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The risk of TESE-induced hypogonadism: a systematic review and meta-analysis

Jitske Eliveld, Madelon van Wely, Andreas Meißner, Sjoerd Repping, Fulco van der Veen, and Ans M.M. van Pelt *

Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, 1105AZ Amsterdam, The Netherlands

*Correspondence address. Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, 1105AZ Amsterdam, The Netherlands. E-mail: a.m.vanpelt@amc.uva.nl orcid.org/0000-0001-9155-548X

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TABLE OF CONTENTS

BACKGROUND: Testicular sperm extraction (TESE) is a surgical procedure to retrieve spermatozoa from the testes of men with azoospermia to help them achieve biological parenthood. Although effective, the surgical procedure is not without complications and haematoma, devascularization, inflammation and a decrease in testosterone levels have been described as such. The prevalence and duration of hypogonadism and associated symptoms after TESE have not been studied systematically.

OBJECTIVE AND RATIONALE: In this systematic review we addressed the following research questions: Are serum testosterone levels decreased after TESE and, if so, do these levels recover over time? What is the prevalence of symptoms and signs related to hypogonadism after TESE and are they related to testosterone levels?

SEARCH METHODS: We searched the databases Pubmed and Embase from 1 January 1993 to 26 June 2017. We combined subject headings with terms in title and/or abstract for participants, intervention and outcomes. We included all studies that reported on TESE, regardless

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of the specific technique used, that measured testosterone and/or LH, and/or had information on signs or symptoms related to hypogonadism as defined by hypogonadism guidelines. An additional inclusion criterion was that studies described these measurements both before and after TESE. The quality of the included studies was assessed using the Risk Of Bias In Non-randomized Studies—of Interventions tool.

OUTCOMES: We identified 15 studies reporting on total testosterone levels of which five studies also reported on testicular volume and one study on erectile dysfunction. Men with Klinefelter syndrome and men with non-obstructive azoospermia had the strongest decrease in total testosterone levels 6 months after TESE, with a mean decrease of 4.1 and 2.7 nmol/l, respectively, which recovered again to baseline levels 26 and 18 months after TESE, respectively. At 6 months after TESE, some studies reported serum total testosterone concentrations below a cut-off value of 12 nmol/l, where symptoms and signs related to hypogonadism may appear. Furthermore, an increased prevalence of erectile dysfunction related to decreased total testosterone levels 6 months after TESE was reported. Also, in some men a decrease in testicular volume was reported. However, it is not clear if this is related to low testosterone levels.

WIDER IMPLICATIONS: The transient, but statistically significant, decrease in total testosterone levels indicates that men are at risk of developing a temporary hypogonadism after TESE, but there is insufficient evidence for whether patients actually experience clinical symptoms in case of decreased serum testosterone levels. To be able to properly counsel TESE patients, more large-scale monitoring on signs and symptoms of hypogonadism, in combination with testosterone measurements, needs to be performed in men undergoing TESE.

Key words: testosterone / azoospermia / assisted reproduction / testicular sperm extraction / hypogonadism / erectile dysfunction / non-obstructive azoospermia / Klinefelter syndrome / sperm retrieval

Introduction

Testicular sperm extraction (TESE) is a surgical procedure to assist men with azoospermia to achieve biological parenthood. With this technique spermatozoa can be retrieved directly from the testicular tissue and subsequently used for ICSI. In 1993, the first successful fertilization, and soon after that the first pregnancy, with testicular sperm after ICSI were described (Craft *et al.*, 1993; Schoysman *et al.*, 1993). Since then, the technique developed further and at present various TESE techniques have been reported, such as multibiopsy/conventional TESE, microdissection TESE and testicular sperm aspiration (TESA) (Craft *et al.*, 1993; Bourne *et al.*, 1995; Schlegel and Li, 1998). Evidence that one technique should be preferred over the other based on sperm retrieval rates, pregnancy rates, live birth rates and complications are limited (Donoso *et al.*, 2007; Van Peperstraten *et al.*, 2008). Although a higher sperm retrieval rate is described for men with non-obstructive azoospermia (NOA) after microdissection TESE compared to conventional TESE, this is not true for all men and depends on the type and cause of azoospermia (Deruyver *et al.*, 2014).

Currently, TESE is the only possible therapy for men with NOA to obtain spermatozoa to father their own genetic children. In case of obstructive azoospermia (OA), microsurgical epididymal sperm aspiration or percutaneous epididymal sperm aspiration is the preferred method to retrieve spermatozoa (Van Wely *et al.*, 2015). If unsuccessful, a subsequent TESE procedure could also be offered to these men. TESE is now routinely offered worldwide to men with azoospermia with a success rate of retrieval of spermatozoa of ~50% for men with NOA and Klinefelter syndrome, and can go up to 100% for men with OA (Chan and Schlegel, 2000; Cissen *et al.*, 2016; Corona *et al.*, 2017). The described live birth rates for ICSI with testicular retrieved spermatozoa vary from 10 to 45% per cycle for all types of azoospermia (Bocca *et al.*, 2017; Corona *et al.*, 2017; Esteves and Agarwal, 2013; Meijerink *et al.*, 2016).

In the TESE procedure a small biopsy of testicular tissue is obtained via dissection followed by tissue resection, while with TESA small pieces of tissue are aspirated to extract spermatozoa. Post-operative complications, such as haematoma, devascularisation and inflammation, have been described, eventually leading to scars and calcification (Schlegel and Su, 1997; Donoso *et al.*, 2007). Furthermore, a decrease in serum testosterone levels after a TESE procedure has been described (Donoso *et al.*, 2007; Shin and Turek, 2013). Decreased testosterone levels can subsequently lead to hypogonadism. Symptoms of hypogonadism have been described to occur more often when total testosterone levels are below a threshold of 12 nmol/l, and the lower the total testosterone values, the more frequent the symptoms of hypogonadism will be present (Bhasin *et al.*, 2011; Dohle *et al.*, 2015; Zitzmann *et al.*, 2006).

According to various guidelines on hypogonadism, the diagnosis is based on the presence of one or more signs or symptoms. Symptoms can be—among others—erectile dysfunction (ED), decreased muscle strength and loss of libido. Additional signs, for example, decrease in bone mineral density and a decrease in testicular volume can occur (Table I) (Wang *et al.*, 2009; Bhasin *et al.*, 2010; Jungwirth *et al.*, 2015). Because many of these clinical manifestations are not specific for hypogonadism, the diagnosis should always be confirmed by measuring serum testosterone levels (Bhasin *et al.*, 2010; Dohle *et al.*, 2015).

