup>[2]</sup> It occurs in about 3% of infertile males and among almost 12% of azoospermic ones.<sup>[3]</sup> Most patients (85%) present in the nonmosaic form (i.e. 47, XXY) or mosaic (i.e. 47, XXY/46, XY) forms.<sup>[4]</sup> These patients usually presented with infertility with low testosterone level despite they have normal puberty or normal libido.<sup>[5]</sup> They have smaller testis, firm inconstancy, androgen deficiency, including female hair distribution, scant body hair, and long arms and legs due to late epiphyseal closure. Leydig cell function is commonly impaired in men with Klinefelter syndrome.<sup>[6]</sup>

In patients with azoospermia, microscopic testicular sperm extraction (M-TESE) can be proposed as a therapeutic option since spermatozoa can be recovered in about 30% of cases.<sup>[7]</sup> Testicular biopsy can be part of intra-cytoplasmic sperm injection treatment in patients with nonobstructive azoospermia.<sup>[8]</sup> Despite the common belief that male patients with Klinefelter syndrome are always sterile. Pregnancy has been reported by favor of advances in assisted reproductive techniques.<sup>[5]</sup> Testicular histopathological examination often reveals germ cell atrophy with fibrosis, Leydig cell hyperplasia, and hyalinized seminiferous tubules.<sup>[9]</sup>

This study aimed to assess the sperm retrieval rate in patients with Klinefelter syndrome.

## MATERIALS AND METHODS

This study followed a retrospective study design. Conducted in King Faisal Specialist Hospital and Research Center, Riyadh. Hospital records of 32 patients with Klinefelter syndrome who underwent m-TESE under general anesthesia were reviewed and analyzed.

All patients had two sets of semen analysis after 3–5 days abstinence of ejaculation with further study of semen by *in vitro* fertilization (IVF) wash (centrifugation). The hormonal analysis was studied for E2, FSH, LH, prolactin, and testosterone levels. Ultrasonography of testes was assessed preoperatively to rule out any abnormality and to assess

testicular size. Biopsies of testicular tubules were sent to an IVF laboratory and for histopathology looking for sperms.

All patients underwent M-TESE under general anesthesia. Both testes were examined under the microscope. Testicular tubules were sent to the IVF laboratory in multipurpose human solutions were studied under the microscope with  $\times 400$  looking for sperms. Some testicular tissues were sent for histopathology diagnosis, normal spermatogenesis, hypospermatogenesis, maturation arrest, and Sertoli-cell-only syndrome.

Both testes were opened transversely, dissected under the microscope then closed with either 3-0 vicryl or 5-0 maxone (according to the surgeon preference). Dartos was closed with 3-0 vicryl and the skin was closed with 4-0 vicryl.

## RESULTS

A total of 32 patients who were diagnosed to have Klinefelter syndrome with nonobstructive azoospermia underwent m-TESE. Their mean age was  $34.9 \pm 6.0$  years. Hormonal analyses of E2, FSH, LH, prolactin, and testosterone showed that the mean levels were  $96.0 \pm 22.0$  pmol/L,  $29.8 \pm 5.4$  IU/L,  $19.0 \pm 2.9$  IU/L,  $15.4 \pm 3.6$  ug/L, and  $10.0 \pm 1.9$  nmol/L, respectively [Tables 1 and 2].

There were two mosaic Klinefelter syndrome patients (6.25%). Both had histopathology of Sertoli-cell-only and were negative for sperms. On the other hand, 30 patients had a nonmosaic form (93.75%), as shown in Figure 1 and Table 3.

