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# Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis

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**BACKGROUND:** Specific factors underlying successful surgical sperm retrieval rates (SRR) or pregnancy rates (PR) after testicular sperm extraction (TESE) in adult patients with Klinefelter syndrome (KS) have not been completely clarified.

**OBJECTIVE AND RATIONALE:** The aim of this review was to meta-analyse the currently available data from subjects with KS regarding SRRs as the primary outcome. In addition, when available, PRs and live birth rates (LBRs) after the ICSI technique were also investigated as secondary outcomes.

**SEARCH METHODS:** An extensive Medline, Embase and Cochrane search was performed. All trials reporting SRR for conventional-TESE (cTESE) or micro-TESE (mTESE) and its specific determinants without any arbitrary restriction were included.

[†]These authors equally contributed to the article.

**OUTCOMES:** Out of 139 studies, 37 trials were included in the study, enrolling a total of 1248 patients with a mean age of 30.9 ± 5.6 years. The majority of the studies ($n$ = 18) applied mTESE, 13 applied cTESE and in one case testicular sperm aspiration (TESA) was used. Additionally, four studies used a mixed approach and in one study, the method applied for sperm retrieval was not specified. Overall, a SRR per TESE cycle of 44[39;48]% was detected. Similar results were observed when mTESE was compared to cTESE (SRR 43[35;50]% vs 45[38;52]% for cTESE vs micro-TESE, respectively; $Q = 0.20$, $P = 0.65$). Meta-regression analysis showed that none of the parameters tested, including age, testis volume and FSH, LH and testosterone (T) levels at enrollment, affected the final SRR. Similarly, no difference was observed when a bilateral procedure was compared to a unilateral approach. No sufficient data were available to evaluate the effect of previous T treatment on SRR. Information on fertility outcome after ICSI was available for 29 studies. Overall a total of 218 biochemical pregnancies after 410 ICSI cycles were observed (PR = 43[36;50]%). Similar results were observed when LBR was analyzed (LBR = 43[34;53]%). Similar to what was observed for SRR, no influence of KS age, mean testis volume, LH, FSH or total T levels on either PR and LBR was observed. No sufficient data were available to test the effect of the women's age or other female fertility problems on PR and LBR. Finally, no difference in PR or LBR was observed when the use of fresh sperm was compared to the utilization of cryopreserved sperm.

**WIDER IMPLICATIONS:** The present data suggest that performing TESE/micro-TESE in subjects with KS results in SRRs of close to 50%, and then PRs and LBRs of close to 50%, with the results being independent of any clinical or biochemical parameters tested.

**Key words:** Klinefelter syndrome / fertility / non-obstructive azoospermia / testicular sperm extraction / assisted reproductive techniques / intra-cytoplasmic sperm injection

# Introduction

Klinefelter syndrome (KS) is the most frequent abnormality of sex chromosomes with an estimated prevalence raging from 1:500 to 1:700 newborn males (Lanfranco et al., 2004). KS represents a group of chromosomal disorders in which there is at least one extra X chromosome, added to the male karyotype, 46,XY (Lanfranco et al., 2004). In the vast majority of cases, KS patients show a 47,XXY karyotype, although mosaicisms or, more rarely, other chromosome aneuploidies can be detected (Lanfranco et al., 2004).

Because of the genetic alteration, there is progressive testicular damage leading to impaired sperm production and infertility (Aksglaede and Juul, 2013). The degree of androgenization reflects the number and residual function of Leydig cells but usually at least two-thirds of adult (20–40 years old) men with KS show normal testosterone (T) concentrations (Aksglaede et al., 2007). Accordingly, despite its high incidence, it is common for the majority of cases of KS to remain undiagnosed (Bojesen et al. 2003; Herlihy et al. 2011). Hence, it is more common to diagnose KS in subjects seeking medical care for hypogonadism, couple infertility, and/or sexual dysfunction (Foresta et al., 1999; Corona et al., 2010; Forti et al., 2010; Vignozzi et al., 2010).

Infertility in men with KS has remained an untreatable disease for a long time. However, recent data have emphasized that subjects with KS may benefit from ART due to the presence of residual foci with preserved spermatogenesis (Foresta et al., 1999, see for review Aksglaede and Juul, 2013). It is still unclear whether the residual spermatogenesis originates from 47,XXY spermatogonia or from euploid germ cells (Foresta et al., 1999; Sciurano et al., 2009) and the higher frequency of sperm aneuploidy reported in KS does not clarify this aspect. In fact, this condition could be related both to aneuploid stem cells and to meiotic errors due to a deleterious testicular environment, as demonstrated in non-obstructive azoospermic patients. In this regard, some authors have provided arguments for offering preimplantation genetic diagnosis or prenatal diagnosis for patients with non-obstructive azoospermia (Vialard et al., 2012).

A recent overview of the published studies on success rates and predictors of sperm retrieval by conventional testicular sperm extraction (cTESE) and by microsurgical TESE (micro-TESE) in men with KS, reported an average sperm retrieval rate (SRR) of 50% (Aksglaede and Juul, 2013). So far, at least 149 healthy live born babies have been conceived after TESE combined with intra-cytoplasmic sperm injection in couples including a 47,XXY father (Aksglaede and Juul, 2013). The specific predictors of this approach are, however, still conflicting. Hormonal parameters, including levels of FSH, inhibin B, T and oestradiol (E2), as well as testicular volume seem not to be predictive factors for sperm recovery in males with KS (Aksglaede and Juul, 2013). Some authors have emphasized that KS subjects of a younger age (below 35 years) have a better chance of positive TESE (Vernaeve et al., 2004; Okada et al., 2005a; Bakircioglu et al., 2006, 2011; Kyono et al., 2007; Ferhi et al., 2009; Ramasamy et al., 2009). However, other authors have not confirmed these results (Plotton et al., 2015). In addition, no information on fertility rate and its predictions after TESE/ICSI in KS is available. Finally, another controversial topic is related to the utility of an early T treatment on SRR outcome (Gies et al., 2014). Mehta et al. (2013) previously described a better SRR at TESE in a small group of adolescents and young adults, with KS, who first received a T supplementation in combination with an aromatase inhibitor therapy for several years (1–5 years). However, at present, there are not enough data to suggest this approach.

