Erectile dysfunction and decreased libido in Klinefelter syndrome: a prevalence meta-analysis and meta-regression study

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Running title: Sexual dysfunction in Klinefelter syndrome

ABSTRACT

Introduction: Only few studies have assessed sexual dysfunctions in men with Klinefelter syndrome (KS).

Aim: To define pooled prevalence estimates and correlates of erectile dysfunction (ED) and decreased libido (DL) in KS.

Methods: A thorough search of Medline, Embase and Web of Science was carried out to identify suitable studies. Quality of the articles was scored using the Assessment Tool for Prevalence Studies. Data were combined using random effects models and the between-studies heterogeneity was assessed by the Cochrane's Q and I^2. The sources of heterogeneity were investigated by meta-regression and sub-group analyses. Funnel plot, Begg's rank correlation test and trim-and-fill test were used to assess publication bias.

Main Outcome Measure: The pooled prevalence of ED and DL in KS as well as 95% confidence intervals (CIs) were estimated from the proportion of cases of sexual dysfunctions and the sample size. Variables that could affect the estimates were identified by linear meta-regression models.

Results: Sixteen studies included collectively gave information about ED and DL in 482 and 368 KS men, respectively, resulting in a pooled prevalence of 28% (95% CI: 19-36%) for ED and 51% (95% CI: 36-66%) for DL, with a large heterogeneity. The trim-and-fill adjustment for publication bias produced a negligible effect on the pooled estimates. At the meta-regression analyses, higher prevalence of ED was significantly associated with older age but not with lower testosterone levels: in series with a mean age >35 years, the ED prevalence estimate increased up to 38% (95% CI: 31-

44%) with no heterogeneity (I^2=0.0%, P=0.6). On the contrary, the prevalence of DL increased significantly as testosterone levels decreased, without significant relationship with age.

Clinical Implications: While DL would reflect an androgen deficiency, in older men with KS, erectile function should be assessed irrespective of testosterone levels.

Strength & Limitations: This is the first meta-analysis defining pooled prevalence estimates and correlates of sexual dysfunctions in KS. Nevertheless, caution is required when interpreting results, due to the high risk of bias in many studies, as well as the dearth of data about psychologic/psychosexological variables and age at the diagnosis.

Conclusions: ED and DL represent common clinical complaints in KS. While the prevalence of ED would increase with age, DL gets more common as serum testosterone decreases. Further studies are warranted to elucidate the pathogenetic mechanism(s) underlying the age-dependent increase in the prevalence of ED, apparently unrelated to the androgenic status.

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Key Words: sexual dysfunction, erectile dysfunction, impotence, decreased libido, XXY, hypogonadism.

INTRODUCTION

Although Klinefelter syndrome (KS) represents the most frequent sex chromosome aneuploidy among males,[1,2] there is still a relative lack of knowledge about its features, since over 50% of individuals with the classic (47,XXY) karyotype are deemed to remain undiagnosed throughout their life.[3] A wide spectrum of clinical characteristics is associated to KS, including endocrinological,[4] cardiovascular[5] and metabolic abnormalities,[6] along with a variable degree of psychological involvement.[7-9] Nevertheless, the reproductive defects are still considered the “hallmarks” of this condition. Indeed, small testes, non-obstructive azoospermia, and a variable onset of hypergonadotropic hypogonadism characterize KS.[10,11] As for men with impaired testis function,

sexual dysfunction would be expected among the clinical features. Surprisingly, a limited number of studies so far have been designed to assess sexual health among KS men, where the severity of sexual symptoms does not necessarily reflect the extent of androgen deficiency. Corona and colleagues[12] described a significant reduction of erectile function and sexual desire among 23 KS young adults. More recently, Ferlin and colleagues[13] analyzed 62 young non-mosaic KS men, reporting a high prevalence of sexual dysfunction along with poorer scores in sexual desire, intercourse satisfaction and overall satisfaction domains of the International Index of Erectile Function-15 (IIEF-15) questionnaire. As uncertainty remains concerning the prevalence rates of sexual dysfunctions in KS, in this study, we aimed to define pooled prevalence estimates and correlates of erectile dysfunction (ED) and decreased libido (DL) in KS using a meta-analytic approach.