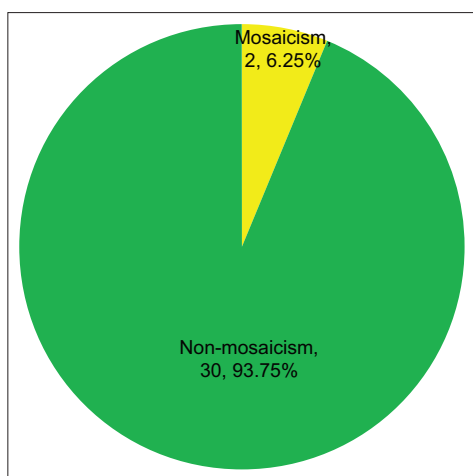
The overall sperm retrieval rate was 37.5% (12/32), as shown in Figure 2 and Table 4. All patients had small bilateral testes, with mean testicular size measured by ultrasound on the right and left sides were  $2.36 \pm 0.76$  mL and  $2.39 \pm 0.76$  mL, respectively [Tables 5 and 6].

Table 7 shows that apart from two patients whose data were missing, three patients with low spermatogenesis sperm retrieval could be done. One patient (3.3%)

**Table 1: Age and results of hormonal analysis (mean $\pm$ standard deviation) of Klinefelter's syndrome patients**

Variables	n	Mean $\pm$ SD
Age (years)	32	34.9 $\pm$ 6.0
Estrogen (pmol/L)	21	96.0 $\pm$ 48.3
Follicle stimulating hormone (IU/L)	32	29.8 $\pm$ 15.0
Luteinizing hormone (IU/L)	32	19.0 $\pm$ 8.0
Prolactin (ug/L)	32	15.4 $\pm$ 10.0
Testosterone (nmol/L)	32	10.0 $\pm$ 5.4

SD: Standard deviation



**Figure 1:** Mosaicism form of patients with Klinefelter's syndrome

was diagnosed with maturation arrest which was successful for sperm retrieval. Four patients (13.3%) had complete hyalinization of testicular tissues with complete failure to retrieve sperms. The rest of the patients (22, 73.3%) had a Sertoli-cell-only pattern. Eight out of these 22 patients (36.4%) had successful sperm retrieval.

Looking at wives' records of 12 patients, the ovulation cycle and intra-cytoplasmic sperm injection were done among six, which yielded three successful pregnancies. Therefore, the pregnancy rate after intra-cytoplasmic sperm injection was 50%. Four records were missing, and the rest were waiting for starting cycles.

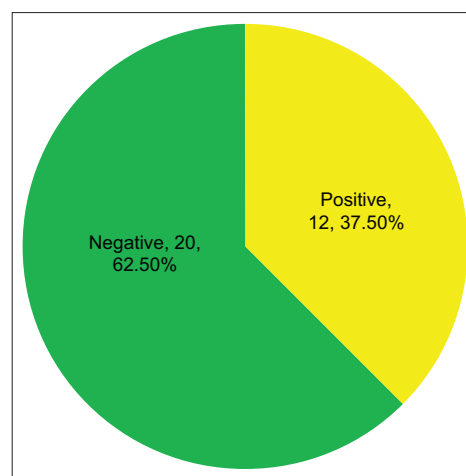
## DISCUSSION

With the advent of testicular sperm extraction and intracytoplasmic sperm injection, Klinefelter syndrome patients may frequently achieve their reproductive potential.<sup>[5]</sup>

This study included the data of 32 Klinefelter syndrome patients with nonobstructive azoospermia. Genetic analysis showed revealed that two were mosaic Klinefelter syndrome patients (6.25%), whereas 30 patients (93.75%) had nonmosaic form. The results of their hormonal analyses were disturbed.

Friedler *et al.* (2001)<sup>[4]</sup> stated that Klinefelter syndrome presents itself in nonmosaic or mosaic forms, with about 85% of Klinefelter patients having a nonmosaic karyotype.

McLachlan *et al.* (2007)<sup>[10]</sup> noted that the management of nonobstructive azoospermia is by assessment of the



**Figure 2:** Overall sperm retrieval

male partner with a hormonal assay and genetic analyses. However, as endocrine tests cannot always distinguish normal from impaired spermatogenesis or predict retrieval of mature sperm for intracytoplasmic sperm injection, an isolated diagnostic testicular biopsy is commonly sent for histopathological confirmation of sperm production before sperm retrieval is attempted.

