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

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(Article begins on next page)

1 **Title: Sperm recovery and ICSI outcomes in Klinefelter Syndrome: a meta-analysis**

3 **Running title: Fertility and Klinefelter**

5 Giovanni Corona^{1,*}, Alessandro Pizzocaro^{2*}, Fabio Lanfranco^{3*}, Andrea Garolla^{4*}, Fiore
6 Pelliccione², Linda Vignozzi⁵, Alberto Ferlin⁴, Carlo Foresta⁴, Emmanuele A, Jannini⁶, Mario
7 Maggi⁵, Andrea Lenzi⁷, Daniela Pasquali⁸, Sandro Francavilla^{9§} On behalf of the Klinefelter Italian
8 Group (KING)

10 ¹Endocrinology Unit, Medical Department, Endocrinology Unit, Azienda Usl Bologna Maggiore-
11 Bellaria Hospital, Bologna, Italy; ²Endocrinology Unit, IRCCS, Humanitas Research Hospital,
12 Rozzano (Milan), Italy; ³Department of Medical Sciences, Division of Endocrinology, Diabetology
13 and Metabolism, University of Torino, Turin, Italy; ⁴Department of Medicine, Andrology and
14 Reproductive Medicine Unit, University of Padova, Padova, Italy; ⁵Sexual Medicine and Andrology
15 Unit, Department of Experimental and Clinical Biomedical Sciences, Sexual Medicine and
16 Andrology Unit, University of Florence, Florence, Italy; ⁶Department of Systems Medicine, Tor
17 Vergata University of Rome, Rome, Italy; ⁷Department of Experimental Medicine, Sapienza
18 University of Rome, Rome, Italy; ⁸Department of Cardiothoracic and Respiratory Sciences,
19 Endocrine Unit, Second University of Naples, Naples, Italy; ⁹Department of Life, Health and
20 Environmental Sciences, University of L'Aquila, L'Aquila, Italy

22 *These authors equally contributed to the paper

24 §Correspondence address. Prof Sandro Francavilla. Department of Internal Medicine, Andrology
25 Unit, University of L'Aquila, Via Vetoio, 67100 L'Aquila, Italy. Tel: +39-0862-368338; Fax: +39-
26 0862-338342; E-mail: sandro.francavilla@univaq.it

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37 **DiscussionAbstract**

38 **Background:** Specific factors underlying successful surgical sperm retrieval rate (SRR) or
39 pregnancy rate (PR) after testicular sperm extraction (TESE) in adult patients with Klinefelter
40 syndrome (KS) are not completely clarified.

41 **Objective and rationale:** To meta-analyze currently available data regarding SRR in subject with
42 KS. In addition, when available, PR and live birth rate (LBR) after intracytoplasmatic sperm
43 injection (ICSI) technique have been also investigated.

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

47 **Outcomes:** Out of 139 studies, 37 trials were included in the study enrolling 1248 patients with a
48 mean age of 30.9 ± 5.6 years. The majority of the studies ($n=18$) applied mTESE, 13 cTESE and in
49 one case testicular sperm aspiration (TESA) was used. Finally, 4 studies used a mixed approach and
50 in 1 study the method applied for sperm retrieval was not specified. Overall, a SRR per TESE cycle
51 of 44[39-48] % was detected. In addition, similar results were observed when mTESE was
52 compared to cTESE, (SRR 43[35;50] % vs 45[28-52] % for cTESE vs micro-TESE, respectively;
53 $Q=0.20$, $p=0.65$). Meta-regression analysis showed that none of the parameters tested, including
54 age, testis volume as well as FSH, LH and testosterone (T) levels at enrolment, affects final SRR.
55 Similarly, no difference was observed when a bilateral procedure was compared to a unilateral
56 approach. No sufficient data were available to evaluate the effect of previous T treatment on SRR.
57 Information on fertility outcome after ICSI was available for 29 trials. Overall a total of 218
58 biochemical pregnancies after 410 ICSI cycles were observed ($PR=43[36;50]\%$). Similar results
59 were observed when LBR was analyzed. Similar to what observed for SRR no influence of KS age,
60 mean testis volume, LH, FSH and total T levels on both PR and LBR were observed. No sufficient
61 data were available to test the effect of women age or other women fertility problems on PR and

62 LBR. Finally, no difference in PR or LBR was observed when the use of fresh sperms was
63 compared to the utilization of cryopreserved ones.