The prevalence and duration of hypogonadism and associated symptoms as a result of the TESE procedure have not been studied systematically. This information is necessary to properly counsel men who qualify for TESE on the potential risk of developing hypogonadism after TESE. We therefore systematically reviewed the literature and included all studies that measured testosterone levels, LH levels and/or signs and symptoms related to hypogonadism before and after TESE. In this systematic review we addressed the following research questions: Are serum total testosterone levels decreased after TESE and, if so, do these levels recover over time? What is the prevalence of symptoms and signs related to hypogonadism after TESE and are they related to total testosterone levels?

Methods

This review is reported according to the PRISMA statement (The Prisma Group from Moher D, Liberati A, Tetzlaff J, 2009). Prior to the search, a

Table I Signs and symptoms associated with androgen deficiency in men.

Small testes/shrinking testes
Erectile dysfunction/decreased spontaneous erections/fewer and diminished nocturnal erections
Gynaecomastia/breast discomfort
Decrease in lean body mass and muscle strength/reduced muscle bulk
Visceral obesity/increased body fat, BMI
Decrease in bone mineral density (osteoporosis) with low trauma fractures/height loss
Reduced sexual desire and sexual activity/reduced libido
Decreased body hair/loss of body (axillary and pubic) hair
Hot flushes/sweats
Changes in mood and anger/decreased motivation, initiative, and self-confidence, feeling sad or blue, depressed mood, dysthymia
Decreased energy/fatigue/sleep disturbances/increased sleepiness
Metabolic syndrome
Insulin resistance and type-2 diabetes mellitus
Diminished cognitive function/poor concentration and memory, diminished physical or work performance
Mild anaemia

plan regarding the study design, search methods and inclusion and exclusion criteria was written and approved by all authors. We registered the protocol at Prospero with registration number CRD42016052596 and is available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016052596.

Search and study selection

We searched the databases Pubmed and Embase from 1 January 1993, when the first TESE procedure was described, to 26 June 2017. We combined subject headings with terms in title and/or abstract for the intervention, outcomes and participants. The details of the search are displayed in Supplementary Tables SI and SII. Two authors (J.E. and A.v.P.) screened the articles independently on title and abstract for eligibility based on predefined inclusion criteria. Then both authors carefully read the full text independently to further exclude studies which did not meet the inclusion criteria. When there was discrepancy between the authors, articles were discussed until agreement was achieved.

Eligibility criteria

We included all original English, peer-reviewed articles, irrespective of study-design. There were no restrictions in TESE techniques. We included all studies which measured testosterone, LH, or signs or symptoms related to hypogonadism (Table I), defined according to international hypogonadism guidelines (Bhasin *et al.*, 2010; Dohle *et al.*, 2015; Wang *et al.*, 2009). To assess the association between TESE and hypogonadism and its associated signs and symptoms, we only included the studies that performed the measurements before and after TESE. Furthermore, we excluded studies that included prepubertal boys because of the difference in baseline characteristics and testosterone and LH levels compared to adult men.

Data extraction

One author (J.E.) extracted data from the included articles. The following data were extracted when available: first author, year of publication, study design, TESE technique, number of participants, age of participants, type of azoospermia, type of outcome and outcome in mean values with SD or prevalence of the outcome. When data of the outcomes were not available we contacted the corresponding authors to ask for the data. If we did not receive the raw data, but they were displayed in graphs, we extracted the data from the graphs.

Quality assessment

Two authors (J.E. and M.v.W.) assessed the quality of the included studies using the Risk Of Bias In Non-randomized Studies—of Interventions tool (Sterne *et al.*, 2016). With this tool we estimated the risk of bias due to confounding, selection of participants into the study, classification of intervention, deviations from intended interventions, missing data, measurements of outcomes and selection of the reported results.

Data analyses

To study the effect of TESE on testosterone levels over time we used the mean testosterone levels from individual studies to perform meta-regression analyses using STATA 14.2 software (StataCorp, College Station, TX, USA). Where possible we identified different patient groups based on their type of azoospermia, namely, men with OA, men with NOA and men with Klinefelter syndrome. To calculate the individual odds ratios (OR) and individual and pooled mean differences with corresponding 95% CI we used Review Manager 5.3 (The Cochrane Collaboration, The Nordic Cochrane Centre, Copenhagen, Denmark). Binary outcomes were expressed as OR and continuous outcomes as mean differences. To calculate mean differences we compared the outcomes after TESE with outcomes before TESE. A random effect model was standardly applied. Data were pooled based on the patient groups described above and on follow-up time. We classified follow-up time of 1–3 months as short term, 6 months as intermediate, 9–12 months as intermediate-long, and when the follow-up was more than 12 months this was classified as long term.

Results

Study selection

With our systematic search we identified 611 reports from PubMed and 1011 from Embase. After removing duplicates, 1255 reports

remained for screening of eligibility in title and abstract. Based on our inclusion criteria, we included 47 reports for reading the full text, of which we excluded 32 studies that did not meet the inclusion criteria. Eventually, we included 15 studies for our systematic review. The corresponding flowchart is depicted in Fig. 1.

Study characteristics

All 15 included studies reported on serum levels of total testosterone before and after TESE. Nine studies also reported serum LH levels and six studies reported on symptoms or signs of hypogonadism in addition to the hormone levels (Table II). The 15 studies were published between 1998 and 2017. The study with the smallest number of patients included 15 men and the study with the largest number of patients included 435 men, although they only reported testosterone levels before and after TESE from 142 men. The age of the men varied between 25 and 61 years. In all studies men with NOA were included; five studies also included men with OA and eight studies also included men with Klinefelter syndrome. In some of these studies the data for the various types of azoospermia were not separated. The TESE techniques used varied between studies (TESA, multiple biopsy/conventional, microdissection TESE and Trucut needle). The follow up of the men varied between 1 month and 46 months after TESE.

