

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

Giovanni Corona<sup>1,†</sup>, Alessandro Pizzocaro<sup>2,†</sup>, Fabio Lanfranco<sup>3,†</sup>,  
Andrea Garolla<sup>4,†</sup>, Fiore Pelliccione<sup>2</sup>, Linda Vignozzi<sup>5</sup>,  
Alberto Ferlin<sup>4</sup>, Carlo Foresta<sup>4</sup>, Emmanuele A. Jannini<sup>6</sup>,  
Mario Maggi<sup>5</sup>, Andrea Lenzi<sup>7</sup>, Daniela Pasquali<sup>8</sup>, Sandro Francavilla<sup>9,\*</sup>,  
and On behalf of the Klinefelter ItaliaN Group (KING)

<sup>1</sup>Endocrinology Unit, Medical Department, Endocrinology Unit, Azienda Usl Bologna Maggiore-Bellaria Hospital, Via Altura 3, 40139 Bologna, Italy <sup>2</sup>Endocrinology Unit, IRCCS, Humanitas Research Hospital, Rozzano, Via Manzoni 56, 20089 Milan, Italy <sup>3</sup>Department of Medical Sciences, Division of Endocrinology, Diabetology and Metabolism, University of Torino, Corso Dogliotti 14, 10126 Turin, Italy <sup>4</sup>Department of Medicine, Andrology and Reproductive Medicine Unit, University of Padova, Via Giustiniani 2, 35100 Padova, Italy <sup>5</sup>Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences, Sexual Medicine and Andrology Unit, University of Florence, Largo Brambilla 3, 50134 Florence, Italy <sup>6</sup>Department of Systems Medicine, Tor Vergata University of Rome, Via Montpellier 1, 00166 Rome, Italy <sup>7</sup>Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy <sup>8</sup>Department of Cardiothoracic and Respiratory Sciences, Endocrine Unit, Second University of Naples, Via Pansini 5, 80131 Naples, Italy <sup>9</sup>Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio, 67100 L'Aquila, Italy

\*Correspondence address. Department of Internal Medicine, Andrology Unit, University of L'Aquila, Via Vetoio, 67100 L'Aquila, Italy. Tel: +39-0862-368338; Fax: +39-0862-338342; E-mail: sandro.francavilla@univaq.it

Submitted on January 23, 2017; resubmitted on March 8, 2017; editorial decision on March 21, 2017; accepted on March 27, 2017

## TABLE OF CONTENTS

- Introduction
- Methods
  - Search strategy
  - Study selection
  - Outcome and quality assessment
  - Statistical analysis
- Results
  - Sperm retrieval outcome
  - Fertility outcome
- Discussion

**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.

<sup>†</sup>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  $30.9 \pm 5.6$  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 ranging 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; Sciarano 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 (Vernaev 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