METHODS

The study was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P).[14] It also complies with the guidelines of Meta-Analyses and Systematic Reviews of Observational Studies (MOOSE).[15] The PRISMA-P and MOOSE checklists have been presented as **Supplementary Table 1 and Table 2, respectively**. The study is registered in the PROSPERO (International Prospective Register of Systematic Reviews) with the number CRD42020190798 (https://www.crd.york.ac.uk/PROSPERO/).

Systematic search strategy

A systematic search was performed in PubMed, EMBASE and Web of Science, including the following free and vocabulary terms: 'sexual', 'erection', 'erectile', 'impotence', 'libido', 'sexual desire', 'Klinefelter', 'XXY', 'XXYY', 'XXXY', using the Boolean functions AND/OR. The search was restricted to English-language studies enrolling human participants, published up to July 2020. If it was not clear from the abstract whether the study contained relevant data, the full text was retrieved. The identification of eligible studies was performed by two authors independently (A.B, S.D.A.), and

disagreements resolved by the other investigators. No search software was employed. The reference lists of the identified articles were also scrutinized to find possible additional pertinent studies.

Inclusion and exclusion criteria

Eligible studies were identified according to a PECOS (Population, Exposure, Comparison/Comparator, Outcomes, Study design) model (**Supplementary Table 3**).

Studies were included in quantitative analysis if they reported the prevalence (or information for its calculation) of any diagnosis of ED and/or DL (according to different diagnosis, see **Table 1**) in subjects with documented diagnosis of KS recruited from the general population or from cohorts of patients. Observational studies (case-control, cross-sectional, prospective and series of cases), as well as intervention studies, were screened for eligibility. Only information about cases (men with KS) was extracted from case-control studies; only baseline information was extracted from intervention studies assessing the effects of testosterone treatment in men with KS. Duplicates were rigorously checked and removed. Commentaries/letters to editor, case reports, reviews, studies with missing/incomplete or unsuitable data, studies lacking to assess the outcomes of interest or enrolling populations other than KS, were excluded. Two independent reviewers (A.B. and S.D.A.) evaluated the full text of all selected studies for eligibility, and, where disagreement occurred, a third reviewer (S.F.) took a decision after open discussion.

Data extraction

Data were extracted from the selected studies by three independent reviewers (A.B., A.P. and W.V.) by including the first author, publication year, country/geographic region, study design, the total number of men with KS and the number of those complaining of ED and/or DL and the diagnostic tool for sexual dysfunction. The mean value of total testosterone levels and age of the participants were also extracted, when available.

When summary statistics were not fully reported, these were calculated, whenever possible.[16] Where data were missing, incomplete or inconsistent, the authors were contacted to obtain necessary information.

Quality assessment

Quality of the studies was assessed using an adapted Assessment Tool for Prevalence Studies.[17] This tool, designed to assess the risk of bias in prevalence studies, takes into account ten different items, including representativeness and selection of the study population, likelihood of non-response bias, process of data collection, appropriateness of the definition of cases (subjects with ED and/or DL), as well as of the measurement of the parameter of interest (prevalence of ED and DL). Response options for individual items were either low or high risk of bias and a summary assessment of the overall risk of bias was based on the subjective judgment attributed to the 10 items: 7-10 items with 'low risk' judgment indicated an overall low risk of bias; 4-6 items with 'low risk' judgment indicated an overall moderate risk of bias; 0-3 items with 'low risk' judgment indicated an overall high risk of bias.

Quality assessment was performed independently by two reviewers (W.V. and S.D.A.) and any disagreement was resolved by involving a third reviewer (A.B.) who re-evaluated the original study.

Statistical analysis

The pooled prevalence of ED and DL was estimated by a random-effects model, which assumes that the included studies have varying effect sizes, thus providing a conservative estimate of the overall effect. The 95% confidence intervals (CIs) of the prevalence reported in individual studies were estimated from the proportion of cases of ED or DL and the sample size, using the binomial Clopper-Pearson exact method. After ascertaining the non-normal distribution of the original data sets (by the Shapiro-Wilk test), the Freeman-Tukey double arcsine transformation was applied to the primary study data to approximate normality. The final pooled results and 95% CIs were then back

transformed and expressed as percentages for an easier interpretation. An inverse variance method was used for weighting each study in the pooled estimates. The Cochran's Chi square (Cochran's Q) test and the I^2 test were used to analyze the statistical heterogeneity between the results of different studies: a I^2 >50% and/or p <0.05 indicated substantial heterogeneity.[18]

