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

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

Running title: Fertility and Klinefelter

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DiscussionAbstract

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

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

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

Outcomes: Out of 139 studies, 37 trials were included in the study enrolling 1248 patients with a mean age of 30.9±5.6years. The majority of the studies (n=18) applied mTESE, 13 cTESE and in one case testicular sperm aspiration (TESA) was used. Finally, 4 studies used a mixed approach and in 1 study the method applied for sperm retrieval was not specified. Overall, a SRR per TESE cycle of 44[39-48] % was detected. In addition, similar results were observed when mTESE was compared to cTESE, (SRR 43[35;50] % vs 45[28-52] % 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 as well as FSH, LH and testosterone (T) levels at enrolment, affects 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 trials. 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. Similar to what observed for SRR no influence of KS age, mean testis volume, LH, FSH and total T levels on both PR and LBR were observed. No sufficient data were available to test the effect of women age or other women fertility problems on PR and

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

Wider implications: Present data suggest that performing TESE/micro-TESE in subjects with KS provide a SRR, PR or LBR of about 50% 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 47,XXY with an estimated prevalence raging from 1:500 to 1:700 new born 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 a progressive testicular damage leading to impaired sperm production and infertility (Aksglaede and Juul, 2013). The degree of androgenization reflects 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 there is common agreement that the majority of cases of KS remain undiagnosed (Bojesen et al. 2003; Herlihy et al. 2011). Therefore, KS is most often diagnosed in adulthood, when men are evaluated for symptomatic hypogonadism, infertility, and/or sexual dysfunction (Foresta et al., 1999; Corona et al., 2010; Forti et al., 2010; Vignozzi et al., 2010).

Until recently, infertility was considered an untreatable condition in KS. However, it has been shown that azoospermic men with KS may have single residual foci with preserved spermatogenesis (Foresta et al., 1999, see for review Aksglaede and Juul 2013) and they may benefit from assisted reproductive techniques (ART). A recent overview of the published studies on success rates and predictors of sperm retrieval by conventional testicular sperm extraction (cTESE) and by microsurgical testicular sperm extraction (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 were conceived after TESE combined with intra-cytoplasmic sperm injection (ICSI) from 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 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 (Vernaeve 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

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].

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

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

Study selection

All observational trials reporting SRR in azoospermic subjects with KS without any arbitrary restriction (see also Figure 1 and Table 1) were included. Case reports or trials reporting sperm retrieval in non KS were excluded from the analysis (see Figure 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, when available, PR and LBR after ICS were also investigated. The quality of trials included was assessed using the Cochrane criteria (Higgins et al., 2008).

Statistical analysis

Heterogeneity in sperm retrieval rate 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 1). The study flow is summarized in Figure 1. The majority of the studies (n=18) applied cTESE , 13mTESE, and in one case TESA was used (Table 1). Finally, 4 studies used a mixed approach and in 1 study the method applied for sperm retrieval was not specified. Surgical approach included a bilateral procedure in 23 and monolateral method in 3 studies, respectively (Table 1). The latter information was not available in 6 cases and in 5 studies a mixed approach was reported (Table 1). In addition, multiple biopsies were performed in 30 cases whereas 3 studies used a single biopsy (Table 1). The latter information was not available in 4 cases (Table 1). The characteristics of the retrieved trials (including parameters on trial quality) are reported in Tables 1 and 2. Retrieved trials included 1248 patients with a mean age of 30.9±5.6years. 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 1). The I^2 in trials assessing overall SRR per TESE cycle was 50.44 ($p<0.001$). Overall a SRR per TESE cycle of 44[39;48]% was detected (Figure 2 and Supplementary figure 1). Funnel plot and Begg adjusted rank correlation test (Kendall's τ: 0.12; $p=0.30$) suggested no publication bias. Data were confirmed in sensitivity analysis when the trial 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 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 enrolment (Figure 3, panel A-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 1). In these trials, women mean age was 29.5±2.9 years. In addition, ICSI procedure was performed either with cryopreserved or fresh sperms in 7 and 11 trials respectively (Table 1). Eight studies applied a mixed approach using both cryopreserved or fresh sperm whereas this information was not available in 3 cases (Table 1). I^2 in trials assessing overall pregnancy rate was 35.40 ($p<0.05$). Overall a total of 218 biochemical pregnancies after 410 ICSI cycles were observed (PR=43[36;50]%; see also Figure 4, panel A). Funnel plot and Begg adjusted rank correlation test (Kendall's τ: -0.01; p=0.93) suggested the absence of publication bias. Similar results were observed when LBR per ISCI cycle was analyzed: 211 live births (LBR=43[34;53]%; see also Figure 4, panel B). Similar to what observed for SRR no influence of KS age, mean testis volumeLH and total T levels on both PR and LBR per ICSI cycle were observed (not shown). However, FSH levels at enrolment 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). No sufficient data were available to test the effect of women age or other women fertility problems on PR and LBR.