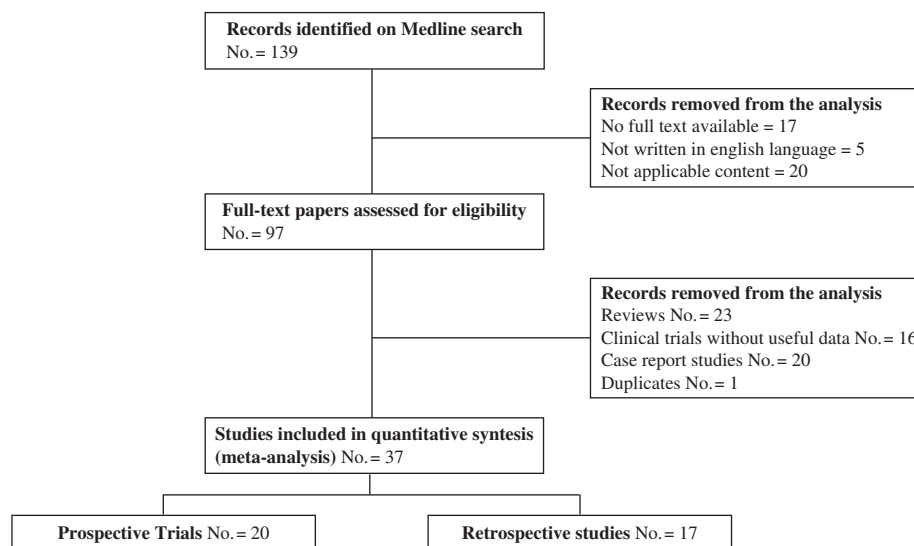
Heterogeneity in SRR was assessed using  $I^2$  statistics. Even when low heterogeneity was detected, a random-effect model was applied, because the validity of tests of heterogeneity can be limited with a small number of component studies. We used funnel plots and the Begg adjusted rank correlation 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  $30.9 \pm 5.6$  years. Mean testicular volume was  $3.9 \pm 1.6$  ml and mean hormonal parameters reflect the condition of primary or compensated hypogonadism (FSH =  $36.0 \pm 7.0$  U/L, LH  $18.4 \pm 4.3$  U/L, total testosterone  $10.3 \pm 4.0$  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 1** 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)
<a href="#">Tournaye et al. (1996)</a>	9	10	cTESE	Yes	Yes	4	Fresh	3	0	0	31.9	NR	NM	40	26.4	9.5	3.1
<a href="#">Palermo et al. (1998)</a>	2	2	cTESE	Yes	Yes	2	Fresh	3	2	3	33.0	32.5	NM	NR	NR	NR	NR
<a href="#">Reubinoff et al. (1998)</a>	7	9	TESA	Yes	Yes	4	Fresh	5	1	1	30.4	26.2	NM	44.4	26.6	13.2	NR
<a href="#">Levron et al. (2000)</a>	20	20	cTESE	NR	NR	8	Mixed	8	4	7	NR	NR	NM	26.1	16.1	11.8	NR
<a href="#">Friedler et al. (2001)</a>	12	12	cTESE	Yes	Yes	5	Mixed	10	5	6	28.	26.4	NM	38.3	NR	25.3	3
<a href="#">Poulakis et al. (2001)</a>	2	2	cTESE	Yes	NR	2	Fresh	2	2	2	34	28.5	NM	16.4	16.5	7.4	5.5
<a href="#">Westlander et al. (2001)</a>	19	19	cTESE	Yes	Yes	4	NR	4	4	NR	33.	NR	NM	30.5	NR	11.2	3.2
<a href="#">Bergère et al. (2002)</a>	4	4	cTESE	Yes	Yes	3	CP	4	1	1	NR	NR	NM	26-33.7	NR	NR	4-6
<a href="#">Madgar et al. (2002)</a>	20	NR	cTESE	NR	NR	9	NR	NR	NR	NR	32.2	NR	NM	33.6	18.5	8.6	6.6
<a href="#">Yamamoto et al. (2002)</a>	24	24	cTESE	No	No	12	Fresh	12	4	5	23-4	NR	NM	14-56	NR	NR	NR
<a href="#">Staessen et al. (2003)</a>	19	19	cTESE	Yes	Yes	17	Mixed	31	7	4	NR	29.5	NM	NR	NR	NR	NR
<a href="#">Westlander et al. (2003)</a>	18	18	cTESE	Yes	Yes	5	CP	5	2	NR	33.4	NR	NM	NR	NR	NR	2-5
<a href="#">Ulug et al. (2003)</a>	11	11	cTESE	No	Yes	6	Fresh	6	2	1	33.4	30.4	NM	42.6	27.3	10.2	4.2
<a href="#">Seo et al. (2004)</a>	25	25	cTESE	Yes	Yes	4	Fresh	4	2	1	31.6	NR	NM	31.4	NR	9.4	4.5
<a href="#">Vernaev et al. (2004)</a>	50	50	cTESE	Yes	Yes	24	Mixed	NR	NR	NR	31.2	NR	NM	36.0	NR	10.91	3.9
<a href="#">Gonsalves et al. (2005)</a>	4	4	NR	NR	NR	4	CP	4	3	6	33.2	NR	NM	NR	NR	NR	NR
<a href="#">Okada et al. (2005a)</a>	10	10	mTESE	Yes	Yes	6	CP	10	4	3	NR	27.3	NM	NR	NR	NR	NR
<a href="#">Okada et al. (2005b)</a>	51	51	Mixed	Yes	Yes	26	Mixed	26	12	12	34.4	NR	NM	28.0	15.6	8.9	2.8
<a href="#">Schiff et al. (2005)</a>	42	54	mTESE	Yes	Yes	29	Fresh	39	19	21	32.8	33.2	3 M	33.2	NR	9.8	2.5