Sensitivity analyses were performed with the leave-one-out cross-validation procedure, by the sequential omission of individual studies to determine the contribution of each study to the pooled estimates, thus evaluating the stability and reliability of the results. The results were shown according to a previously published graphic presentation.[19-21]

Publication bias was explored through funnel plots[22] and the Begg adjusted rank correlation test.[23] To correct for publication bias, Duval and Tweedie's 'trim-and-fill' analysis was carried out.[24] In the presence of asymmetric funnel shape, this test detects putative missing studies to rebalance the distribution and provides an adjusted pooled estimate taking the additional studies into account, thus correcting the analysis for publication bias.

Covariates that could affect the estimates, such as publication year, mean values of age and total testosterone levels of the study populations, were included in linear meta-regression models. When data allowed, an additional subgroup analysis was conducted, according to the meta-regression results, to detect the possible source of the between-study heterogeneity.

Data were analyzed and graphed using the packages 'metafor' and 'ggplo2' of the R statistical software (version 3.6.3, 2020; The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study selection and quality assessment

From the electronic search, we retrieved a total of 1103 studies and five additional records were found by manual search. After removal of duplicates, 713 studies were left; of which, 655 were excluded as irrelevant based on title and abstract. Hence, as shown in **Figure 1**, 58 studies were identified, of which 16 met the inclusion criteria: six studies provided information about the

prevalence of ED,[13,25-29] three studies reported the prevalence of DL.[30-32] In the remaining seven studies, information for calculating both outcomes was available.[12,33-38] Details of the studies included in the quantitative synthesis are summarized in **Table 1**.

Quality assessment of the selected studies is shown in **Table 2**. Ten studies were considered at low/moderate risk of bias, whereas an overall high risk of bias was attributed to the remaining 6 studies (mostly the older ones).

Synthesis of results and sensitivity analysis

As shown in **Figure 2**, the included studies collectively gave information about ED and DL in 482 and 368 KS men, respectively, resulting in a pooled ED prevalence of 28% (95% CI: 19-36%; Panel A) and a pooled prevalence of DL of 51% (95% CI: 36-66%; Panel B). However, a large heterogeneity between studies was found (I^2 = 79.0% and 87.6%, both P <0.0001, for ED and DL, respectively).

A sensitivity analysis was therefore performed, by the leave-one-out cross-validation procedure to assess the contribution of individual studies to the overall estimates. As shown in **Supplementary Figure 1**, the pooled prevalence and 95% CIs were not remarkably affected by the exclusion of any study, thus indicating the high degree of stability of the results.

Publication bias

Although the Begg's rank correlation test suggested a not significant asymmetry in funnel plot of ED (Kendall's τ = 0.0903, p = 0.6688) and DL (Kendall's τ = 0.2697, p = 0.2812), the trim-and-fill analysis identified one putative 'missing study' on the left side of both distributions (**Supplementary Figure 2**). Nevertheless, when the funnel plot distributions were rebalanced by including these putative additional studies, the adjustment for publication bias produced a negligible effect on the pooled prevalence estimate for both ED (adjusted pooled prevalence: 26.4%, 95% CI: 18.0-34.8%) and DL (adjusted pooled prevalence: 47.7%, 95% CI: 32.6-62.8%).

Meta-regressions and subgroup analysis

Meta-regression analyses were performed to find out covariates that could affect the prevalence estimates.

No significant relationship was found between study publication year and either ED prevalence [S = 0.002 (95% CI: -0.004, 0.008), p = 0.48; I = -3.43 (95% CI: -14.65, 7.78), p = 0.55] or DL prevalence [S = -0.002 (95% CI: -0.011, 0.007), p = 0.70; I = 4.23 (95% CI: -13.50, 21.96), p = 0.64].