Quality assessment

We assessed the risk of bias on confounding as low in all studies since we only included studies comparing data after TESE with that before TESE. We assessed the risk of bias on selection of participants as unclear in studies that did not describe men with OA as a separate group from men with NOA ($n = 3$), and high in the studies that included men with Klinefelter syndrome together with men with NOA ($n = 5$). In studies in which men who had undergone TESE for the second or third time were included, we determined the risk of bias on selection of participants as unclear ($n = 3$). We determined the risk of bias in classification of intervention as unclear in studies in which different TESE techniques were applied but assessed the outcome as one group ($n = 2$) and in studies in which one testis was biopsied in some men but both testes in others ($n = 1$). Some studies did not describe all men in the follow up, which we assessed as an unclear ($n = 8$) or high ($n = 4$) risk of bias on missing data. We assigned an unclear bias in measurements of outcome to studies that did not report at what time during the day blood samples were taken

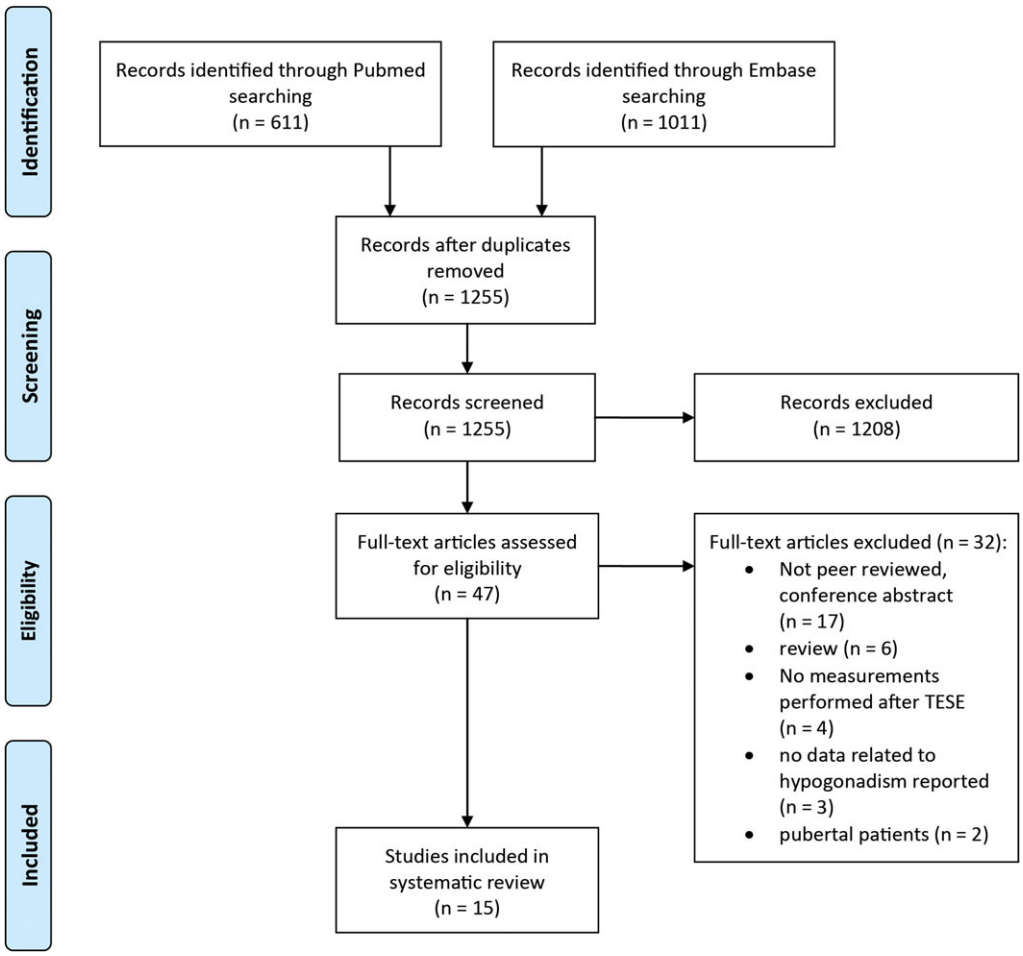

Figure 1 Flowchart of the search and selection of literature on induced hypogonadism after testicular sperm extraction in men. TESE, testicular sperm extraction.

Table II Basic characteristics of studies on testicular sperm extraction included in this review.

Study characteristics			Intervention		Patient characteristics			Outcome
First author	Year	Study design	TESE technique	Size biopsy	Number participants	Age (years: mean ± SD (range))	Type azoospermia	Type of measurement
Manning	1998	Cohort	Multiple biopsy TESE	Not described	15	Not reported	NOA	Testosterone
Westlander	2001	Prospective cohort	TESA	Not described	35	33.3 ± 5.52 (26–48)	NOA (n = 10) and OA (n = 25)*	Testosterone Testicular volume
Steele	2001	Prospective cohort	Trucut needle testicular biopsy	Not described	20	Not reported	NOA and OA*	Testosterone LH
Okada	2002	Retrospective cohort	Conventional TESE Microdissection TESE	150–450 mg 100–450 mg	146	Not reported	NOA (n = 18), KF (n = 6) and OA (n = 22)* NOA (n = 63), KF (n = 11) and OA (n = 26)*	Testosterone Testicular volume
Schill	2003	Prospective cohort	Open biopsy TESE	Two biopsies	40	36 (29–53)	NOA (n = 31) and OA (n = 9)*	Testosterone Testicular volume
Komori	2004	Cohort	Conventional multiple TESE Microdissection TESE	150 mg 60–120 mg	25	35.4 ± 6.4 (29–49) 31.8 ± 4.7 (27–42)	NOA (n = 13) NOA (n = 10) and KF (n = 2)*	Testosterone
Ramasamy	2005	Retrospective cohort	Conventional multiple biopsy TESE Microdissection TESE	500 mg Not described	435	38 ± 1 36 ± 0.3	NOA	Testosterone LH
Everaert	2006	Retrospective cohort	Microsurgical TESE	Not described	48	34 ± 7	NOA (n = 47 and KF (n = 1)*	Testosterone LH
Takada	2008	Cohort	Microdissection TESE	Not described	69	33.9 ± 0.5 years	NOA (n = 60) and KF (n = 9)	Testosterone LH
Ishikawa	2009	Retrospective cohort	Microdissection TESE	Not described	140	34.8 ± 5.2 (24–57)	NOA (n = 100) and KF (n = 40)	Testosterone LH
Akbal	2010	Cohort	Microdissection TESE	Not described	66	34.8 (24–53)	NOA	Testosterone LH Erectile dysfunction
Ozturk	2011	Prospective cohort	Microdissection TESE	Not described	37	32.8 ± 6.7	NOA	Testosterone Testicular volume
Bobjer	2012	Retrospective cohort	Multiple biopsy TESE	Not described	45	36 ± 6.3 (25–61)	NOA (n = 40) and KF (n = 5)	Testosterone LH
Altinkilic	2017	Prospective cohort	OA: conventional trifocal TESE NOA: combined Trifocal/ microdissection TESE	Not described Not described	78	34 ± 6	NOA (n = 48), KF (n = 6) and OA (n = 24) *	Testosterone LH Testicular volume
Binsaleh	2017	Retrospective cohort	Microdissection TESE	Not described	255	35.8 ± 7.2	NOA (n = 244) KF (n = 11)*	Testosterone LH

*Data not described separately for the different patient groups.
TESE, testicular sperm extraction; NOA, non-obstructive azoospermia; OA, obstructive azoospermia; KF, Klinefelter syndrome.

for hormone levels (n = 4), or studies that did not report cut-off values on low total testosterone (n = 1). In studies that reported a different follow-up time per man we also assessed the risk of bias in measurements of outcome as unclear (n = 3). We assessed the risk of bias selection of reported results as unclear in 13 out of 15 studies (87%). The detailed information on the quality assessed per study can be found in Fig. 2.