<a href="#">Bakircioglu et al. (2006)</a>	74	74	mTESE	Yes	Yes	42	NA	NA	NA	NA	33.1	NR	NM	36.4	21.5	8.2	2.9
<a href="#">Kyono et al. (2007)</a>	17	17	cTESE	Yes	Yes	6	Mixed	9	7	8	35.0	30.6	NM	35.7	12.0	8.6	2.4
<a href="#">Koga et al. (2007)</a>	26	26	mTESE	Mixed	Yes	13	NR	NR	4	2	36.0	NR	NM	40.3	18.7	7.5	3.0
<a href="#">Takada et al. (2008)</a>	9	9	mTESE	NR	Yes	4	NR	NR	NR	NR	33.9	NR	NM	42.7	17.3	9.7	3.5
<a href="#">Ferhi et al. (2009)</a>	27	27	Mixed	Yes	No	8	CP	NR	4	5	32.3	NR	NM	38.3	NR	NR	2.04
<a href="#">Ramasamy et al. (2009)</a>	68	91	mTESE	Yes	Yes	45	Fresh	NR	33	28	33	NR	NM	34.4	16.3	6.0	3.5
<a href="#">Yarali et al. (2009)</a>	33	39	mTESE	NR	Yes	22	Fresh	39	7	5	32	NR	NM	NR	NR	NR	NR
<a href="#">Bakircioglu et al. (2011)</a>	106	106	mTESE	Yes	Yes	50	Fresh	49	26	29	34.3	NR	NM	NR	14.8	NR	NR
<a href="#">Greco et al. (2013)</a>	38	38	Mixed	Yes	Yes	15	Mixed	26	15	16	35.3	33.7	NM	30.1	15.1	11.3	3.9
<a href="#">Mehta et al. (2013)</a>	10	10	mTESE	Mixed	Yes	7	CP	NR	NR	NR	15.5	NR	NM	18.5	NR	5.1	3.8
<a href="#">Rives et al. (2013)</a>	5	5	cTESE	Yes	No	1	CP	NA	NA	NA	15.8	NR	NM	41.8	15.9	6.6	2.3
<a href="#">Haliloglu et al. (2014)</a>	18	18	mTESE	NR	Yes	3	NR	3	1	1	30.3	NR	NM	39.4	21.6	6.4	2.09
<a href="#">Madureira et al. (2014)</a>	65	65	cTESE	Mixed	Yes	25	Mixed	37	16	17	33.8	NR	NM	30.5	16.4	19.3	7.7
<a href="#">Sabbaghian et al. (2014)</a>	134	134	mTESE	Mixed	Yes	38	CP	18	4	5	32.6	15.9	NM	34.5	17.9	9.2	NA
<a href="#">Plotton et al. (2015) y</a>	25	25	cTESE	Yes	Yes	13	CP	NA	NA	NA	18.2	NA	NM	47.2	NR	10.7	6.8
<a href="#">Plotton et al. (2015) a</a>	16	16	cTESE	Yes	Yes	6	CP	10	4	3	32.1	NR	NM	43.7	NR	9.1	6.7
<a href="#">Rohayem et al. (2015) y</a>	50	50	mTESE	Yes	Yes	45	NA	NA	NA	NA	NR	NR	NM	32.4	12.9	10.8	5.3
<a href="#">Rohayem et al. (2015) a</a>	50	85	mTESE	Yes	Yes	45	NA	NA	NA	NA	NR	NR	NM	33.5	17.9	10.7	4.6
<a href="#">Nahata et al. (2016)</a>	10	10	mTESE	No	Yes	5	NA	NA	NA	NA	17.6	NR	NM	36.2	NR	12.8	2.3
<a href="#">Vicdan et al. (2016)</a>	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
Tourmaye 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	??
Vernaev 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

Continued

**Table II** Continued

Study	Selection bias	Study design	Data collection	Global rating
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

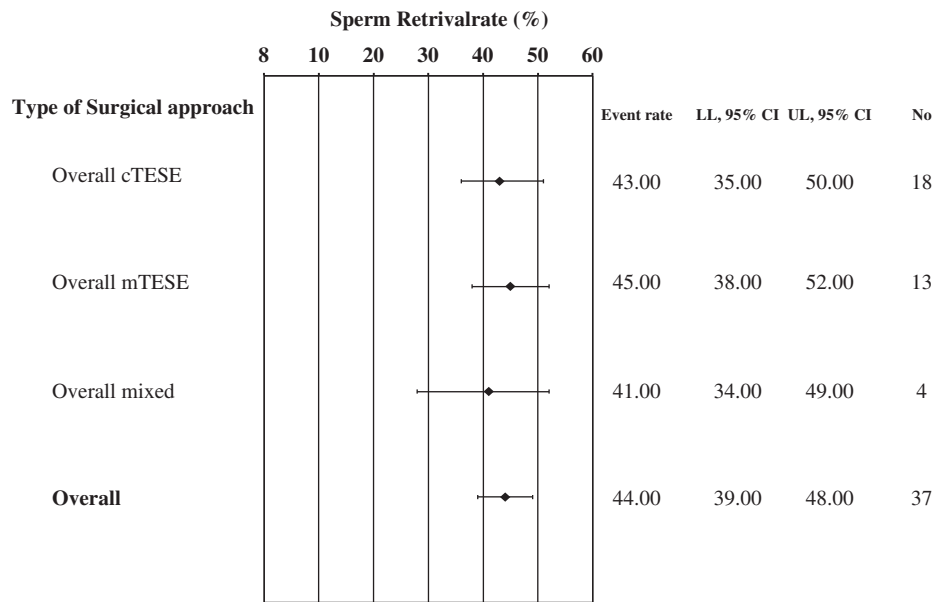
(Fig. 2;  $Q = 0.20$ ,  $P = 0.65$ ). Finally, no differences were observed when SRR per patient was considered (SRR of 45[40;51]%).

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  $29.5 \pm 2.9$  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 I).  $I^2$  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;  $Q = 0.78$ ,  $P = 0.38$ ).