The aim of this comprehensive review was to meta-analyse the currently available data regarding SRR and its predictors in subject with KS. In addition, where available, pregnancy rate (PR) and live birth rate (LBR) after ICSI were also investigated.

# Methods

This meta-analysis was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines.

## Search strategy

An extensive Medline, Embase and Cochrane search was performed, including the following words: 'klinefelter syndrome'[MeSH Terms] OR ('klinefelter'[All Fields] AND 'syndrome'[All Fields]) OR ('klinefelter syndrome'[All Fields]) AND ('fertility'[MeSH Terms] OR 'fertility'[All Fields]).

The search, which accrued data from January 1st, 1969 up to November 5th, 2016, was restricted to English-language articles and studies including human participants. The identification of relevant studies was performed independently by three of the authors (A.P., A.G. and F.L.), and conflicts were resolved by the fourth investigator (G.C.). We did not employ search software but hand-searched bibliographies of retrieved papers for additional references. The main source of information was derived from published articles.

## Study selection

All observational trials reporting SRR in azoospermic subjects with KS without any arbitrary restriction (Fig. 1 and Table I) were included. Case reports or trials reporting sperm retrieval in non-KS patients were excluded from the analysis (Fig. 1).

## Outcome and quality assessment

The principal outcome was the analysis of SRR in azoospermic subjects with KS. Secondary outcomes included the comparison of SRR according to different surgical techniques including cTESE, micro-TESE (mTESE) and testicular sperm aspiration (TESA). In addition, where available, PR and LBR after ICSI were also investigated. When possible both per cycle or cumulative rates were calculated. The quality of trials included was assessed using the Cochrane criteria (Higgins and Green, 2008).

## Statistical analysis

Heterogeneity in SRR was assessed using $I^2$ statistics. Even when low heterogeneity was detected, a random-effect model was applied, because the validity of tests of heterogeneity can be limited with a small number of component studies. We used funnel plots and the Begg adjusted rank correlation test to estimate possible publication or disclosure bias (Begg and Mazumdar, 1994), however, undetected bias may still be present because these tests have low statistical power when the number of trials is small. In addition, a meta-regression analysis was performed to test the effect of different parameters on SRR, PR and LBR.

# Results

## Sperm retrieval outcome

Out of 139 retrieved articles, 37 were included in the study (Table I). The study flow is summarized in Fig. 1. The majority of the studies ($n = 18$) applied cTESE, 13 applied mTESE, and in one case TESA was used (Table I). Additionally, four studies used a mixed approach and in one study the method applied for sperm retrieval was not specified. Surgical approaches included a bilateral procedure in 23 cases and a monolateral method in three studies, respectively (Table I). The latter information was not available in six cases, and in five studies a mixed approach was reported (Table I). In addition, multiple biopsies were performed in 30 cases whereas three studies used a single biopsy (Table I). The latter information was not available in four cases (Table I). The characteristics of the retrieved trials, including parameters on trial quality, are reported in Tables I and II. Retrieved trials included a total of 1248 patients with a mean age of 30.9 ± 5.6 years. Mean testicular volume was 3.9 ± 1.6 ml and mean hormonal parameters reflect the condition of primary or compensated hypogonadism (FSH = 36.0 ± 7.0 U/L, LH 18.4 ± 4.3 U/L, total testosterone 10.3 ± 4.0 nM). All studies, except two, included non-mosaic KS (Table I). The $I^2$ in trials assessing overall SRR per TESE cycle was 50.44 ($P < 0.001$). A cumulative SRR per TESE cycle of 44[39;48] % was determined (Fig. 2 and Supplementary Figure 1). A funnel plot and Begg adjusted rank correlation test (Kendall's $\tau$: 0.12; $P = 0.30$) suggested no publication bias. Data were confirmed in sensitivity analysis when the trials enrolling mosaic KS subjects was excluded from the analysis (SRR of 43[39;48] %). In addition, similar results were observed when micro-TESE was compared to cTESE,