An older age of the participants was significantly associated with a higher prevalence of ED [S = 0.01 (95% CI: 0.003, 0.03), p = 0.01; I = 0.01 (95% CI: -0.41, 0.43), p = 0.96; **Figure 3A**]; whereas, no significant association was revealed between ED prevalence and total testosterone levels [S = -0.04 (95% CI: -0.32, 0.23), p = 0.75; I = 0.65 (95% CI: -0.05, 1.35), p = 0.07]. To substantiate the impact of the age as a source of the between-study heterogeneity, in a subgroup analysis, pooled estimates were calculated separately for studies enrolling KS men below and above 35 years of age. Dichotomization value was chosen, according to the distribution of mean ages among the study populations. As shown in **Figure 4**, when the analysis was restricted to series with a mean age >35 years, the prevalence estimate for ED increased up to 38% (95% CI: 31-44%) with no heterogeneity (I^2 = 0.0%, P = 0.6). On the contrary, studies on younger participants (mean age <35 years) collectively produced a pooled ED prevalence estimate of 17% (95% CI: 6-27%) with a large heterogeneity (I^2 = 81.2%, P = 0.0005). In this latter subgroup (mean age <35 years), the meta-regression analysis did not find significant association between total testosterone levels and ED prevalence [S = 0.02 (95% CI: -0.72, 0.77), p = 0.95; I = 0.38 (95% CI: -1.60, 2.35), p = 0.71].

As far as the prevalence of DL was concerned, unlike ED, a statistically significant negative linear trend was revealed in meta-regression to explain effect size variation by total testosterone levels (S = -0.55 (95% CI: -0.85, -0.25), p = 0.0003; I = 1.97 (95% CI: 1.27, 2.69), p <0.0001; **Figure 3B**), but not by the mean age of the study populations (S = 0.01 (95% CI: -0.02, 0.04), p = 0.44; I = 0.38

(95% CI: -0.60, 1.38), p = 0.44). Unfortunately, the limited number of studies did not allow to perform subgroup analyses for the prevalence of DL according to total testosterone levels.

DISCUSSION

Although it is commonly assumed that men with KS usually suffer from sexual dysfunctions,[39-42] only few studies have specifically investigated this issue. In a consecutive series of 1386 males attending an outpatient clinic for sexual disorders, Corona and colleagues[12] found a relatively higher prevalence (1.7%) of KS than that reported in the general population, thus confirming that sexual dysfunction is a common feature of KS. Noteworthy, in that series, 21.7% of KS men suffered from severe ED, which was defined as erection not sufficient for penetration in more than 75% of cases.[12] In the present meta-analysis of 16 carefully selected studies, the crude overall prevalence estimates for ED and DL reached 28% and 51%, respectively. Interestingly, at meta-regression analyses, ED appeared to be significantly associated with age but not with testosterone levels. Indeed, in individual series of KS men, the prevalence of ED largely varied from 2%[36] up to 56%[35] (**Figure 2**) and the enrollment of series with different mean age could account for the large between-study heterogeneity. At a sub-group analysis restricted to men with mean age >35 years, ED prevalence estimate increased up to 38%, without heterogeneity.

In KS, an age-dependent androgen deficiency of various intensity is well documented. At the time of puberty, approximately 60% of KS boys experience a normal development of secondary sexual characteristics, with testosterone levels within the normal range.[43,44] However, from early puberty onward, increasing serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) reflect a latent or subclinical endocrine testicular dysfunction. The overt clinical and biochemical primary androgen deficiency usually occurs either during late adolescence or at an undefined time point in adulthood.[1,45,46] Although sexual symptoms, such as DL, decreased sexual thoughts, and ED represent the complaints more specifically associated with male hypogonadism,[47] the order of their onset would reflect the extent of the testosterone decrease: while

the loss of libido represents one of the earliest symptoms, severe ED only occurs when testosterone falls in the range of overt hypogonadism (below 8 nmol/L).[48] Accordingly, in the present meta-analysis, the prevalence of DL was significantly associated with testosterone levels but not with age, suggesting that this symptom would commonly occur even among young KS men with an early mild decrease in serum testosterone levels, reaching an overall prevalence estimate of 51% (**Figure 2**). Consistent with this finding, in the aforementioned study by Corona and colleagues,[12] although a high proportion of men with KS reported hypoactive sexual desire (60.9%) and severe ED (21.7%), only the association between KS and hypoactive sexual desire was confirmed after adjustment for age. Furthermore, when men with KS were compared with testosterone-matched controls, even the association of KS with hypoactive sexual desire disappeared.[12]

It could be hypothesized that in KS the worsening of androgen deficiency with age could also contribute to the onset of ED once very low testosterone levels are reached, thus partially mediating the here revealed positive association between ED prevalence and age. Nevertheless, the lack of significant association of ED with testosterone levels at meta-regression analysis would suggest a preeminent contribution of other age-related pathogenic factors. In this light, while a number of not well defined psychologic, psychosexological and psychiatric involvements cannot be ruled out,[49-51] risk factors for cardio-vascular disease (CVD) are likely to play a major role.