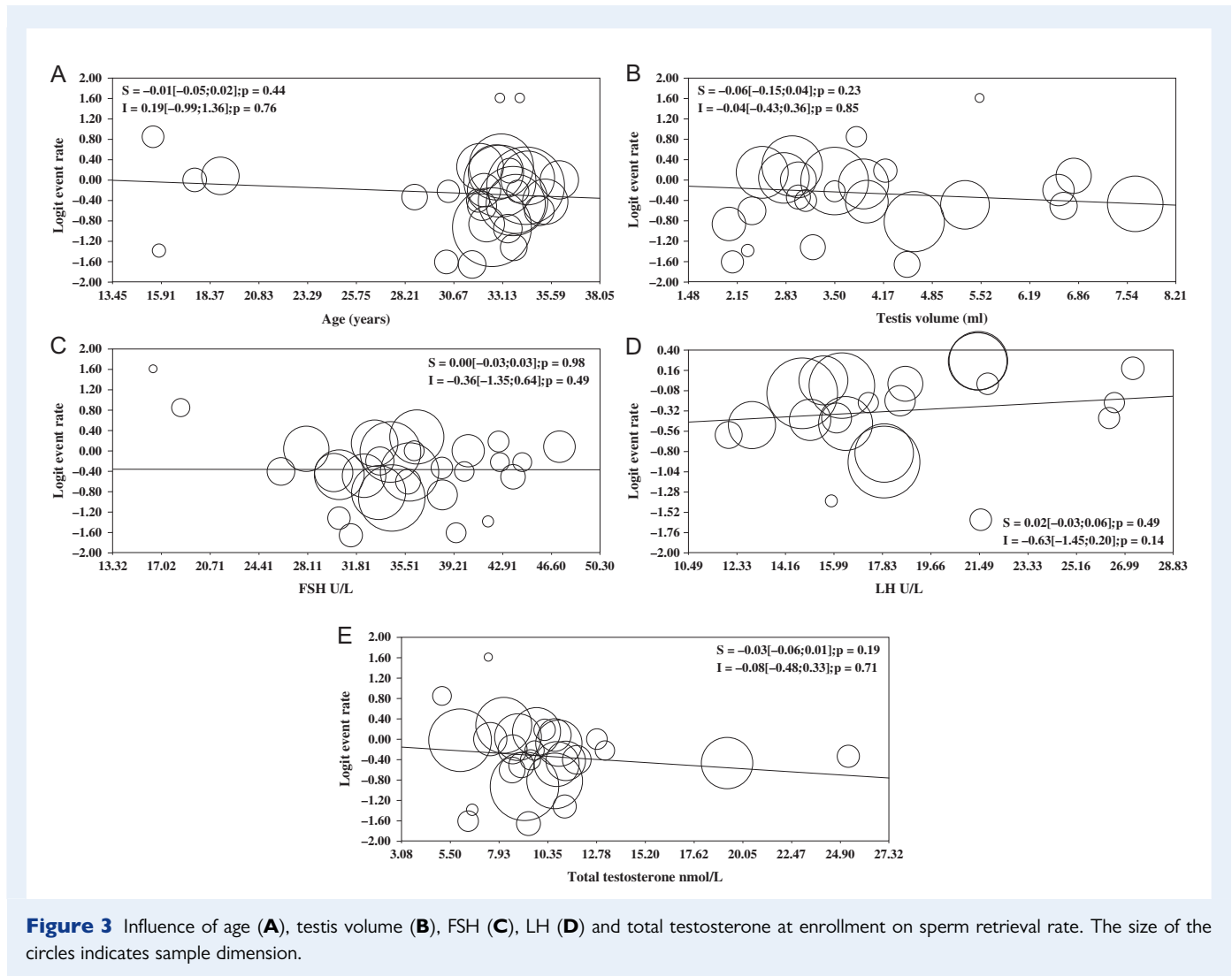
Finally, when cumulative LBR was calculated according to the number of biochemical pregnancies obtained, a limited abortion rate was detected (15[10;23]%).