Testosterone levels after TESE

From the 15 studies that measured serum total testosterone levels before and after TESE, one study only reported the testosterone levels of the men with new-onset of ED after TESE. In these men, serum total testosterone levels were significantly decreased after TESE (Akbal *et al.*, 2010). Another study reported a decrease of

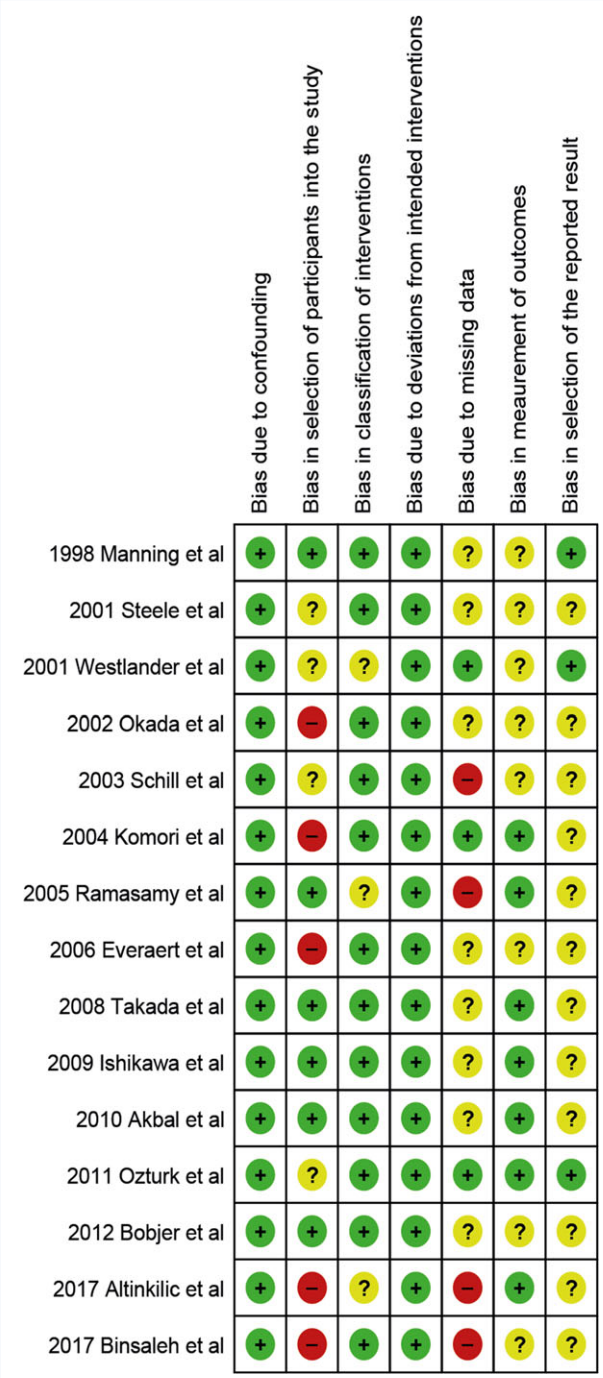

Figure 2 Risk of bias summary for the 15 studies selected from the literature review.

serum testosterone levels 3–6 months after TESE, and a recovery of these levels after 18 months in both microdissection and conventional TESE, but did not report SDs (Ramasamy *et al.*, 2005). We did not include these two studies in our meta-analyses.

Mean testosterone levels after TESE

Twelve studies compared mean total testosterone levels before and after TESE (Table III). Meta-regression analysis showed a significant decrease in total testosterone levels at 3, 6, 9 and 12 months after TESE compared to before, which recovered to normal values again at 18 months (Fig. 3A). There was a lot of variation in testosterone levels between studies before TESE. To reduce this heterogeneity we analysed two patient groups separately, i.e. men with Klinefelter syndrome (three studies) and men with NOA (five studies). It was not possible to identify separate data for men with OA.

For men with Klinefelter syndrome, in total data for 54 men were available (Takada *et al.*, 2008; Ishikawa *et al.*, 2009; Bobjer *et al.*, 2012). There was a significant decrease in total testosterone levels after TESE, but these levels recovered to baseline values after 26 months (Fig. 3B). In one out of three studies, the mean testosterone levels in men with Klinefelter syndrome were above the threshold for hypogonadism of 12 nmol/l at baseline (Bobjer *et al.*, 2012). The meta-analysis using mean differences shows the strongest mean decrease in total testosterone at 6 months after TESE with a mean decrease of 4.13 nmol/l (95% CI: −5.86, −2.40) (Supplementary Fig. S1A). The mean total testosterone levels rose at long-term follow-up (>12 months) to a mean difference of −2.28 nmol/l (95% CI: −4.03, −0.53), but the total testosterone levels remained significantly lower compared to the levels before TESE.

For men with NOA we were able to include five studies with a total of 252 men (Manning *et al.*, 1998; Takada *et al.*, 2008; Ishikawa *et al.*, 2009; Ozturk *et al.*, 2011; Bobjer *et al.*, 2012). In these men we found variations in baseline mean total testosterone levels with some mean total testosterone levels below the threshold for hypogonadism of 12 nmol/l (Fig. 3C). We found the largest decrease in mean total testosterone levels 6 months after TESE, of 2.72 nmol/l (95% CI: −5.02, −0.41) (Supplementary Fig. S1B). Our meta-analysis shows a significant decrease in mean total testosterone levels at 9–12 months after TESE, which disappears at long-term follow-up.