Data from large registry-based studies indicated a significant increase in CVD mortality in men with KS,[52,53] who exhibit higher rates of CVD,[54,55] visceral obesity, metabolic syndrome[55] and diabetes,[54] when compared to the general population. Androgen deficiency can represent a major determinant of body composition changes, visceral obesity and metabolic syndrome,[56] thus contributing to cardiometabolic risk in this population.[55,57] However, it cannot be ruled out that visceral obesity precedes androgen deficiency in KS, where metabolic syndrome could occur even independently from testosterone levels. Indeed, cardiovascular abnormalities in KS seem to be both unrelated to testosterone levels[58,59] and unresponsive to testosterone replacement therapy (TRT).[28,58,60-63] In a series of 221 men with KS and 77 age-matched controls, epicardial fat thickness

(EFT), a cardiac marker of visceral adiposity, was similar in hypogonadal KS men and in either KS men under TRT or obese controls, suggesting that KS itself and BMI represent the major determinants of EFT, independently from androgenic status.[64] In a recent randomized, double-blind, placebo-controlled, BMI-matched, cross-over study on 13 men with KS, TRT did not affect insulin sensitivity, as assessed by euglycemic clamp.[65] Taken together these findings point to a genetic, rather than hormonal basis, of KS-associated metabolic derangements. This hypothesis seems to be supported by results from studies on infants and on prepubertal boys with KS, who display higher adiposity and a significantly higher prevalence of metabolic syndrome features, with respect to their healthy peers, in spite of comparable physiologically low serum testosterone levels.[66-69] Therefore, as an unfavorable metabolic profile would be present early on in life, i.e. during infancy and childhood, hypogonadism might not be the only link between KS and metabolic syndrome.[71,72] In this scenario, an androgen-independent increase in cardio-metabolic risk with age[69] could explain the here revealed association of ED with age, but not with testosterone levels. In this light, as ED itself represents a marker of early systemic endothelial damage, a key determinant of atherosclerosis,[72] a screening for coronary artery disease in the presence of ED could be especially advisable in KS men who exhibit an early-onset combination of clinically relevant CVD risk factors. On the other hand, in younger KS men (<35 years of age) the pathogenic contribution of different non-CVD-related factors could get prevalent, thus resulting in a lower, other than variable, prevalence of ED, resulting in a pooled estimate burdened by large between-study heterogeneity (**Figure 4**). Consistent with a higher prevalence of androgen deficiency in older age groups, testosterone levels did not significant contribute to this heterogeneity, as in the subgroup with mean age <35 years, the meta-regression analysis did not reveal significant association between testosterone levels and ED prevalence. Considering that different psychiatric conditions, including depression and anxiety, are often associated to the syndrome,[49] it is possible that these psychological derangements could facilitate the establishment of ED, along with the aforementioned organic factors.

This meta-analysis has some limitations. First, only a few studies were included in quantitative syntheses, overall accounting for a relatively small number of participants. This resulted, indeed, from a strict screening and selection of the literature on this poorly investigated topic. Second, a high risk of bias was attributed to 6 out of the 16 selected studies (**Table 2**). Actually, most of these old studies were carried out before validated tools for assessing sexual dysfunctions were made available: this necessarily imposed the inclusion criterion of any diagnosis of ED and DL. Furthermore, in some old series, institutionalized KS men were enrolled,[31,33] thus generating a possible representativeness bias. Overall, the most recent studies generally used validated tools for assessing sexual dysfunction as a primary end point (**Table 1**) and reached higher quality scores than older reports (**Table 2**). Nevertheless, at the meta-regression analysis, no significant association was found between study publication year and either ED or DL prevalence. Third, information about testosterone levels was lacking in many series (**Table 1**), thus restricting the number of studies suitable for meta-regression analysis on DL, the results of which, hence, should be interpreted cautiously. Similarly, the large unavailability of data about CVD risk factors prevented us from checking a possible association of an altered cardio-metabolic profile with a higher prevalence of ED. Finally, psychosocial variables and age at the diagnosis could also affect sexual function, behavior, and comorbidities in KS. In particular, a delayed diagnosis can result in a higher severity of some KS clinical features.[73] Unfortunately, the dearth of data about psychological variables and age at the diagnosis did not allow their inclusion in the quantitative analyses.