## 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



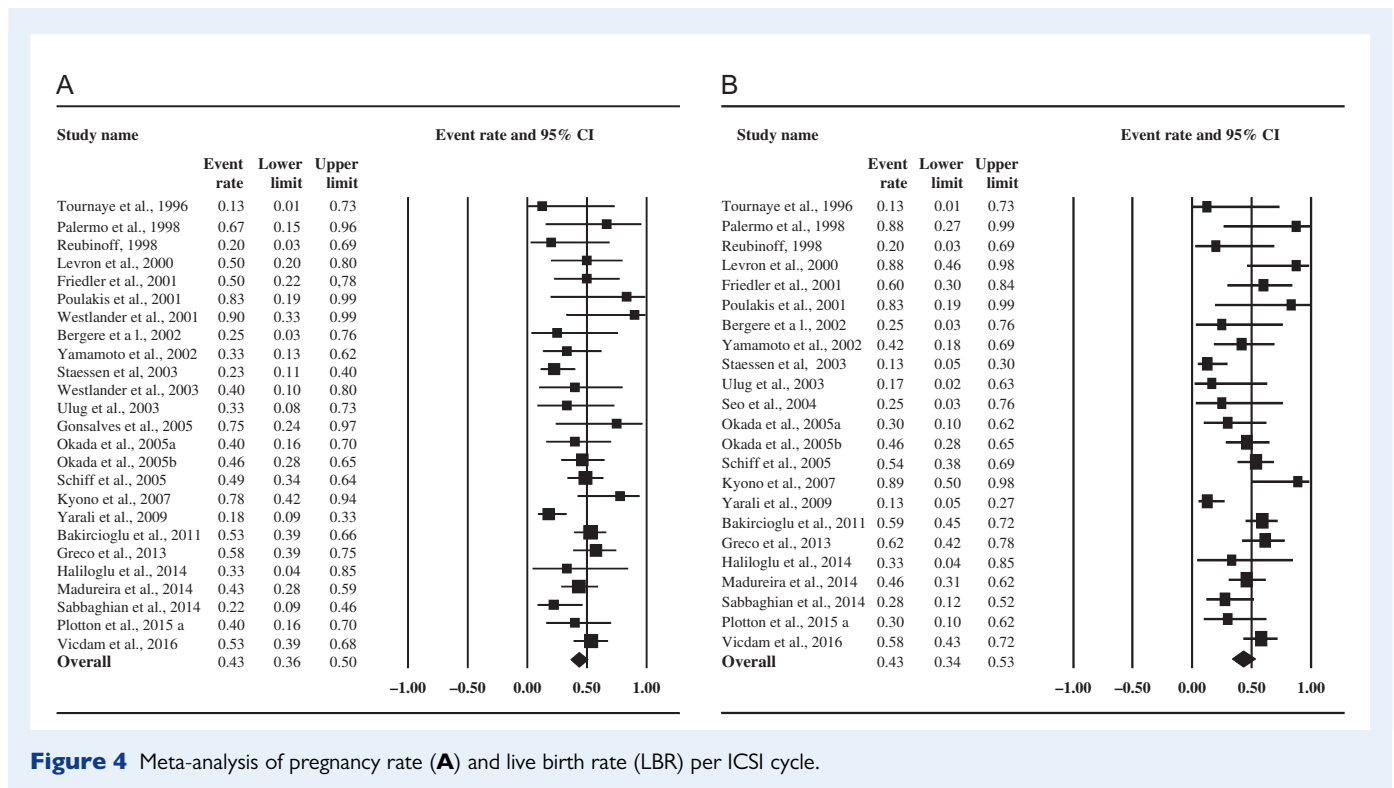
hormone pattern and testicular volume have been advocated as possible prognostic values for successful SRR in KS patients (Forti et al., 2010; Aksglaede and Juul, 2013; Franik et al., 2016; Gies et al., 2016). Rohayem et al. (2015) reported that the combination of total serum testosterone above 7.5 nmol/l 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.

## Acknowledgements

This study was performed on behalf of the Klinefelter ItaliaN Group (KING). Coordinators: Giancarlo Balercia (Ancona), Marco Bonomi (Milan), Aldo Calogero (Catania), Giovanni Corona (Bologna), Andrea Fabbri (Rome), Alberto Ferlin (Padua), Felice Francavilla (L'Aquila), Vito Giagulli (Conversano, Bari), Fabio Lanfranco (Turin), Mario Maggi (Florence), Daniela Pasquali (Naples), Rosario Pivonello (Naples), Alessandro Pizzocaro (Milan), Antonio Radicioni (Rome), Vincenzo Rochira (Modena), Linda Vignozzi (Florence); Members:

Giacomo Accardo (Naples), Biagio Cangiano (Milan), Rosita A. Condorelli (Catania), Giuliana Cordeschi (L'Aquila), Settimio D'Andrea (L'Aquila), Antonella Di Mambro (Padua), Daniela Esposito (Naples), Carlo Foresta (Padua), Sandro Francavilla (L'Aquila), Mariano Galdiero (Naples), Andrea Garolla (Padua), Lara Giovannini (Ancona), Antonio R.M. Granata (Modena), Sandro La Vignera (Catania), Giovanna Motta (Turin), Luciano Negri (Milan), Fiore Pelliccione (Milan), Luca Persani (Milan), Ciro Salzano (Naples), Daniele Santi (Modena), Riccardo Selice (Padua), Manuela Simoni (Modena), Carla Tatone (L'Aquila), Giacomo Tirabassi (Ancona), Alberto Stefano Tresoldi (Milan) and Enzo Vicari (Catania). The KING belongs to the Italian Society of Andrology and Sexual Medicine (SIAMS) and aims to promote all the activities, clinical, research, and informative, concerning KS in Italy. The KING is made up of highly-specialized endocrinology and andrology units, either academic or institutes for treatment and research (IRCCS), located throughout Italy.