In most studies LH levels were associated with testosterone levels; when a decrease in total testosterone was reported, a significant increase in LH was found (Takada *et al.*, 2008; Altinkilic *et al.*, 2017; Binsaleh *et al.*, 2017) and when no decrease in total testosterone was reported, also no increase in LH was found (Steele *et al.*, 2001; Everaert *et al.*, 2006; Bobjer *et al.*, 2012). In one study no decrease in serum testosterone after TESE in men with NOA and OA was reported, but an increase in LH levels after TESE was seen (Ishikawa *et al.*, 2009). In the same study (Ishikawa *et al.*, 2009) in men with Klinefelter syndrome a decrease in testosterone after TESE was reported, but no increase in LH levels.

One study examined Leydig cell function by performing an hCG test within a period of 4–32 months after TESE (Schill *et al.*, 2003). Out of 13 men with NOA with low serum testosterone levels measured before or after TESE, three men had insufficient increase in testosterone levels after stimulation with hCG (<1.5-fold increase). In addition, 6 out of 15 men with NOA with normal testosterone levels before and after TESE showed insufficient testosterone increase after hCG stimulation, indicating a disturbed functioning of Leydig cells.

Prevalence of low testosterone after TESE

Five studies described the prevalence of low total testosterone levels after TESE, using thresholds varying from 5 to 12 nmol/l, or this was not specified. In total, 229 men were involved (Table III). The ORs per study show that there is a trend towards an increased risk for low total testosterone levels after TESE (Fig. 4). Because of the

Table III Characteristics of studies that measured testosterone levels after TESE.

First author (year of publication)	Technique testosterone measurement	Time of blood samples	Time after TESE measured	Threshold used for low testosterone levels	Number participants with data available	Type of outcome
Manning (1998)	Not described	08.00 AM	6 and 12 months	Not described	15 (12 months $n = 8$)	Mean testosterone levels and prevalence low testosterone
Westlander (2001)	Not described	Not described	3 and 6 months	Not described	35	Mean testosterone levels
Steele (2001)	Radioimmunoassay by Coat-A-Count technology.	Not described	4 weeks	≤12 nmol/l	8	Mean testosterone levels and individual testosterone levels
Okada (2002)*	Radioimmunoassay	Not described	6 months	Significant decrease was defined as: When testosterone level was normal before TESE and after TESE <1.4 ng/ml or a decrease >1 ng/ml for men with hypogonadism before TESE	Conventional TESE: $n = 40$ microdissection TESE: $n = 80$	Prevalence significant decrease in testosterone levels
Schill (2003)	Competitive enzyme immunoassay, part of an automatic measuring apparatus, SR1	08.00–10.00 AM	Average 18 months (4 to 32)	<12 nmol/l	Before TESE: $n = 26$ after TESE: $n = 39$	Mean testosterone levels and prevalence low testosterone
Komori (2004)	Not described	09.00–11.00 AM	1, 6 and 12 months	Not described	Multiple TESE: $n = 13$ microdissection TESE: $n = 12$	Mean total testosterone levels
Ramasamy (2005)*	Not described	07.00–10.00 AM	3, 6, 12 and 18 months	Not described	3–6 months: $n = 142$ 12 months: $n = 88$ 18 months: $n = 53$	Mean testosterone levels
Everaert (2006)	Radioimmunoassay	08.00–10.00 AM	2.4 years ± 1.1 years	<280 ng/dl	Before TESE: $n = 45$ after TESE: $n = 31$	Mean testosterone levels and number of men with de novo androgen deficiency
Takada (2008)	Solid-phase [125]I radioimmunoassay kit Coat-A-Count	08.00–11.00 AM	3, 6, and 12 months	Not described	KF: $n = 9$ NOA: $n = 60$	Mean total testosterone levels
Ishikawa (2009)	Not described	09.00–10:00 AM	1, 3, 6, 9, 12, and 18 months	Not described	KF: $n = 40$ NOA: $n = 100$	Changes in testosterone levels relative to baseline testosterone levels
Akbal (2010)*	Not described	09.00–11.00 AM	6 months	Not described	Data of men with new-onset ED: $n = 13$	Mean total testosterone levels
Ozturk (2011)	Not described	09.00–11.00 AM	3 and 12 months	Not described	37	Mean total testosterone levels
Bobjer (2012)	Competitive immunoassay	Before 11.00 AM	Average 2.2 years ± 1.6 (0.2–5.4)	≤10 nmol/l	KF: $n = 5$ NOA: $n = 40$	Mean testosterone levels and prevalence low testosterone
Altinkilic (2017)	Not described	08.00–10.00 AM	6 weeks	Not described	before TESE: $n = 78$ after TESE: $n = 67$	Mean testosterone levels
Binsaleh (2017)	Not described	Not reported	3 months and more than 1 year	Not described	111	Mean testosterone levels

*Not included in meta-regression analysis.

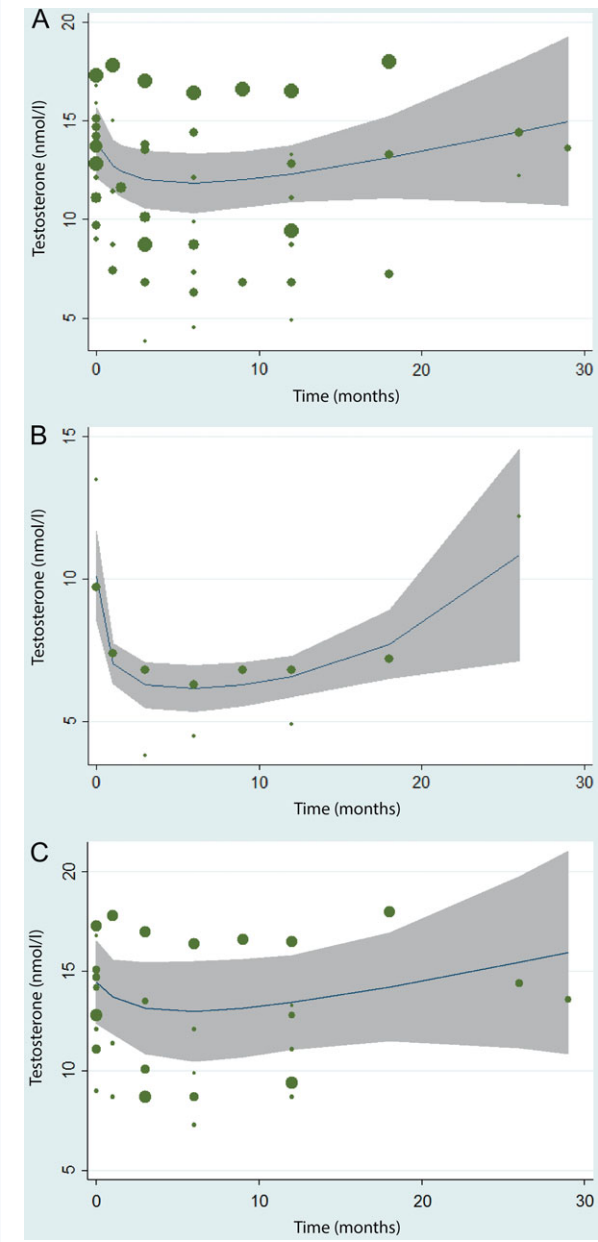

Figure 3 Overview of mean serum total testosterone levels in men after TESE over time. Total testosterone levels are shown in (**A**) all men, (**B**) men with Klinefelter syndrome and (**C**) men with non-obstructive azoospermia.

differences in follow-up time and in thresholds used for low testosterone levels, pooling of the data was not informative.