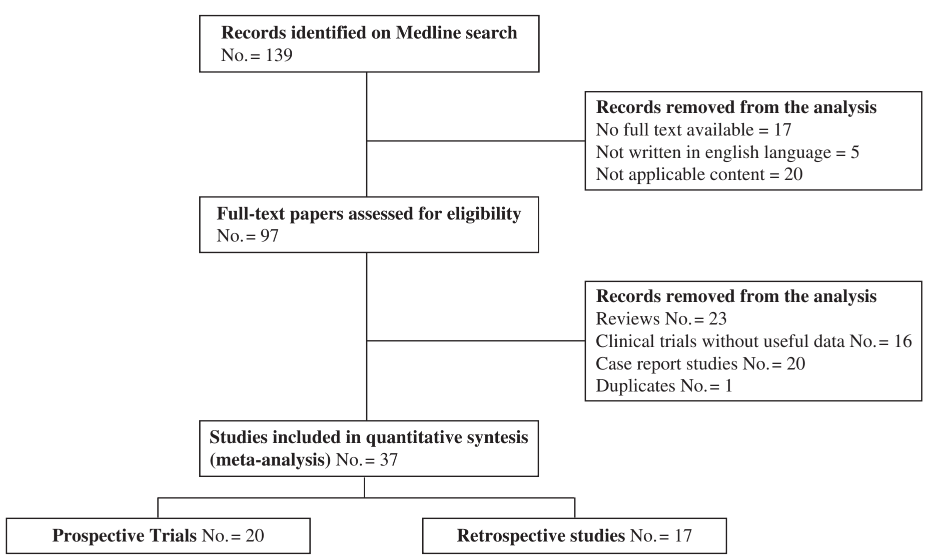


**Figure 1** Trial flow diagram.

**Table I** Characteristics of the clinical studies included in the meta-analysis.

| Study | No. pts | No. total of procedure | Type of surgical procedure | Bilateral approach | Multiple biopsy approach | Success SR | Type of sperm used for ICSI | No. of ICSI cycles | Clinical pregnancies | Live born children | Age (years) | Women age | Mosaic /non-Mosaic | FSH (U/L) | LH (U/L) | Total T (nM) | Testis volume (ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tournaye et al. (1996) | 9 | 10 | cTESE | Yes | Yes | 4 | Fresh | 3 | 0 | 0 | 31.9 | NR | NM | 40 | 26.4 | 9.5 | 3.1 |
| Palermo et al. (1998) | 2 | 2 | cTESE | Yes | Yes | 2 | Fresh | 3 | 2 | 3 | 33.0 | 32.5 | NM | NR | NR | NR | NR |
| Reubinoff et al. (1998) | 7 | 9 | TESA | Yes | Yes | 4 | Fresh | 5 | 1 | 1 | 30.4 | 26.2 | NM | 44.4 | 26.6 | 13.2 | NR |
| Levron et al. (2000) | 20 | 20 | cTESE | NR | NR | 8 | Mixed | 8 | 4 | 7 | NR | NR | NM | 26.1 | 16.1 | 11.8 | NR |
| Friedler et al. (2001) | 12 | 12 | cTESE | Yes | Yes | 5 | Mixed | 10 | 5 | 6 | 28. | 26.4 | NM | 38.3 | NR | 25.3 | 3 |
| Poulakis et al. (2001) | 2 | 2 | cTESE | Yes | NR | 2 | Fresh | 2 | 2 | 2 | 34 | 28.5 | NM | 16.4 | 16.5 | 7.4 | 5.5 |
| Westlander et al. (2001) | 19 | 19 | cTESE | Yes | Yes | 4 | NR | 4 | 4 | NR | 33. | NR | NM | 30.5 | NR | 11.2 | 3.2 |
| Bergère et al. (2002) | 4 | 4 | cTESE | Yes | Yes | 3 | CP | 4 | 1 | 1 | NR | NR | NM | 26-33.7 | NR | NR | 4-6 |
| Madgar et al. (2002) | 20 | NR | cTESE | NR | NR | 9 | NR | NR | NR | NR | 32.2 | NR | NM | 33.6 | 18.5 | 8.6 | 6.6 |
| Yamamoto et al. (2002) | 24 | 24 | cTESE | No | No | 12 | Fresh | 12 | 4 | 5 | 23-4 | NR | NM | 14-56 | NR | NR | NR |
| Staessen et al. (2003) | 19 | 19 | cTESE | Yes | Yes | 17 | Mixed | 31 | 7 | 4 | NR | 29.5 | NM | NR | NR | NR | NR |
| Westlander et al. (2003) | 18 | 18 | cTESE | Yes | Yes | 5 | CP | 5 | 2 | NR | 33.4 | NR | NM | NR | NR | NR | 2-5 |
| Ulug et al. (2003) | 11 | 11 | cTESE | No | Yes | 6 | Fresh | 6 | 2 | 1 | 33.4 | 30.4 | NM | 42.6 | 27.3 | 10.2 | 4.2 |
| Seo et al. (2004) | 25 | 25 | cTESE | Yes | Yes | 4 | Fresh | 4 | 2 | 1 | 31.6 | NR | NM | 31.4 | NR | 9.4 | 4.5 |
| Vernaeve et al. (2004) | 50 | 50 | cTESE | Yes | Yes | 24 | Mixed | NR | NR | NR | 31.2 | NR | NM | 36.0 | NR | 10.91 | 3.9 |
| Gonsalves et al. (2005) | 4 | 4 | NR | NR | NR | 4 | CP | 4 | 3 | 6 | 33.2 | NR | NM | NR | NR | NR | NR |
| Okada et al. (2005a) | 10 | 10 | mTESE | Yes | Yes | 6 | CP | 10 | 4 | 3 | NR | 27.3 | NM | NR | NR | NR | NR |
| Okada et al. (2005b) | 51 | 51 | Mixed | Yes | Yes | 26 | Mixed | 26 | 12 | 12 | 34.4 | NR | NM | 28.0 | 15.6 | 8.9 | 2.8 |