## 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.

## Funding

This research project did not receive any funding.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

Aksglaede L, Andersson AM, Jørgensen N, Jensen TK, Carlsen E, McLachlan RI, Skakkebaek NE, Petersen JH, Juul A. Primary testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing hormone-testosterone reference charts. *Clin Endocrinol (Oxf)* 2007;**66**:276–281.

Aksglaede L, Skakkebaek NE, Almstrup K, Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. *Acta Paediatr* 2011;**100**:793–806.

Aksglaede L, Juul A. Testicular function and fertility in men with Klinefelter syndrome: a review. *Eur J Endocrinol* 2013;**168**:R67–R76.

Amer M, Ateyah A, Hany R, Zohdy W. Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: follow-up by serial ultrasound examinations. *Hum Reprod* 2000;**15**: 653–656.

Bakircioglu EM, Erden HF, Kaplancan T, Ciray N, Bener F, Bahceci M. Aging may adversely affect testicular sperm recovery in patients with Klinefelter syndrome. *Urology* 2006;**68**:1082–1086.

Bakircioglu ME, Ulug U, Erden HF, Tosun S, Bayram A, Ciray N, Bahceci M. Klinefelter syndrome: does it confer a bad prognosis in treatment of nonobstructive azoospermia? *Fertil Steril* 2011;**95**:1696–1699.

Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;**50**:1088–1101.

Bergère M, Wainer R, Nataf V, Bailly M, Gombault M, Ville Y, Selva J. Biopsied testis cells of four 47,XXY patients: fluorescence in-situ hybridization and ICSI results. *Hum Reprod* 2002;**17**:32–37.

Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab* 2003;**88**:622–626.

Cissen M, Meijerink AM, D'Hauwers KW, Meissner A, van der Weide N, Mochtar MH, de Melker AA, Ramos L, Repping S, Braat DD, et al. Prediction model for obtaining spermatozoa with testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 2016;**31**:1934–1941.

Corona G, Petrone L, Paggi F, Lotti F, Boddi V, Fisher A, Vignozzi L, Balercia G, Sforza A, Forti G, et al. Sexual dysfunction in subjects with Klinefelter's syndrome. *Int J Androl* 2010;**33**:574–580.

Ferhi K, Avakian R, Griveau JF, Guille F. Age as only predictive factor for successful sperm recovery in patients with Klinefelter's syndrome. *Andrologia* 2009;**41**:84–87.

Foresta C, Galeazzi C, Bettella A, Marin P, Rossato M, Garolla A, Ferlin A. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. *J Clin Endocrinol Metab* 1999;**84**:3807–3810.

Forti G, Corona G, Vignozzi L, Krausz C, Maggi M. Klinefelter's syndrome: a clinical and therapeutical update. *Sex Dev* 2010;**4**:249–258.

Franik S, Hoeijmakers Y, D'Hauwers K, Braat DD, Nelen WL, Smeets D, Claahsen-van der Grinten HL, Ramos L, Fleischer K. Klinefelter syndrome and fertility: sperm preservation should not be offered to children with Klinefelter syndrome. *Hum Reprod* 2016;**31**:1952–1959.

Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. *Hum Reprod* 2001;**16**:2616–2620.

Gies I, Unuane D, Velkeniers B, De Schepper J. Management of Klinefelter syndrome during transition. *Eur J Endocrinol* 2014;**171**:R67–R77.

Gies I, Oates R, De Schepper J, Tournaye H. Testicular biopsy and cryopreservation for fertility preservation of prepubertal boys with Klinefelter syndrome: a pro/con debate. *Fertil Steril* 2016;**105**:249–255.

Gonsalves J, Turek PJ, Schlegel PN, Hopps CV, Weier JF, Pera RA. Recombination in men with Klinefelter syndrome. *Reproduction* 2005;**130**:223–229.

Greco E, Scarselli F, Minasi MG, Casciani V, Zavaglia D, Dente D, Tesarik J, Franco G. Birth of 16 healthy children after ICSI in cases of nonmosaic Klinefelter syndrome. *Hum Reprod* 2013;**28**:1155–1160.

Haliloglu AH, Tangel S, Gulpinar O, Onal K, Pabuccu R. Should repeated TESE be performed following a failed TESE in men with Klinefelter syndrome? *Andrology* 2014;**2**:42–44.

Herlihy AS, Halliday JL, Cock ML, McLachlan RI. The prevalence and diagnosis rates of Klinefelter syndrome: an Australian comparison. *Med J Aust* 2011;**194**:24–28.

Hessel M, Robben JC, D'Hauwers KW, Braat DD, Ramos L. The influence of sperm motility and cryopreservation on the treatment outcome after intracytoplasmic sperm injection following testicular sperm extraction. *Acta Obstet Gynecol Scand* 2015;**94**:1313–1321.