Risk of symptoms and signs related to hypogonadism after TESE

Our systematic literature search revealed that only six studies reported on symptoms or signs associated with hypogonadism after TESE (Table IV). The reported symptoms and signs are limited to ED (Akbal *et al.*, 2010) and changes in testicular size (Altinkilic *et al.*, 2017; Esteves, 2002; Okada *et al.*, 2002; Ozturk *et al.*, 2011; Schill *et al.*, 2003).

Risk of ED after TESE

The risk of ED after TESE, one of the symptoms of hypogonadism, was described in one study in which 13 out of 66 men with new-onset ED were reported 6 months after TESE (Akbal *et al.*, 2010): men with NOA and undergoing microdissection TESE were included. Overall, 13 men had a score of ≥22 according to the International Index of Erectile Function-5 (IIEF-5) questionnaire, indicating no ED, before TESE and ≤21, indicating ED, after TESE. However, a score of ≤21 was reported in 28 men before TESE and 35 men after TESE, respectively. Although ED was subdivided into mild, moderate and severe depending on the score, no details were reported on the severity of ED in each patient. Out of the 13 men with new-onset ED, one man had successful retrieval of sperm, while from the other 12 men no sperm was retrieved.

Hormonal levels were measured in 36 men, but only the data of the 13 men with new-onset ED are reported. In these men the mean total testosterone level was significantly decreased from 27.1 to 9.7 nmol/l. LH was increased in these men, but not significantly. All 13 men reported depression and anxiety after TESE, assessed with the Hospital Anxiety and Depression Scale. This was only measured after TESE and not before and therefore it is unclear if this depression is a consequence of the TESE procedure or a cause of the ED.

Risk of decrease in testis volume after TESE

One of the signs associated with hypogonadism is a decrease in testis volume. Five studies reported on this sign before and after TESE (Altinkilic *et al.*, 2017; Okada *et al.*, 2002; Ozturk *et al.*, 2011; Schill *et al.*, 2003; Westlander *et al.*, 2001). Because all studies used different time-points and TESE techniques, we were not able to combine the data. Therefore, we will describe these studies one by one.

One study measured testicular volume in 35 men with NOA and OA after TESA (Westlander *et al.*, 2001). Here the testis is punctured with a 19-gauge needle with suction, to retrieve sperm. The aspiration was performed 3–5 times per testis. Three months after TESA the mean volume was the same as before TESA.

In the second study, a 0.3 and 0.6 ml decrease in mean testicular volume 3 and 12 months after microdissection TESE, respectively, was found (Ozturk *et al.*, 2011). In the 37 men with NOA included in this study, the amount of biopsied tissue was determined at the surgery site and biopsies were taken until it was thought more biopsies would impair the blood supply.

The third study evaluated testicular volume in men undergoing conventional TESE (n = 40, including men with NOA, OA and Klinefelter) or microdissection TESE (n = 80, including men with NOA, OA and Klinefelter) (Okada *et al.*, 2002). Measurements of testicular volume were performed at 2 weeks, 1 month and 6 months after TESE but only the data of 6 months after TESE of 120 men were reported. Six months after TESE a decreased testicular volume of at least 2 ml was observed in 25% of the men after conventional TESE and in 2.5% of the men after microdissection TESE. The amount of tissue removed in microdissection TESE was comparable of that of the conventional TESE and ranged between 100 and 300 mg.

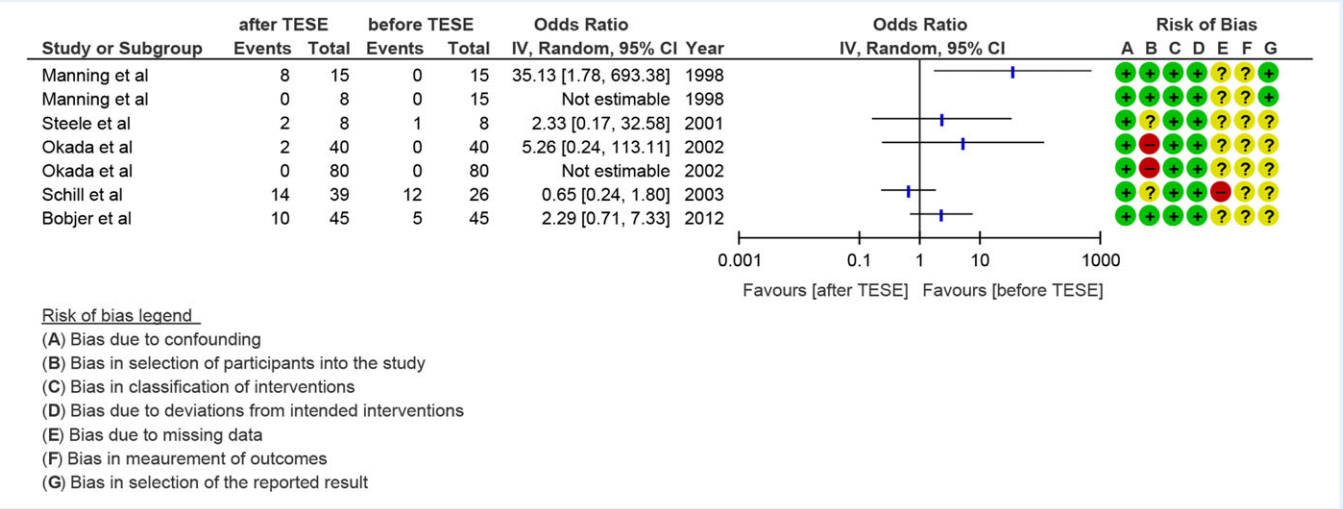

Figure 4 Forest plot with odds ratio of low total testosterone in men after TESE.