| Schiff et al. (2005) | 42 | 54 | mTESE | Yes | Yes | 29 | Fresh | 39 | 19 | 21 | 32.8 | 33.2 | 3 M | 33.2 | NR | 9.8 | 2.5 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bakircioglu *et al.* (2006) | 74 | 74 | mTESE | Yes | Yes | 42 | NA | NA | NA | NA | 33.1 | NR | NM | 36,4 | 21.5 | 8.2 | 2.9 |
| Kyono *et al.* (2007) | 17 | 17 | cTESE | Yes | Yes | 6 | Mixed | 9 | 7 | 8 | 35.0 | 30.6 | NM | 35.7 | 12.0 | 8.6 | 2.4 |
| Koga *et al.* (2007) | 26 | 26 | mTESE | Mixed | Yes | 13 | NR | NR | 4 | 2 | 36.0 | NR | NM | 40.3 | 18.7 | 7.5 | 3.0 |
| Takada *et al.* (2008) | 9 | 9 | mTESE | NR | Yes | 4 | NR | NR | NR | NR | 33.9 | NR | NM | 42.7 | 17.3 | 9.7 | 3.5 |
| Ferhi *et al.* (2009) | 27 | 27 | Mixed | Yes | No | 8 | CP | NR | 4 | 5 | 32.3 | NR | NM | 38.3 | NR | NR | 2.04 |
| Ramasamy *et al.* (2009) | 68 | 91 | mTESE | Yes | Yes | 45 | Fresh | NR | 33 | 28 | 33 | NR | NM | 34.4 | 16.3 | 6.0 | 3.5 |
| Yarali *et al.* (2009) | 33 | 39 | mTESE | NR | Yes | 22 | Fresh | 39 | 7 | 5 | 32 | NR | NM | NR | NR | NR | NR |
| Bakircioglu *et al.* (2011) | 106 | 106 | mTESE | Yes | Yes | 50 | Fresh | 49 | 26 | 29 | 34.3 | NR | NM | NR | 14.8 | NR | NR |
| Greco *et al.* (2013) | 38 | 38 | Mixed | Yes | Yes | 15 | Mixed | 26 | 15 | 16 | 35.3 | 33.7 | NM | 30.1 | 15.1 | 11.3 | 3.9 |
| Mehta *et al.* (2013) | 10 | 10 | mTESE | Mixed | Yes | 7 | CP | NR | NR | NR | 15.5 | NR | NM | 18.5 | NR | 5.1 | 3.8 |
| Rives *et al.* (2013) | 5 | 5 | cTESE | Yes | No | 1 | CP | NA | NA | NA | 15.8 | NR | NM | 41.8 | 15.9 | 6.6 | 2.3 |
| Haliloglu *et al.* (2014) | 18 | 18 | mTESE | NR | Yes | 3 | NR | 3 | 1 | 1 | 30.3 | NR | NM | 39.4 | 21.6 | 6.4 | 2.09 |
| Madureira *et al.* (2014) | 65 | 65 | cTESE | Mixed | Yes | 25 | Mixed | 37 | 16 | 17 | 33.8 | NR | NM | 30.5 | 16.4 | 19.3 | 7.7 |
| Sabbaghian *et al.* (2014) | 134 | 134 | mTESE | Mixed | Yes | 38 | CP | 18 | 4 | 5 | 32.6 | 15.9 | NM | 34.5 | 17.9 | 9.2 | NA |
| Plotton *et al.* (2015) y | 25 | 25 | cTESE | Yes | Yes | 13 | CP | NA | NA | NA | 18.2 | NA | NM | 47.2 | NR | 10.7 | 6.8 |
| Plotton *et al.* (2015) a | 16 | 16 | cTESE | Yes | Yes | 6 | CP | 10 | 4 | 3 | 32.1 | NR | NM | 43.7 | NR | 9.1 | 6.7 |
| Rohayem *et al.* (2015) y | 50 | 50 | mTESE | Yes | Yes | 45 | NA | NA | NA | NA | NR | NR | NM | 32.4 | 12.9 | 10.8 | 5.3 |
| Rohayem *et al.* (2015) a | 50 | 85 | mTESE | Yes | Yes | 45 | NA | NA | NA | NA | NR | NR | NM | 33.5 | 17.9 | 10.7 | 4.6 |
| Nahata *et al.* (2016) | 10 | 10 | mTESE | No | Yes | 5 | NA | NA | NA | NA | 17.6 | NR | NM | 36.2 | NR | 12.8 | 2.3 |
| Vicdan *et al.* (2016) | 83 | 88 | Mixed | Yes | Yes | 35 | Mixed | 43 | 23 | 25 | 33.7 | NR | 6M | 35.9 | NR | NR | NR |