64 **Wider implications:** Present data suggest that performing TESE/micro-TESE in subjects with KS
65 provide a SRR, PR or LBR of about 50% independent of any clinical or biochemical parameters
66 tested.

67

68

69 **Key words:** Klinefelter Syndrome, fertility, non-obstructive azoospermia, testicular sperm
70 extraction, assisted reproductive techniques, intra-cytoplasmic sperm injection

71 **Introduction**

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

78 Because of the genetic alteration, there is a progressive testicular damage leading to impaired
79 sperm production and infertility (Aksglaede and Juul, 2013). The degree of androgenization reflects
80 number and residual function of Leydig cells but, usually, at least two-thirds of adult (20–40 years
81 old) men with KS, show normal testosterone (T) concentrations (Aksglaede et al., 2007).
82 Accordingly, despite its high incidence there is common agreement that the majority of cases of KS
83 remain undiagnosed (Bojesen et al. 2003; Herlihy et al. 2011). Therefore, KS is most often
84 diagnosed in adulthood, when men are evaluated for symptomatic hypogonadism, infertility, and/or
85 sexual dysfunction (Foresta et al., 1999; Corona et al., 2010; Forti et al., 2010; Vignozzi et al.,
86 2010).

87 Until recently, infertility was considered an untreatable condition in KS. However, it has been
88 shown that azoospermic men with KS may have single residual foci with preserved
89 spermatogenesis (Foresta et al., 1999, see for review Aksglaede and Juul 2013) and they may
90 benefit from assisted reproductive techniques (ART). A recent overview of the published studies on
91 success rates and predictors of sperm retrieval by conventional testicular sperm extraction (cTESE)
92 and by microsurgical testicular sperm extraction (micro-TESE) in men with KS, reported an
93 average sperm retrieval rate (SRR) of 50% (Aksglaede and Juul 2013). So far, at least 149 healthy
94 live born babies were conceived after TESE combined with intra-cytoplasmic sperm injection
95 (ICSI) from couples, including a 47,XXY father (Aksglaede and Juul 2013). The specific predictors
96 of this approach are, however, still conflicting. Hormonal parameters, including levels of follicular

stimulating hormone (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 emphasized that KS subjects with younger age (below 35 years) have a better chance of positive TESE (Vernaev et al., 2004; Okada et al., 2005a; Kyono et al., 2007; Ferhi et al., 2009; Ramasamy et al., 2009; Bakircioglu et al., 2006; 2011). However, other authors did not confirm 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 conflicting 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 received a T supplementation in combination with an aromatase inhibitor therapy for several years (1–5 years). However, at present, there are no enough data to suggest this approach.

The aim of this comprehensive review is to meta-analyze currently available data regarding SRR and its predictors in subject with KS. In addition, when available, pregnancy rate (PR) and live birth rate (LBR) after ICSI will be also investigated

111

112 **Methods**

This meta-analysis was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline [see Supplementary file 1].

115 *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 5th2016, 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 forth investigator (G.C). We did not employ search software but hand-searched

123 bibliographies of retrieved papers for additional references. The principal source of information was
124 derived from published articles.

125

126 *Study selection*

127 All observational trials reporting SRR in azoospermic subjects with KS without any arbitrary
128 restriction (see also Figure 1 and Table 1) were included. Case reports or trials reporting sperm
129 retrieval in non KS were excluded from the analysis (see Figure 1)

130

131 *Outcome and quality assessment*

132 The principal outcome was the analysis of SRR in azoospermic subjects with KS. Secondary
133 outcomes included the comparison of SRR according to different surgical techniques including
134 cTESE, micro-TESE (mTESE) and testicular sperm aspiration (TESA). In addition, when available,
135 PR and LBR after ICS were also investigated. The quality of trials included was assessed using the
136 Cochrane criteria (Higgins et al., 2008).