Table IV Basic characteristics of studies that measured symptoms or signs related to hypogonadism after TESE.

First author (year of publication)	Type of measurement	Technique of measurement	Time after TESE	Number participants with data available	Outcome (mean ± SD)
Akbal (2010)	Erectile dysfunction	IIEF-5 questionnaire	6 months	66	Prevalence erectile dysfunction (score < 22) before TESE: 28 out of 66 after TESE: 35 out of 66 *However: 13 out of 66 with new-onset ED*
Westlander (2001)	Testicular volume	Physical examination	3 months	35	Mean testicular volume before TESE: 17.1 ± 4.24 ml after TESE: 17.1 ± 4.27 ml
Okada (2002)	Testicular volume	Orchidometer	6 months	Conventional TESE: 40 microdissection TESE: 80	Prevalence of decrease >2 ml in testicular volume conventional TESE: before TESE: 0 out of 40 6 months: 10 out of 40 microdissection TESE: before TESE: 0 out of 80 6 months: 2 out of 80
Schill (2003)	Testicular volume	Ultrasound	Average 18 months (4–32)	Before TESE: 26 after TESE: 39	Mean testicular volume before TESE: 17 ml after TESE: 21.3 ml
Ozturk (2011)	Testicular volume	Physical examination	3 and 6 months	37	Mean testicular volume before TESE: 9.8 ± 1.29 ml 3 months: 9.5 ± 1.35 ml 6 months: 9.2 ± 0.94 ml
Altinkilic (2017)	Testicular volume	Ultrasound	24 h and 6 weeks	Before TESE: 78 24 h: 71 6 weeks: 67	Mean testicular volume before TESE left: 8.2 ± 4.2 ml, right: 9 ± 4.8 ml 24 h left: 8.8 ± 4.9 ml, right: 9.4 ± 5.0 ml 6 weeks left: 6.3 ± 3.7 ml, right: 7.5 ± 4.6 ml

The fourth study measured the testicular volume on average 18 months after TESE in 39 men (Schill *et al.*, 2003). An increase of 4.3 ml in volume is described. However, the volume was measured in only 26 men before TESE.

The fifth study measured the bilateral change in testicular volume 6 weeks after the surgery in 67 men with NOA, OA or Klinefelter (Altinkilic *et al.*, 2017). At this time point, the mean testicular volume was decreased with a difference of 1.5–1.9 ml for, respectively, right or left testis but it is not described if the TESE procedure was performed left, right or bilateral. The volume of the biopsies was not reported.

Taken together, although different results were found in each study, three out of five studies show a decrease in testicular volume

6 weeks to 12 months after TESE. The study that did not see any changes was the only study performing TESA (Westlander *et al.*, 2001). Another study that reported an increase in the testicular volume after TESE did not measure the testis volume in all men before TESE.

Discussion

In this review we evaluated hypogonadism in men with azoospermia after TESE surgery and showed that few high quality data are available from literature. We found there is a temporary decrease in serum total testosterone levels after TESE, for at least 1 year, with levels that may decrease below 12 nmol/l, a threshold level for risk of becoming hypogonadal. The available information on signs and symptoms of hypogonadism after TESE suggests that some men may experience ED, together with depression and anxiety, and a decrease in testicular size.

Testosterone as a measure for hypogonadism

All included studies presented data on serum total testosterone, one of the measures to determine hypogonadism. The decrease in mean serum total testosterone levels was most profound 6 months after TESE and recovered again to baseline levels after 18 months in men with NOA as well as in men with Klinefelter syndrome. Overall, we saw a greater effect of TESE on mean total testosterone levels in men with Klinefelter syndrome compared to men with NOA. Men with Klinefelter syndrome have decreased basal testosterone levels during and after puberty (Rohayem *et al.*, 2016). Because of their low baseline levels, these men might be more sensitive to a decrease in testosterone levels after TESE. Another explanation is that in men with Klinefelter syndrome the testicular volume is small (Rohayem *et al.*, 2016). Because of this smaller testis the percentage of tissue removed during the TESE biopsy is relatively high compared to men with a bigger testis and subsequently the area of tissue that is damaged will be higher.

Interpretation of the consequence of low testosterone levels is not straightforward in view of the different cut-off values suggested by different professional societies and expert groups. According to the Endocrine Society, levels lower than 10.4 nmol/l can be regarded as associated with hypogonadism (Bhasin *et al.*, 2010). On the other hand, the European Association of Urology suggests that levels below 12 nmol/l are associated with symptoms related to hypogonadism (Dohle *et al.*, 2015). At 6 months after TESE, the mean total testosterone levels of men with Klinefelter syndrome were 4.5–6.3 nmol/l and for men with NOA, 8.7–16.4 nmol/l. This implies that in men with Klinefelter and in a proportion of men with NOA total testosterone levels drop below the threshold of 12 nmol/l and thus have a risk of developing symptoms and signs associated with hypogonadism after TESE.

We expect the highest prevalence of low total testosterone levels 6 months after TESE, because of the fact that we see the most profound decrease in mean total testosterone levels at this time point. Two out of five studies measured the prevalence at 6 months and these two studies found low testosterone levels in 53 and 5% of the men (Manning *et al.*, 1998; Okada *et al.*, 2002). However, Manning *et al.* (1998) did not report the threshold used, while Okada *et al.* (2002) used a very low threshold of 4.9 nmol/l. The other studies that measured prevalence of low testosterone used a follow-up time varying from 1 to 26 months after TESE.

Clinical signs and symptoms of hypogonadism

Patients will experience hypogonadism by their symptoms rather than on serum testosterone levels. Although cut-off values of serum testosterone levels help to diagnose hypogonadism after TESE, it is not clear whether the TESE-induced decrease in total testosterone levels, irrespective of the levels of serum total testosterone before TESE, might lead to hypogonadism. Therefore, symptoms are more clinically relevant in order to diagnose patients for hypogonadism. New-onset ED was measured in 13 out of 66 men at 6 months after TESE. This was accompanied by a significant decrease in mean total testosterone levels below 12 nmol/l. This suggests that ED after TESE might be explained by hypogonadism, although we cannot exclude that ED is caused by psychological reasons instead of decreased total testosterone levels. ED was especially found in the group of men with unsuccessful sperm retrieval, depression and anxiety, and these factors may have resulted in the ED. However, depression and anxiety can, in addition to causing ED, also be symptoms of hypogonadism. Another possible explanation of the higher prevalence of ED in the group with unsuccessful sperm retrieval is that possibly more biopsies are taken from these men. This might contribute to a higher risk of a decrease in testosterone levels and therefore ED (Manning *et al.*, 1998).