y, young ; a, adult ; cTESE, conventional TEsticular Sperm Extraction; mTESE, microsurgical TEsticular Sperm Extraction; TESA, TEsticular Sperm Aspiration; NR, not reported; NA, not available; NM, non-mosaic; M, mosaic; FSH, follicular stimulating hormone; LH, Luteinizing hormone; CP, cryopreserved.

**Table II** Quality assessment of the clinical studies included in the meta-analysis.

| Study | Selection bias | Study design | Data collection | Global rating |
|---|---|---|---|---|
| Tournaye et al. (1996) | Moderate | Observational Single center | Strong | Moderate |
| Palermo et al. (1998) | Moderate | Retrospective (CASE REPORT) Single center | Moderate | Moderate |
| Reubinoff et al. (1998) | Weak | Prospective Single center | Strong | Strong |
| Levron et al. (2000) | Moderate | Prospective Single center | Strong | Moderate |
| Friedler et al. (2001) | Weak | Prospective Single center | Strong | Strong |
| Poulakis et al. (2001) | Moderate | Retrospective (CASE REPORT) Single center | Moderate | Moderate |
| Westlander et al. (2001) | Weak | Prospective Single center | Strong | Strong |
| Bergère et al. (2002) | Weak | Prospective Single center | Strong | Strong |
| Madgar et al. (2002) | Weak | Prospective Single center | Strong | Strong |
| Yamamoto et al. (2002) | Weak | Prospective Single center | Strong | Strong |
| Staessen et al. (2003) | Weak | Prospective Single center | Strong | Strong |
| Westlander et al. (2003) | Weak | Prospective Single center | Strong | Strong |
| Ulug et al. (2003) | Moderate | Retrospective Single center | Moderate | Moderate |
| Seo et al. (2004) | Weak | Prospective Single center | Strong | ?? |
| Vernaeve et al. (2004) | Weak | Retrospective Single center | Strong | Strong |
| Gonsalves et al. (2005) | Weak | Prospective Single center | Strong | Strong |
| Okada et al. (2005a) | Weak | Prospective Multi-center | Strong | Strong |
| Okada et al. (2005b) | Moderate | Retrospective (CASE REPORT) Single center | Strong | Strong |
| Schiff et al. (2005) | Weak | Retrospective Single center | Strong | Strong |
| Bakircioglu et al. (2006) | Weak | Prospective Single center | Moderate | Moderate |
| Kyono et al. (2007) | Weak | Prospective Single center | Strong | Strong |
| Koga et al. (2007) | Weak | Prospective Single center | Strong | Strong |
| Takada et al. (2008) | Weak | Prospective Single center | Strong | Strong |
| Ferhi et al. (2009) | Weak | Retrospective Single center | Strong | Strong |
| Ramasamy et al. (2009) | Weak | Retrospective Single center | Strong | Strong |
| Yarali et al. (2009) | Weak | Retrospective Single center | Strong | Strong |
| Bakircioglu et al. (2011) | Weak | Retrospective Single center | Strong | Strong |
| Greco et al. (2013) | Weak | Prospective Multi-center | Strong | Strong |
| Mehta et al. (2013) | Weak | Retrospective Single center | Strong | Strong |
| Rives et al. (2013) | Moderate | Retrospective Single center | Moderate | Weak |
| Haliloglu et al. (2014) | Moderate | Retrospective Single center | Moderate | Moderate |
| Madureira et al. (2014) | Weak | Retrospective Single center | Strong | Strong |
| Sabbaghian et al. (2014) | Weak | Retrospective Single center | Moderate | Moderate |
| Plotton et al. (2015) | Weak | Prospective Single center | Strong | Strong |
| Rohayem et al. (2015)W | Weak | Retrospective Single center | Strong | Strong |
| Nahata et al. (2016) | Weak | Prospective Single center | Strong | Moderate |
| Vicdan et al. (2016) | Weak | Retrospective Single center | Strong | Strong |

(Fig. 2; $Q = 0.20$, $P = 0.65$). Finally, no differences were observed when SRR per patient was considered (SRR of 45[40;51]%).

Meta-regression analysis showed that SRR per cycle was independent of age, testis volume and hormonal parameters at enrollment (Fig. 3A–E). Accordingly, no difference in SRR per cycle was observed when studies enrolling patients <20 years were compared to the rest of the sample (SRR 43[35;51] vs 43[38;49]% $Q = 0.01$; $P = 0.95$). Similarly, no difference was observed according to year of study publication (not shown).

When sensitivity analysis was performed according to the type of surgical approach, no difference was observed when a bilateral procedure was compared to a unilateral approach (SRR 51[37;65] vs 44 [38;49]%, $Q = 0.91$, $P = 0.34$). No sufficient data were available to evaluate the effect of previous testosterone treatment on SRR.

## Fertility outcome

Among the studies included in the SRR analysis, information on fertility outcome after ICSI were available for 29 trials (Table I). In these trials, the mean age of women was 29.5 ± 2.9 years. In addition, the ICSI procedure was performed with either cryopreserved or fresh sperm in seven and eleven trials, respectively (Table I). Eight studies applied a mixed approach using both cryopreserved or fresh sperm

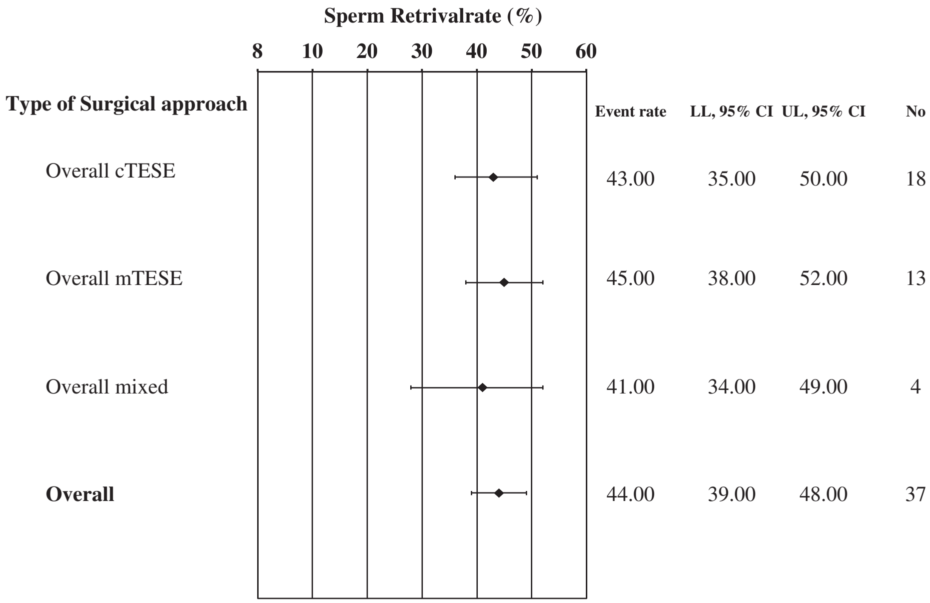


**Figure 2** Sperm retrieval rate (SRR) per testicular sperm extraction (TESE) cycle according to the type of surgical approach. cTESE = conventional TESE; mTESE = microsurgical-TESE.