137

138 *Statistical analysis*

139 Heterogeneity in sperm retrieval rate was assessed using I^2 statistics. Even when low heterogeneity
140 was detected, a random-effect model was applied, because the validity of tests of heterogeneity can
141 be limited with a small number of component studies. We used funnel plots and the Begg adjusted
142 rank correlation test to estimate possible publication or disclosure bias (Begg and Mazumdar,
143 1994), however, undetected bias may still be present because these tests have low statistical power
144 when the number of trials is small. In addition, a meta-regression analysis was performed to test the
145 effect of different parameters on SRR, PR and LBR.

146

147 **Results**

148 *Sperm retrieval outcome*

149 Out of 139 retrieved articles, 37 were included in the study (Table 1). The study flow is summarized
150 in Figure 1. The majority of the studies (n=18) applied cTESE, 13mTESE, and in one case TESA
151 was used (Table 1). Finally, 4 studies used a mixed approach and in 1 study the method applied for
152 sperm retrieval was not specified. Surgical approach included a bilateral procedure in 23 and
153 monolateral method in 3 studies, respectively (Table 1). The latter information was not available in
154 6 cases and in 5 studies a mixed approach was reported (Table 1). In addition, multiple biopsies
155 were performed in 30 cases whereas 3 studies used a single biopsy (Table 1). The latter information
156 was not available in 4 cases (Table 1). The characteristics of the retrieved trials (including
157 parameters on trial quality) are reported in Tables 1 and 2. Retrieved trials included 1248 patients
158 with a mean age of 30.9 ± 5.6 years. Mean testicular volume was 3.9 ± 1.6 ml and mean hormonal
159 parameters reflect the condition of primary or compensated hypogonadism (FSH= 36.0 ± 7.0 U/L,
160 LH 18.4 ± 4.3 U/L, total testosterone 10.3 ± 4.0 nM). All studies, except two included non-mosaic KS
161 (Table 1). The I^2 in trials assessing overall SRR per TESE cycle was 50.44 ($p < 0.001$). Overall a
162 SRR per TESE cycle of 44[39;48]% was detected (Figure 2 and Supplementary figure 1). Funnel
163 plot and Begg adjusted rank correlation test (Kendall's τ : 0.12; $p = 0.30$) suggested no publication
164 bias. Data were confirmed in sensitivity analysis when the trial enrolling mosaic KS subjects was
165 excluded from the analysis (SRR of 43[39;48]%). In addition, similar results were observed when
166 micro-TESE was compared to cTESE, (Figure 2; $Q = 0.20$, $p = 0.65$). Finally, no differences were
167 observed when SRR per patient was considered (SRR of 45[40;51]%).

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

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

177

178 *Fertility outcome*

179 Among the studies included in the SRR analysis, information on fertility outcome after ICSI were
180 available for 29 trials (Table 1). In these trials, women mean age was 29.5 ± 2.9 years. In addition,
181 ICSI procedure was performed either with cryopreserved or fresh sperms in 7 and 11 trials
182 respectively (Table 1). Eight studies applied a mixed approach using both cryopreserved or fresh
183 sperm whereas this information was not available in 3 cases (Table 1). I^2 in trials assessing overall
184 pregnancy rate was 35.40 ($p<0.05$). Overall a total of 218 biochemical pregnancies after 410 ICSI
185 cycles were observed (PR=43[36;50]%; see also Figure 4, panel A). Funnel plot and Begg adjusted
186 rank correlation test (Kendall's τ : -0.01; $p=0.93$) suggested the absence of publication bias. Similar
187 results were observed when LBR per ICSI cycle was analyzed: 211 live births (LBR=43[34;53]%;
188 see also Figure 4, panel B). Similar to what observed for SRR no influence of KS age, mean testis
189 volumeLH and total T levels on both PR and LBR per ICSI cycle were observed (not shown).
190 However, FSH levels at enrolment showed a trend toward an inversely significant association with
191 LBR per ICSI cycle ($S=-0.056[-0.117;0.004]$; $p=0.06$ and $I=1.883[-0.132;3.899]$; $p=0.06$). No
192 sufficient data were available to test the effect of women age or other women fertility problems on
193 PR and LBR.