In conclusion, ED and DL represent quite common clinical complaints in KS. The prevalence of ED, apparently unrelated to the androgenic status, would increase with age, reaching up to 38% above the age of 35 years, when, from a clinical point of view, erectile function should be assessed irrespective of testosterone levels. Meanwhile, DL, overall involving half of the patients, gets more common as serum testosterone levels decrease. Further studies are warranted to elucidate the pathogenic mechanism(s) underlying the age-dependent increase in the prevalence of ED.

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FIGURE LEGENDS

Figure 1. Flow diagram showing an overview of the study selection process.

Figure 2. Forest plots depicting the pooled prevalence estimate for (**A**) erectile dysfunction (ED) and (**B**) decreased libido in Klinefelter syndrome. Diamonds indicate the overall summary estimates and width of the diamonds represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled results.

Figure 3. Sensitivity analysis of the leave-one-out cross-validation procedure showing the influence of each individual study on the pooled prevalence with 95% confidence interval (CI) of (**A**) erectile dysfunction (ED) and (**B**) decreased libido: values are pooled prevalence estimate (95% CI) produced by the exclusion of the corresponding study.

Figure 4. Meta-regression bubble plots: prevalence of (**A**) erectile dysfunction (ED) and (**B**) decreased libido in Klinefelter syndrome as a function of the mean age and total testosterone levels, respectively. The predicted effects (solid line) with corresponding confidence intervals (gray range) are also shown. CI, confidence interval; I, Intercept; S, slope.

Figure 5. Forest plots depicting the results of the subgroup analysis of the prevalence of erectile dysfunction (ED) in Klinefelter syndrome (KS) by mean age. The pooled prevalence estimate was calculated separately for studies enrolling KS men (**A**) below and (**B**) above 35 years of age. Diamonds indicate the overall summary estimates and width of the diamonds represents the 95% confidence interval (CI); boxes indicate the weight of individual studies in the pooled results.

Supplementary Figure 1. Funnel plots of results from studies assessing the prevalence of (**A**) erectile dysfunction (ED) (Kendall's $\tau = 0.0903$, $p = 0.6688$) and (**B**) decreased libido (Kendall's $\tau = 0.2697$, $p = 0.2812$). The trim-and-fill analysis identified one putative missing study (white circle) on the left side of both distributions.

Table 1. Characteristics of the included studies

Study	Country or region	Study design	KS number	KS with SD	Diagnosis of SD	SD as primary end point	Mean age (years)	Mean age at the KS diagnosis (years)	Mean TT levels (ng/mL)
Kvale & Fishman 1965[33]	USA	Case series	12	ED: 4 DL: 4	Self-reported	No	NA	NA	NA
Makino, 1966[25]	Japan	Retrospective case series	27	ED: 1	Not specified	No	21.4	NA	NA
Becker, 1972[30]	USA	Case series	104	DL: 69	Self-reported	No	45.0	NA	NA
Money et al., 1974[31]	USA	Retrospective case series	12	DL: 9	Self-reported	No	25.0	NA	NA
Niermann et al., 1975[34]	Germany	Case-control study	51	ED: 20 DL: 20	Clinical diagnosis	No	35.3	NA	NA
Nicholls & Anderson, 1982[32]	UK	Case series	10	DL: 7	Self-reported	No	37.9	37.9	NA
Wu et al., 1982[26]	UK	Double blind cross-over study with oral TU	4	ED: 1*	Self-reported	Yes	35.2	NA	3.07
Yoshida et al., 1997[36]	Japan	Case-control study	40	ED: 1 DL: 4	Author questionnaire	Yes	33.2	NA	2.70
Meikle et al., 1998[35]	Sweden and USA	Multicentric intervention study with transdermal testosterone	9	ED: 5* DL: 8*	RigiScan, Watts sexual function and Davidson questionnaires	Yes	NA	NA	1.70
Corona et al., 2010[12]	Italy	Case-control study in men with SD	23	Severe ED[§]: 5 DL: 14	SIEDY	Yes	40.6	NA	1.73