whereas this information was not available in three cases (Table 1). $I^2$ in trials assessing overall PR was 35.40 ($P < 0.05$). Overall a total of 218 biochemical pregnancies after 410 ICSI cycles were observed (cumulative PR = 43[36;50]% per ICSI cycle; Fig. 4A). A funnel plot and Begg adjusted rank correlation test (Kendall's $\tau$: −0.01; $P = 0.93$) suggested the absence of publication bias. Similar results were observed when the LBR per ICSI cycle was analyzed from the 211 live births (cumulative LBR = 43[34;53]% per ICSI cycle; Fig. 4B). Similar to what was observed for SRR, no influence of KS age, mean testis volume, or LH or total T levels on both PR and LBR per ICSI cycle were observed (not shown). However, FSH levels at enrollment showed a trend toward an inversely significant association with LBR per ICSI cycle ($S$ = −0.056[−0.117;0.004]; $P$ = 0.06 and $I$ = 1.883 [−0.132;3.899]; $P = 0.06$). Sufficient data were not available to test the effect of women's age or other female fertility problems on PR or LBR.

When sensitivity analysis was performed according to the type of sperm used for ICSI procedure, no difference in cumulative PR per ICSI cycle was observed when the use of fresh sperm was compared to the utilization of cryopreserved sperm (PR = 39[26;53]% vs 36 [23;50]%, respectively; $Q$ = 0.10, $P$ = 0.76). Similar results were observed when the cumulative LBR per ICSI cycle was analyzed (LBR = 39[23;57]% vs 29[17;44]%, respectively; $Q = 0.78$, $P = 0.38$).

Finally, when cumulative LBR was calculated according to the number of biochemical pregnancies obtained, a limited abortion rate was detected (15[10;23]%).

# Discussion

In this study, we systematically reviewed and meta-analyzed for the first time all available information regarding SRR and fertility outcome in subjects with KS. In this specific population, we report an overall SRR of about 40%, which is independent of several clinical and biochemical parameters, including age, testis volume and hormonal status at baseline. In addition, the use of retrieved sperm allows live children to be born in ~40% of ICSI cycles meaning a final LBR of 16% for the couples who initiated the assisted reproductive techniques.

In 1996, Tournaye *et al.* reported a successful recovery of spermatozoa by cTESE in men with azoospermia and KS for the first time. One year later, Palermo *et al.* (1998) documented the first pregnancies in KS after TESE/ICSI. Almost 20 years later, the predictive factors underlying successful TESE in KS are still conflicting. Based on the reported progressive hyalinization of seminiferous tubules observed after puberty in subjects with KS, it has been suggested that performing earlier TESE procedures might result in better outcomes (Franik *et al.*, 2016; Gies *et al.*, 2016). In contrast to this view, the present data show that successful SRR in KS is independent of age. Accordingly, it has been reported that the progressive hyalinization of seminiferous tubules which characterizes KS testes after puberty is not ubiquitous and it is possible to observe tubules with normal residual activity (Franik *et al.*, 2016; Gies *et al.*, 2016). The mechanisms underlying this process are not yet fully known. Recent evidence seems to suggest that the impaired spermatogenesis in KS patients could also be caused by an intrinsic defect of the germ cells, possibly linked to (epi)-genetics of the surplus X chromosome instead of being a result of the hyalinization and fibrosis of the testicular environment (Aksglaede and Juul, 2013; Franik *et al.*, 2016; Gies *et al.*, 2016). The stable SRR of around 40% among KS patients seems to support this view. However, sufficient information on the inactivation pattern of the surplus X chromosome was not available in the studies analyzed in this meta-analysis. Hence, this hypothesis needs to be confirmed in specific trials. Besides age, other factors including