194 When sensitivity analysis was performed according to the type of sperm used for ICSI procedure,
195 no difference in PR per ICSI cycle was observed when the use of fresh sperms was compared to the
196 utilization of cryopreserved ones (PR = 39[26;53]%, vs. 36[23;50]% respectively; $Q= 0.10$,
197 $p=0.76$). Similar results were observed when LBR per ICSI cycle was analyzed (LBR = 39[23;57]%
198 vs. 29[17;44]%, respectively; $Q= 0.78$, $p=0.38$).

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

201

202 **Discussion**

203 In this study we systematically reviewed and meta-analyzed for the first time, all available
204 information regarding SRR and fertility outcome in subjects with KS. In this specific population we
205 report an overall SRR of about 40%, which is independent of several clinical and biochemical
206 parameters including age, testis volume and hormonal status at baseline. In addition, the use of
207 retrieved sperms allows obtaining live children in about 40% of cases meaning a final live birth rate
208 of 16% for the couples who initiated the assisted reproductive techniques.

209 In 1996 Tournaye et al., reported for the first time, successful recovery of spermatozoa by cTESE in
210 men with azoospermia and KS. One year later Palermo et al. (1998) documented the first
211 pregnancies in KS after TESE/ICSI. Almost 20 years later, the predictive factors underlying
212 successful TESE in KS are still conflicting. Based on the reported progressive hyalinization of
213 seminiferous tubules observed after puberty in subjects with KS, it has been suggested that
214 performing earlier TESE procedures might result in better outcomes (Franik et al., 2016; Gies et al.,
215 2016). In contrast to this view, present data show that successful SRR in KS is independent of age.
216 Accordingly, it has been reported that the progressive hyalinization of seminiferous tubules which
217 characterized KS testes after puberty is not, ubiquitous and it is possible to observe tubules with
218 normal residual activity (Franik et al., 2016; Gies et al., 2016). The mechanisms underlying this
219 process are not yet fully known. Recent evidence seems to suggest that the impaired
220 spermatogenesis in KS patients could also be caused by an intrinsic defect of the germ cells,
221 possibly linked to (epi)-genetics of the surplus X chromosome instead of being a result of the
222 hyalinization and fibrosis of the testicular environment (Aksglaede and Juul, 2013; Franik et al.,
223 2016; Gies et al., 2016). The stable sperm retrieval rate of around 40% among KS seems to support
224 this view. However, no sufficient information on the inactivation pattern of the surplus X

225 chromosome was available in the studies analyzed in this meta-analysis. Hence, this hypothesis
226 needs to be confirmed in specific trials. Besides age, other factors including hormone pattern and
227 testicular volume have been advocated as possible prognostic values for successful SRR in KS
228 (Forti et al., 2010; Aksglaede and Juul, 2013; Franik et al., 2016; Gies et al., 2016). Rohayem et al.
229 (2015) reported that the combination of total serum testosterone above 7.5 nmol/l and LH levels
230 below 17.5 U/l resulted in higher retrieval rates of spermatozoa by micro-TESE in both adolescents
231 and adults with KS (Rohayem et al., 2015). Similar results were more recently reported by Cissen et
232 al., 2016. Our data showed that either testicular volume or hormonal pattern did not influence SRR
233 in KS. Interestingly, in line with our data, Rohayem et al., (2016) did not document any clinical
234 difference in non-mosaic KS subjects with or without spermatozoa in seminal fluid. The lack of
235 prognostic value of the FSH levels might be related to the low inhibin B levels to almost
236 undetectable levels during early puberty in all patients with KS not allowing the negative feed back
237 on FSH secretion (Aksglde et al., 2011). Similarly, the testicular growth impairment observed in KS
238 since early infancy might reduce its prognostic value in SRR.