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

Finally, when 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 sperms allows obtaining live children in about 40% of cases meaning a final live birth rate of 16% for the couples who initiated the assisted reproductive techniques.

In 1996 Tournaye et al., reported for the first time, successful recovery of spermatozoa by cTESE in men with azoospermia and KS. 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, 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 characterized 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 becaused by an intrinsic defect of the germ cells, possibly linked to (epi)-genetics of the surplusXchromosomeinstead of being a result of the hyalinizationand fibrosis of the testicular environment(Aksglaede and Juul, 2013; Franik et al., 2016; Gies et al., 2016). The stable sperm retrieval rate of around 40% among KS seems to support this view. However, no sufficient information on the inactivation pattern of the surplus X

chromosome was 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 (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 either testicular volume or hormonal pattern did not influence SRR in KS. 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 seminal fluid. The lack of prognostic value of the FSH levels might be related to the low inhibin B levels to almost undetectable levels during early puberty in all patients with KS not allowing the negative feed back on FSH secretion (Aksglde 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 documented any difference by comparing cTESE to micro-TESE or when bilateral approach was compared to unilateral intervention. This observation confirm the hypothesis of the presence of tubules with normal residual activity despite the progressive testis hyalinization. In addition, 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 postoperative testicular damage leading to a decrease testicular function have been described as a complication of testicular biopsy (Manning et al., 1998). It should be recognized that micro-TESE has been associated with lower incidence of acute and chronic complications when compared to cTESE in subjects with NOA 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, no

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

Fathering is an important issue in subjects with KS. Arecent survey performed in almost 200 Dutch subjects with KS documented that the majority of them and their partners desire to have a 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 can be obtained in about 16% of subjects who underwent TESE approach. Although no studies evaluating face-to face comparisons are available, our rate is similar, although little lower, than that reported in non KS subjects with non-obstructive azoospermia (NOA; 25%; Cissen et al., 2016). In addition, similarly to what 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 sperms was compared to the use of cryopreserved sperms. The latter finding is not surprising and in line with what reported in the general population (Hessel et al., 2015).

Several limitations should be recognized. Meta-analyses are based on the synthetic reports of average results obtained in each study, without access to patient-level data. For this reason, some of the original information of each study is lost in meta-analyses. On the other hand, the possibility of combining a large number of investigations allows for a much greater statistical power, limiting the problem of casual results because of small sample size. It is also possible that some of the results noticed here are caused by the effects of unadjusted confounders. Hence, great caution is required in the interpretation of results, which should be confirmed in large-scale observational studies. Treatment with testosterone has previously been reported to be a negative influence on future fertility of KS (Schiff et al., 2005). Conversely, recent studies described better sperm retrieval rate 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 the limited number of papers reporting SRR in subjects previously treated with testosterone, in this review we cannot drive final conclusions on this topic.

Similarly no sufficient data are available to test the effect of other hormones such as estradiol prolactin and INSL-3 levels as well as to evaluate the effect of cryptorchidism. Finally no sufficient information was available to analyze the incidence of aneuploidies in the obtained children.

In conclusion, present data show that despite KS patients areusually azoospermic theiractual chances of fertility is similar to subjects with NOA and without KS. Even if the conception in KS appear relative safe and the risk of chromosomal abnormalities is similar to that reported in subjects without KS, preimplantation genetic diagnosis should be generally offered to couples with KS who undergo successful TESE and ICSI to avoid transferring abnormal embryos.

Author's 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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