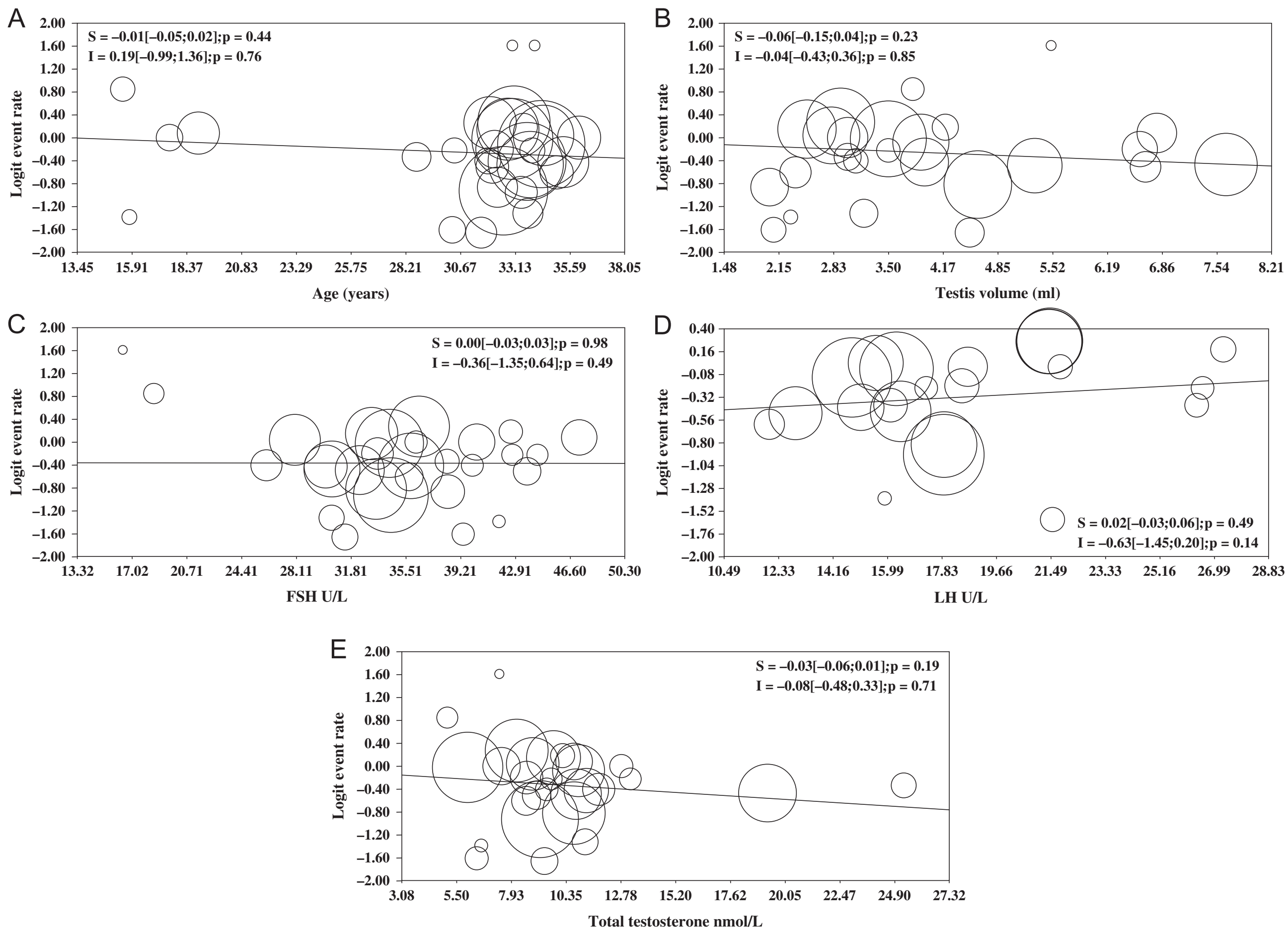


**Figure 3** Influence of age (**A**), testis volume (**B**), FSH (**C**), LH (**D**) and total testosterone at enrollment on sperm retrieval rate. The size of the circles indicates sample dimension.

hormone pattern and testicular volume have been advocated as possible prognostic values for successful SRR in KS patients (Forti et al., 2010; Aksglaede and Juul, 2013; Franik et al., 2016; Gies et al., 2016). Rohayem et al. (2015) reported that the combination of total serum testosterone above 7.5 nmol/l and LH levels below 17.5 U/l resulted in higher retrieval rates of spermatozoa by micro-TESE in both adolescents and adults with KS (Rohayem et al., 2015). Similar results were more recently reported by Cissen et al. (2016). Our data showed that neither testicular volume nor hormonal pattern influenced SRR in KS patients. Interestingly, in line with our data, Rohayem et al. (2016) did not document any clinical difference in non-mosaic KS subjects with or without spermatozoa in the seminal fluid. The lack of prognostic value of the FSH levels might be related to the low inhibin B levels (which is almost undetectable during early puberty) in all patients with KS, which does not allow for the negative feed-back on FSH secretion (Aksglaede et al., 2011). Similarly, the testicular growth impairment observed in KS since early infancy might reduce its prognostic value in SRR.

When the type of surgical procedure was analyzed, we did not document any difference by comparing cTESE to micro-TESE or when a bilateral approach was compared to a unilateral intervention. The reduced testis volume in KS might limit the advantages of micro-TESE in SRR observed in the general population of subjects with azoospermia (Amer et al., 2000). It should be recognized that post-operative testicular damage leading to a decrease in testicular function has been described as a complication of testicular biopsy (Manning et al., 1998). It should be recognized that micro-TESE has been associated with a lower incidence of acute and chronic complications when compared to cTESE in subjects with non-obstructive azoospermia and without KS (Schlegel, 1999; Amer et al., 2000). Similar results have been reported in patients with KS (Okada et al., 2004; Takada et al., 2008; Ishikawa et al., 2009). Unfortunately, sufficient data on complications of surgical approach were not available in the studies included in this meta-analysis.

Fathering is an important issue in subjects with KS. A recent survey performed in almost 200 Dutch subjects with KS documented that the majority of KS patients and their partners would like to have children and have a positive attitude toward TESE–ICSI treatment (Maiburg et al., 2011). The results of the present meta-analysis show that live children could be obtained in about 16% of subjects who