239 When the type of surgical procedure was analyzed, we did not documented any difference by
240 comparing cTESE to micro-TESE or when bilateral approach was compared to unilateral
241 intervention. This observation confirm the hypothesis of the presence of tubules with normal
242 residual activity despite the progressive testis hyalinization. In addition, the reduced testis volume
243 in KS might limit the advantages of micro-TESE in SRR observed in the general population of
244 subjects with azoospermia (Amer et al., 2000). It should be recognized that postoperative testicular
245 damage leading to a decrease testicular function have been described as a complication of testicular
246 biopsy (Manning et al., 1998). It should be recognized that micro-TESE has been associated with
247 lower incidence of acute and chronic complications when compared to cTESE in subjects with
248 NOA and without KS (Schlegel, 1999; Amer et al., 2000). Similar results have been reported in
249 patients with KS (Okada et al., 2004; Takada et al., 2008; Ishikawa et al., 2009). Unfortunately, no

250 sufficient data on surgical approach complications were available in the studies included in this
251 meta-analysis.

252 Fathering is an important issue in subjects with KS. A recent survey performed in almost 200 Dutch
253 subjects with KS documented that the majority of them and their partners desire to have a children
254 and have a positive attitude toward TESE-ICSI treatment (Maiburg et al., 2011). The results of the
255 present meta-analysis show that live children can be obtained in about 16% of subjects who
256 underwent TESE approach. Although no studies evaluating face-to face comparisons are available,
257 our rate is similar, although little lower, than that reported in non KS subjects with non-obstructive
258 azoospermia (NOA; 25%; Cissen et al., 2016). In addition, similarly to what observed for SRR no
259 clinical and biochemical factors influenced the final pregnancy outcome. Finally, no difference in
260 PR and LBR was observed when the use fresh sperms was compared to the use of cryopreserved
261 sperms. The latter finding is not surprising and in line with what reported in the general population
262 (Hessel et al., 2015).

263 Several limitations should be recognized. Meta-analyses are based on the synthetic reports of
264 average results obtained in each study, without access to patient-level data. For this reason, some of
265 the original information of each study is lost in meta-analyses. On the other hand, the possibility of
266 combining a large number of investigations allows for a much greater statistical power, limiting the
267 problem of casual results because of small sample size. It is also possible that some of the results
268 noticed here are caused by the effects of unadjusted confounders. Hence, great caution is required in
269 the interpretation of results, which should be confirmed in large-scale observational studies.
270 Treatment with testosterone has previously been reported to be a negative influence on future
271 fertility of KS (Schiff et al., 2005). Conversely, recent studies described better sperm retrieval rate
272 in a small group of adolescents and young adults with KS, who received testosterone
273 supplementation and aromatase inhibitor therapy for several years before TESE (Paduch et al.,
274 2008; Mehta et al., 2013). Because the limited number of papers reporting SRR in subjects
275 previously treated with testosterone, in this review we cannot drive final conclusions on this topic.

276 Similarly no sufficient data are available to test the effect of other hormones such as estradiol
277 prolactin and INSL-3 levels as well as to evaluate the effect of cryptorchidism. Finally no sufficient
278 information was available to analyze the incidence of aneuploidies in the obtained children.
279 In conclusion, present data show that despite KS patients are usually azoospermic their actual
280 chances of fertility is similar to subjects with NOA and without KS. Even if the conception in KS
281 appear relative safe and the risk of chromosomal abnormalities is similar to that reported in subjects
282 without KS, preimplantation genetic diagnosis should be generally offered to couples with KS who
283 undergo successful TESE and ICSI to avoid transferring abnormal embryos.

284

285 **Author's roles**

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

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

288 Fabio Lanfranco: study design, execution

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

290 Fiore Pelliccione: study design, manuscript drafting

291 Linda Vignozzi: study design, execution

292 Alberto Ferlin: critical discussion

293 Carlo Foresta: critical discussion

294 Emmanuele A, Jannini: critical discussion

295 Mario Maggi: critical discussion

296 Andrea Lenzi: critical discussion

297 Daniela Pasquali: critical discussion

298 Sandro Francavilla: study design, execution, critical discussion

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317

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