All our patients had small testes bilaterally, with mean testicular size on both the right and left sides ( $2.36 \pm 0.76$  mL and  $2.39 \pm 0.76$  mL, respectively).

Several studies indicated that small testicular volumes among patients with Klinefelter syndrome. Ando *et al.* (2013)<sup>[11]</sup> reported a mean testicular volume of  $4.0 \pm 2.1$  mL, and Ozveri *et al.* (2015)<sup>[5]</sup> reported that all their Klinefelter syndrome patients had small-sized testes with their volume ranging from 2 to 5 mL.

The results of the present study showed that the overall sperm retrieval rate among our Klinefelter syndrome patients was 37.5%.

This finding is in accordance with those stated by Madureira *et al.* (2014),<sup>[12]</sup> who reported a sperm retrieval rate of 38.5%, and Ando *et al.* (2013),<sup>[11]</sup> who reported that the sperm retrieval rate from m-TESE among Klinefelter syndrome patients was 42.4%.

The lower rate was stated by Sabbaghian *et al.* (2014)<sup>[13]</sup> and Chehrazhi *et al.* (2017),<sup>[14]</sup> who reported that the sperm retrieval rates were 28.4%. However, higher rates were reported by Kalsi *et al.* (2011),<sup>[15]</sup> and Aksglaede and Juul (2013),<sup>[16]</sup> who reported an overall sperm retrieval rate of 50%, while Ramasamy *et al.* (2009)<sup>[17]</sup> reported a sperm retrieval rate of 60%. Schiff *et al.* (2005)<sup>[18]</sup> reported that the

**Table 2: variables analysis**

Variable	n	Median	Mean±SD	95% CL for mean (lower-upper)	Minimum	Maximum
Age	32	35.0	34.9±6.0	32.7–37.1	21.0	46.0
E2	21	87.0	96.0±48.3	74.0–118.0	27.0	227.0
FSH	32	28.5	29.8±15.0	24.4–35.2	6.2	74.3
LH	32	17.9	19.0±8.0	16.1–21.9	3.2	40.4
PRO	32	12.3	15.4±10.0	11.8–19.0	6.3	51.7
Testosterone	32	10.7	10.0±5.4	8.1–11.9	0.9	20.0

SD: Standard deviation, E2: Estradiol, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, PRO: Prolactin, CL: Confidence level

**Table 3: Mosaicism result of patients with Klinefelter syndrome**

xy_type	Frequency (%)	Cumulative frequency (%)
Mosaicism	2 (6.25)	2 (6.25)
Nonmosaicism	30 (93.75)	32 (100.00)

**Table 4: Sperm retrieval after M.TESE**

Results	Frequency (%)	Cumulative frequency (%)
Negative	20 (62.50)	20 (62.50)
Positive	12 (37.50)	32 (100.00)

**Table 5: Right testicular size assessed by ultrasound**

Ultrasound_right	Frequency (%)	Cumulative frequency (%)
23×9×13	1 (4.55)	1 (4.55)
34×16×25	1 (4.55)	2 (9.09)
10×26×19	1 (4.55)	3 (13.64)
17×7×11	1 (4.55)	4 (18.18)
19×7×10	1 (4.55)	5 (22.73)
20×10×14	1 (4.55)	6 (27.27)
20×11×13	1 (4.55)	7 (31.82)
20×14×7	1 (4.55)	8 (36.36)
20×6×13	1 (4.55)	9 (40.91)
21×7×14	1 (4.55)	10 (45.45)
21×9×14	1 (4.55)	11 (50.00)
21×9×17	1 (4.55)	12 (54.55)
22×11×15	1 (4.55)	13 (59.09)
22×10×14	1 (4.55)	14 (63.64)
22×10×19	1 (4.55)	15 (68.18)
22×24×9	1 (4.55)	16 (72.73)
22×9×18	1 (4.55)	17 (77.27)
23×9×15	1 (4.55)	18 (81.82)
25×6.7×16	1 (4.55)	19 (86.36)
25×9×16	1 (4.55)	20 (90.91)
33×7×16	1 (4.55)	21 (95.45)
5×3×5	1 (4.55)	22 (100.00)

Frequency missing=10

sperm retrieval rate was 72% after m-TESE for Klinefelter syndrome patients.