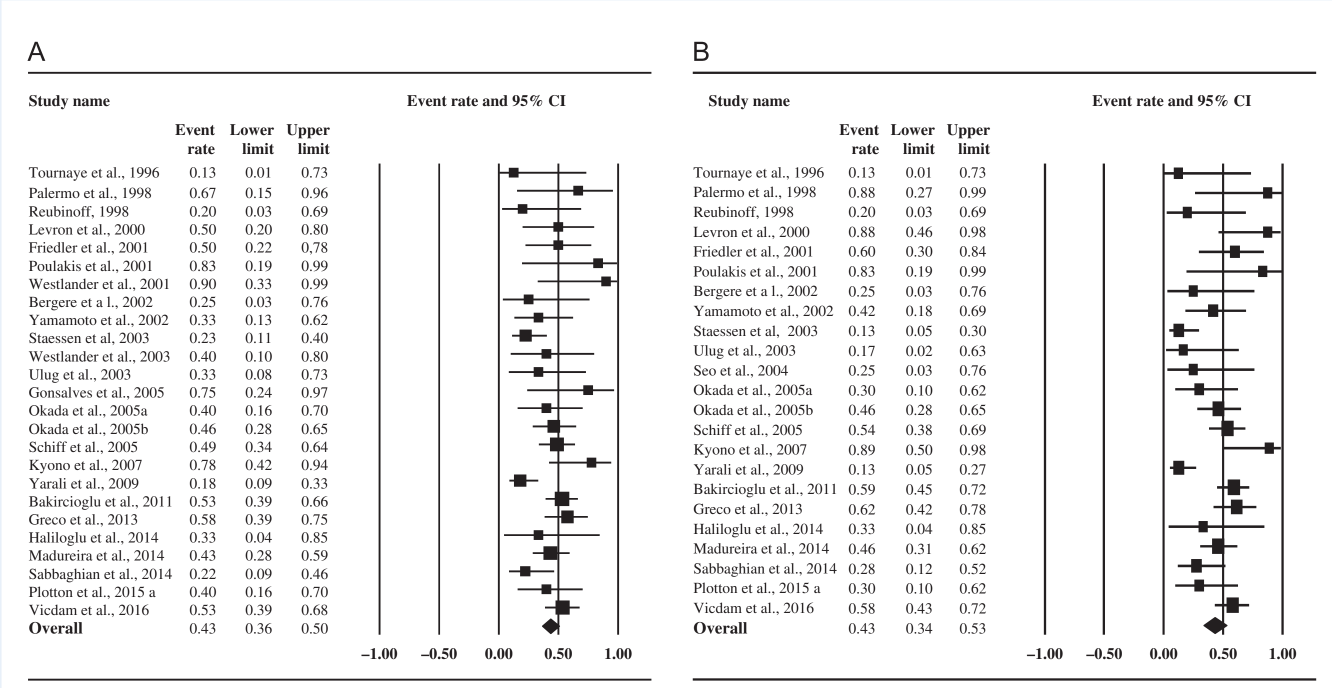


**Figure 4** Meta-analysis of pregnancy rate (**A**) and live birth rate (LBR) per ICSI cycle.

undergo TESE approach. Although no studies evaluating one-to-one comparisons are available, our rate is similar, although a little lower, than that reported in non-KS subjects with non-obstructive azoospermia (25%; Cissen *et al.*, 2016). In addition, similar to what was observed for SRR, no clinical and biochemical factors influenced the final pregnancy outcome. Finally, no difference in PR and LBR was observed when the use fresh sperm was compared to the use of cryopreserved sperm. The latter finding is not surprising and in line with what has been reported in the general population (Hessel *et al.*, 2015).

Several limitations related to this study should be emphasized. The use average results obtained in each study with the absence of patient-level data might represent a first source of bias. Moreover we cannot exclude some selection bias derived from retrospective studies included in this meta-analysis. Meta-analyses allow the combination of a large number of investigations improving the statistical power and reducing the risk of casual results related to small sample size. However, the possibility that some of the obtained results, reported in this study, can be the consequence of the effects of unadjusted confounders cannot be excluded at all. Hence, caution should be used in the interpretation of final results, which should be confirmed in larger trials. Treatment with testosterone has previously been reported to be a negative influence on future fertility of KS (Schiff *et al.*, 2005). Conversely, recent studies have described better SRR in a small group of adolescents and young adults with KS, who received testosterone supplementation and aromatase inhibitor therapy for several years before TESE (Paduch *et al.*, 2008; Mehta *et al.*, 2013). Because of the limited number of papers reporting SRR in subjects previously treated with testosterone, in this review we cannot draw final conclusions on this topic. Similarly, sufficient data are not available to test the effect of other hormones such as estradiol prolactin and INSL-3 levels or to evaluate the effect of cryptorchidism. Finally, sufficient information to analyze the incidence of aneuploidies in the obtained children was not available.

In conclusion, the present data show that despite KS patients usually being azoospermic, their actual chances of fertility is similar to subjects with non-obstructive azoospermia without KS. Even if the conception in KS appears relatively safe and the risk of chromosomal abnormalities is similar to that reported in subjects without KS, it is questionable whether or not preimplantation genetic diagnosis should be offered to couples with KS who undergo successful TESE and ICSI to avoid transferring abnormal embryos.

# Supplementary data

Supplementary data are available at Human Reproduction Update online.

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# Authors' roles

Giovanni Corona: study design, execution, analysis, critical discussion.

Alessandro Pizzocaro: study design, manuscript drafting, critical discussion.

Fabio Lanfranco: study design, execution.

Andrea Garolla: study design, manuscript drafting, critical discussion.

Fiore Pelliccione: study design, manuscript drafting.

Linda Vignozzi: study design, execution.

Alberto Ferlin: critical discussion.

Carlo Foresta: critical discussion.

Emmanuele A. Jannini: critical discussion.

Mario Maggi: critical discussion.

Andrea Lenzi: critical discussion.

Daniela Pasquali: critical discussion.

Sandro Francavilla: study design, execution, critical discussion.

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# Conflict of interest

The authors declare that they have no conflict of interest.

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