Shigehara et al., 2010[27]	Japan	Prospective case series undergoing TESE	12	ED: 5†	IIEF-5	Yes	36.8	NA	2.27
Pacenza et al., 2012[37]	Argentina	Multicentric retrospective case series	54	ED: 16 DL: 15	Clinical inquiry	No	28.4	NA	2.74
Condorelli et al., 2013[28]	Italy	Intervention study with TRT	15	ED: 7*	IIEF-5	No	53.5	NA	3.17
El Bardisi et al., 2017[38]	Qatar	Case-control study	53	ED: 10 DL: 29	ED: IIEF-5 DL: self-reported	Yes	33.0	NA	2.18
Ferlin et al., 2018[13]	Italy	Case-control study	62	ED: 14	IIEF-15	Yes	31.1	NA	2.88
Skakkebæk et al., 2018[29]	Denmark	Case-control study	120	ED: 47	IIEF-15	Yes	44.7	26.5	NA

*At the baseline; §Severe ED = Erection not sufficient for penetration in more than 75% of cases; †Preoperative data; Abbreviations: DL, decreased libido; ED, erectile dysfunction; IIEF, international index of erectile function; KS, Klinefelter syndrome; NA, not available; SD, sexual dysfunction; SIEDY, Structured Interview on Erectile DYsfunction; TESE, testicular sperm extraction; TRT, testosterone replacement therapy; TT, total testosterone; TU, testosterone undecanoate.

Table 2. Quality assessment of the included studies

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	OVERALL
Kvale & Fishman, 1965[33]	H	H	H	L	H	H	H	L	H	L	High risk of bias
Makino, 1966[25]	L	H	H	H	H	H	H	H	H	H	High risk of bias
Becker, 1972[30]	L	H	H	H	H	H	H	H	H	H	High risk of bias
Money et al., 1974[31]	L	H	H	H	L	H	H	H	H	H	High risk of bias
Niermann et al., 1975[34]	L	H	H	H	L	H	H	L	H	L	Moderate risk of bias
Nicholls & Anderson, 1982[32]	H	H	H	L	H	H	H	H	H	L	High risk of bias
Wu et al., 1982[26]	H	H	H	L	L	H	H	L	H	H	High risk of bias
Yoshida et al., 1997[36]	L	H	L	L	L	L	H	L	H	L	Low risk of bias
Meikle et al., 1998[35]	H	H	H	L	L	L	L	L	H	L	Moderate risk of bias
Corona et al., 2010[12]	H	H	L	L	L	L	L	L	L	L	Low risk of bias
Shigehara et al., 2010[27]	H	H	H	L	L	L	L	L	L	L	Low risk of bias
Pacenza et al., 2012[37]	L	L	L	L	L	H	H	L	H	L	Low risk of bias
Condorelli et al., 2013[28]	H	L	H	L	L	L	L	L	L	L	Low risk of bias
El Bardisi et al., 2017[38]	L	L	L	L	L	L	L	L	L	L	Low risk of bias
Ferlin et al., 2018[13]	L	L	L	L	L	L	L	L	L	L	Low risk of bias
Skakkebæk et al., 2018[29]	L	L	L	L	L	L	L	L	L	L	Low risk of bias

H = High risk; L = Low risk

Q1. Was the study's target population a close representation of the national population in relation to relevant variables?

Q2. Was the sampling frame a true or close representation of the target population?
Q3. Was some form of random selection used to select the sample, OR was a census undertaken?
Q4. Was the likelihood of non-response bias minimal?
Q5. Were data collected directly from the subjects (as opposed to a proxy)?
Q6. Was an acceptable case definition used in the study?
Q7. Was the study instrument that measured the parameter of interest (prevalence of sexual dysfunction) shown to have reliability and validity?
Q8. Was the same mode of data collection used for all subjects?
Q9. Was the length of the shortest prevalence period for the parameter of interest appropriate?
Q10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?
OVERALL. Summary item on the overall risk of study bias: 7-10 items with 'low risk' judgment = overall low risk of bias; 4-6 items with 'low risk' judgment = overall moderate risk of bias; 0-3 items with 'low risk' judgment = overall high risk of bias.