Higgins JPT, Green S. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 5.0.1 [updated September 2008]. The Cochrane Collaboration. 2008 Available from <http://www.cochrane-handbook.org> (3 February 2014, date last accessed)

Koga M, Tsujimura A, Takeyama M, Kiuchi H, Takao T, Miyagawa Y, Takada S, Matsumiya K, Fujioka H, Okamoto Y, et al. Clinical comparison of successful and failed microdissection testicular sperm extraction in patients with nonmosaic Klinefelter syndrome. *Urology* 2007;**70**:341–345.

Kyono K, Uto H, Nakajo Y, Kumagai S, Araki Y, Kanto S. Seven pregnancies and deliveries from non-mosaic Klinefelter syndrome patients using fresh and frozen testicular sperm. *J Assist Reprod Genet* 2007;**24**:47–51.

Ishikawa T, Yamaguchi K, Chiba K, Takenaka A, Fujisawa M. Serum hormones in patients with nonobstructive azoospermia after microdissection testicular sperm extraction. *J Urol* 2009;**182**:1495–1499.

- Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. *Lancet* 2004;**364**:273–283.
- Levron J, Aviram-Goldring A, Madgar I, Raviv G, Barkai G, Dor J. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's syndrome. *Fertil Steril* 2000;**74**:925–929.
- Madgar I, Dor J, Weissenberg R, Raviv G, Menashe Y, Levron J. Prognostic value of the clinical and laboratory evaluation in patients with nonmosaic Klinefelter syndrome who are receiving assisted reproductive therapy. *Fertil Steril* 2002;**77**:1167–1169.
- Madureira C, Cunha M, Sousa M, Neto AP, Pinho MJ, Viana P, Gonçalves A, Silva J, Teixeira da Silva J, Oliveira C, et al. Treatment by testicular sperm extraction and intracytoplasmic sperm injection of 65 azoospermic patients with non-mosaic Klinefelter syndrome with birth of 17 healthy children. *Andrology* 2014;**2**:623–631.
- Maiburg MC, Hoppenbrouwers AC, van Stel HF, Giltay JC. Attitudes of Klinefelter men and their relatives towards TESE-ICSI. *J Assist Reprod Genet* 2011;**28**:809–814.
- Manning M, Jünemann KP, Alken P. Decrease in testosterone blood concentrations after testicular sperm extraction for intracytoplasmic sperm injection in azoospermic men. *Lancet* 1998;**352**:37.
- Mehta A, Bolyakov A, Roosa J, Schlegel PN, Paduch DA. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. *Fertil Steril* 2013;**100**:970–974.
- Nahata L, Yu RN, Paltiel HJ, Chow JS, Logvinenko T, Rosoklija I, Cohen LE. Sperm retrieval in adolescents and young adults with Klinefelter syndrome: a prospective, pilot study. *J Pediatr* 2016;**170**:260–265.
- Okada H, Shirakawa T, Ishikawa T, Goda K, Fujisawa M, Kamidono S. Serum testosterone levels in patients with nonmosaic Klinefelter syndrome after testicular sperm extraction for intracytoplasmic sperm injection. *Fertil Steril* 2004;**82**:237–238.
- Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, Koshida M, Horie S. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. *Fertil Steril* 2005a;**84**:1662–1664.
- Okada H, Goda K, Muto S, Maruyama O, Koshida M, Horie S. Four pregnancies in nonmosaic Klinefelter's syndrome using cryopreserved-thawed testicular spermatozoa. *Fertil Steril* 2005b;**84**:1508.e13–e16.
- Paduch DA, Fine RG, Bolyakov A, Kiper J. New concepts in Klinefelter syndrome. *Curr Opin Urol* 2008;**18**:621–627.
- Palermo GD, Schlegel PN, Sills ES, Veeck LL, Zaninovic N, Menendez S, Rosenwaks Z. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with nonmosaic Klinefelter's syndrome. *N Engl J Med* 1998;**338**:588–590.
- Ploton I, Giscard d'Estaing S, Cuzin B, Brosse A, Benchaib M, Lornage J, Ecochard R, Dijoud F, Lejeune H, FERTIPRESERVE group. Preliminary results of a prospective study of testicular sperm extraction in young versus adult patients with nonmosaic 47,XXY Klinefelter syndrome. *J Clin Endocrinol Metab* 2015;**100**:961–967.
- Poulakis V, Witzsch U, Diehl W, de Vries R, Becht E, Trotnow S. Birth of two infants with normal karyotype after intracytoplasmic injection of sperm obtained by testicular extraction from two men with nonmosaic Klinefelter's syndrome. *Fertil Steril* 2001;**76**:1060–1062.
- Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. *J Urol* 2009;**182**:1108–1113.