This study showed that patients' sperm retrieval was successful among patients with hypospermatogenesis or maturation arrest which was successful for sperm retrieval. However, complete failure occurred with patients with complete hyalinization of testicular tissues, while about one-third of patients with Sertoli-cell-only pattern had successful sperm retrieval.

Kalsi *et al.* (2011)<sup>[15]</sup> reported that among cases who underwent m-TESE, successful sperm retrieval occurred

in 42.85% of cases with Sertoli-cell-only pattern, 26.7% of cases with maturation arrest, and 75.86% of cases with hypospermatogenesis. They concluded that m-TESE is the optimum approach to retrieve sperm in patients with nonobstructive azoospermia.

Deruyver *et al.* (2014)<sup>[19]</sup> stated that favorable sperm retrieval is expected for m-TESE in cases of nonobstructive azoospermia, especially in the histological patterns of patchy spermatogenesis, such as Sertoli-cell-only syndrome, while in patients with uniform histological patterns, such as maturation arrest, the outcome of m-TESE seems less favorable.

The present study indicated that the pregnancy rate after intra-cytoplasmic sperm injection was 50%.

This finding is in accordance with that reported by Schiff *et al.* (2005),<sup>[18]</sup> who found that the pregnancy rate after intra-cytoplasmic sperm injection was 46%. However, a lower rate was stated by Sabbaghian *et al.* (2014),<sup>[13]</sup> who reported that the pregnancy rate after intra-cytoplasmic sperm injection was 28%.

Deruyver *et al.* (2014)<sup>[19]</sup> stated that m-TESE in combination with intracytoplasmic sperm injection has become the first-line treatment for patients with nonobstructive azoospermia.

## CONCLUSION

In conclusion, the sperm retrieval rate in patients with Klinefelter syndrome with m-TESE is in accordance with most of those reported in the literature. Regarding histopathology, hypo-spermatogenesis showed a favorable outcome. The pregnancy rate after intra-cytoplasmic sperm injection was 50%.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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**Table 6: Left testicular size assessed by ultrasound**

Ultrasound_left	Frequency (%)	Cumulative frequency (%)
23×10×14	2 (9.09)	2 (9.09)
10×29×18	1 (4.55)	3 (13.64)
15×5×12	1 (4.55)	4 (18.18)
17×8×14	1 (4.55)	5 (22.73)
18×7×10	1 (4.55)	6 (27.27)
18×8×11	1 (4.55)	7 (31.82)
19×10×11	1 (4.55)	8 (36.36)
20×7×15	1 (4.55)	9 (40.91)
20×8×10	1 (4.55)	10 (45.45)
20×9×16	1 (4.55)	11 (50.00)
21×10×15	1 (4.55)	12 (54.55)
22×24×9	1 (4.55)	13 (59.09)
22×7×14	1 (4.55)	14 (63.64)
23×7.6×14	1 (4.55)	15 (68.18)
23×8×13	1 (4.55)	16 (72.73)
24×8×13	1 (4.55)	17 (77.27)
24×9×16	1 (4.55)	18 (81.82)
25×8×13	1 (4.55)	19 (86.36)
28×13×20	1 (4.55)	20 (90.91)
29×8×19	1 (4.55)	21 (95.45)
35×16×23	1 (4.55)	22 (100.00)

Frequency Missing=10

**Table 7: Outcome of sperm retrieval according to histopathology findings (n=30)\***

Histopathology findings	n (%)	Positive, n (%)	Negative, n (%)
Hypospermatogenesis	3 (10.0)	3 (100.0)	0
Maturation arrest	1 (3.3)	1 (100.0)	0
No seminiferous tubules	4 (13.3)	0	4 (100.0)
Sertoli-cell-only	22 (73.3)	8 (36.4)	14 (63.6)

\*Data of two cases were missing

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