- Reubinoff BE, Abelovich D, Werner M, Schenker JG, Safran A, Lewin A. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. *Hum Reprod* 1998;**13**:1887–1892.
- Rives N, Milazzo JP, Perdrix A, Castanet M, Joly-Hélas G, Sibert L, Bironneau A, Way A, Macé B. The feasibility of fertility preservation in adolescents with Klinefelter syndrome. *Hum Reprod* 2013;**28**:1468–1479.
- Rohayem J, Fricke R, Czeloth K, Mallidis C, Wistuba J, Krallmann C, Zitzmann M, Kliesch S. Age and markers of Leydig cell function, but not of Sertoli cell function predict the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome. *Andrology* 2015;**3**:868–875.
- Rohayem J, Nieschlag E, Zitzmann M, Kliesch S. Testicular function during puberty and young adulthood in patients with Klinefelter's syndrome with and without spermatozoa in seminal fluid. *Andrology* 2016. doi:10.1111/andr.12249.
- Sabbaghian M, Modarresi T, Hosseini H, Hosseini J, Farrahi F, Dadkhah F, Chehrizi M, Khalili G, Sadighi Gilani MA. Comparison of sperm retrieval and intracytoplasmic sperm injection outcome in patients with and without Klinefelter syndrome. *Urology* 2014;**83**:107–110.
- Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab* 2005;**90**:6263–6267.
- Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;**14**:131–135.
- Sciarano RB, Luna Hisano CV, Rahn MI, Brugo Olmedo S, Rey Valzacchi G, Coco R, Solari AJ. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod* 2009;**24**:2353–2360.
- Seo JT, Park YS, Lee JS. Successful testicular sperm extraction in Korean Klinefelter syndrome. *Urology* 2004;**64**:1208–1211.
- Staessen C, Tournaye H, Van Assche E, Michiels A, Van Landuyt L, Devroey P, Liebaers I, Van Steirteghem A. PGD in 47,XXY Klinefelter's syndrome patients. *Hum Reprod Update* 2003;**9**:319–330.
- Takada S, Tsujimura A, Ueda T, Matsuoka Y, Takao T, Miyagawa Y, Koga M, Takeyama M, Okamoto Y, Matsumiya K, et al. Androgen decline in patients with nonobstructive azoospermia after microdissection testicular sperm extraction. *Urology* 2008;**72**:114–118.
- Tournaye H, Staessen C, Liebaers I, Van Assche E, Devroey P, Bonduelle M, Van Steirteghem A. Testicular sperm recovery in nine 47,XXY Klinefelter patients. *Hum Reprod* 1996;**11**:1644–1649.
- Ulug U, Bener F, Akman MA, Bahceci M. Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. *Fertil Steril* 2003;**80**:903–906.
- Vialard F, Bailly M, Bouazzi H, Albert M, Pont JC, Mendes V, Bergere M, Gomes DM, de Mazancourt P, Selva J. The high frequency of sperm aneuploidy in Klinefelter patients and in nonobstructive azoospermia is due to meiotic errors in euploid spermatocytes. *J Androl* 2012;**33**:1352–1359.
- Vermaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod* 2004;**19**:1135–1139.
- Vicdan K, Akarsu C, Sözen E, Buluç B, Vicdan A, Yılmaz Y, Biberöglü K. Outcome of intracytoplasmic sperm injection using fresh and cryopreserved-thawed testicular spermatozoa in 83 azoospermic men with Klinefelter syndrome. *J Obstet Gynaecol Res* 2016;**42**:1558–1566.
- Vignozzi L, Corona G, Forti G, Jannini EA, Maggi M. Clinical and therapeutic aspects of Klinefelter's syndrome: sexual function. *Mol Hum Reprod* 2010;**16**:418–424.
- Westlander G, Ekerhovd E, Granberg S, Hanson L, Hanson C, Bergh C. Testicular ultrasonography and extended chromosome analysis in men with nonmosaic Klinefelter syndrome: a prospective study of possible predictive factors for successful sperm recovery. *Fertil Steril* 2001;**75**:1102–1105.
- Westlander G, Ekerhovd E, Bergh C. Low levels of serum inhibin B do not exclude successful sperm recovery in men with nonmosaic Klinefelter syndrome. *Fertil Steril* 2003;**79**:1680–1682.
- Yamamoto Y, Sofikitis N, Kaponis A, Georgiou J, Giannakis D, Mamoulakis Ch, Loutradis D, Yiannakopoulos X, Mio Y, Miyagawa I, et al. Use of a highly sensitive quantitative telomerase assay in intracytoplasmic sperm injection programmes for the treatment of 47,XXY non-mosaic Klinefelter men. *Andrologia* 2002;**34**:218–226.
- Yarali H, Polat M, Bozdog G, Gunel M, Alpas I, Esinler I, Dogan U, Tiras B. TESE-ICSI in patients with non-mosaic Klinefelter syndrome: a comparative study. *Reprod Biomed Online* 2009;**18**:756–760.