An effect of TESE on testicular volume was seen in studies at 6 weeks to 12 months after the procedure, although this effect was less after microdissection TESE. When longer follow-up was reported, no effect was seen of TESE or TESA on testicular volume. This suggests that timing of the follow-up is important.

Strengths of this systematic review

This review is the first to combine all the reported data on TESE-induced hypogonadism. Because we were able to pool data from these studies, leading to a cohort of 54 men with Klinefelter syndrome and 252 men with NOA, we can conclude that serum total testosterone levels decrease after TESE surgery to levels that might be related to symptoms and signs for hypogonadism. This was supported by the prevalence of new-onset ED and the occurrence of decreased testicular volume after TESE. This information can be taken into account in counselling and follow-up of the men with azoospermia after TESE.

Limitations in the interpretation

First and foremost, the data are limited by the low number of studies and, in addition, the small size of the available cohorts. Furthermore, most studies only reported on serum total testosterone levels. Strikingly, although TESE is routinely applied, there are only follow-up data available for 54 men with Klinefelter and 252 men with NOA, suitable for our meta-analysis. Because not all studies used the same follow-up time after TESE, these numbers are not representative for all time-points. Furthermore, although more data on total

testosterone levels after TESE were available, not all studies reported the data separately for men with different types of azoospermia. Therefore, we could not use all the studies in these analyses. In addition, we had to exclude the largest study because no SDs were described in this study (Ramasamy et al., 2005); however, the results of this study are in line with our results of the meta-analysis.The effect of TESE on decreased total testosterone levels is based on mean total testosterone levels and, because of this, the observed effects may be an underestimation in individuals. A decrease in total testosterone levels in individuals could be missed when looking at the mean levels of a cohort. Therefore, normal mean total testosterone levels do not rule out the presence of men with total testosterone levels below 12 nmol/l that are at risk for symptoms of hypogonadism.

Although we were able to do a separate analysis for men with Klinefelter syndrome and men with NOA, we were not able to study the group of men with OA with the available data. It is possible that the risk of hypogonadism differs between various causes of subfertility. In line with this it was shown that the prevalence of low testosterone levels in general in men with NOA is reported to be 45%, while in men with OA this is 16.7% (Sussman et al., 2008). In addition, lower testosterone baseline levels for men with maturation arrest and Sertoli cell only syndrome were found compared to men with hypospermatogenesis (Takada et al., 2008). Therefore, heterogeneity in the mixed population studied might be a potential source of bias. As a results of this possible bias, we tried to separate these data where possible.

In addition we were not able to analyse separately the data for various TESE techniques. While in the conventional/open biopsy technique biopsies are taken randomly from different areas of the testis, in the microdissection TESE the location of the biopsies are determined in a more directed way, with the use of a microscope. Therefore, it might be easier to avoid damage caused by destruction of blood vessels and for that reason it is thought microdissection TESE could cause less damage (Shin and Turek, 2013). With TESA and trucut needle testicular biopsy, a needle is used to puncture the tissue, with or without first puncturing the skin with a scalpel, followed by suction of cells and small pieces of tissue. Although the area of damage might be less when using a needle, the location is less well identified. In the three studies comparing the decrease in total testosterone levels after conventional TESE with that after microdissection TESE, the risk was higher or comparable for conventional TESE (Komori et al., 2004; Okada et al., 2002; Ramasamy et al., 2005). Although the TESE technique is reported as conventional or microdissection TESE, the details of the procedure itself will vary at different institutions. These differences in the TESE procedure might increase the heterogeneity of the data between the studies.

Biological interpretations of the evidence

The effect of TESE on total testosterone can be explained by different mechanisms. The first explanation is that part of the testicular tissue is removed resulting in a lower number of Leydig cells and therefore a lower production of testosterone. However, with decreased serum testosterone levels, the remaining Leydig cells will be stimulated by higher LH levels due to the negative feedback in the hypothalamus–pituitary–gonad axis, and they should become more active in producing testosterone. Nevertheless, it has been shown that stimulation of Leydig cells with hCG after TESE in men with low testosterone levels showed an adequate response, with increased testosterone levels 3 and 4 days after hCG injection. Therefore, these Leydig cells are able to produce more testosterone when they are stimulated, suggesting that in these men a more systemic cause of low LH levels could underlie the hypogonadism after TESE. Indeed, when LH was upregulated immediately after TESE a faster recovery than 18 months was likely expected. Although some studies showed an increase in LH levels after TESE, the normal correlation between LH and total testosterone was not always observed. In one study high LH levels and normal testosterone levels were found in men with NOA and OA (Ishikawa et al., 2009). This combination of high LH and normal testosterone levels is called compensated hypogonadism and might lead to hypogonadism in the future (Tajar et al., 2010). At this point Leydig cells need a higher stimulation to be able to produce normal testosterone levels. However, the number of reports on LH concentrations in relation to testosterone concentrations after TESE is limited and further research would give more insights in the various forms of hypogonadism.

A second explanation of the recovery of total testosterone levels is related to the time to repopulate Leydig cells by stem Leydig cells and therefore restore testosterone production at 18 month after TESE. In the rat testis it takes 21 days for new adult Leydig cells to appear from stem Leydig cells after they were chemically destroyed (Jackson et al., 1986). For human Leydig cells, we expect this to be longer than 21 days, however, it is unknown what the exact time span is for human Leydig cells to repopulate the testis.

A third and more likely possibility is that the number and function of the Leydig cells is not the cause of decreased total testosterone levels after TESE, but that this is induced by vascular damage in the testis. This vascular damage can result in lower stimulation of Leydig cells by LH because it cannot reach the Leydig cells efficiently, or testosterone is produced in the testis at normal levels but cannot be released into the blood circulation. Indeed, several studies have reported ultrasound findings after TESE and document hypoechoic focal lesions, suggesting haematoma up to 1 and 3 months after TESE, and hyporechoic foci suggestive of scar tissue at 3 and 6 months after TESE (Donoso et al., 2007).

Conclusion

Although limited studies were obtained from our systematic search, based on the extracted data we found transient but significantly decreased total testosterone levels after TESE that recover to baseline levels after 18–26 months. This effect was most profound in men with Klinefelter syndrome. The number of studies reporting on symptoms and signs of hypogonadism associated with TESE is very limited, showing some risk of ED, which seems to be related to a decreased total testosterone and/or possible depression and anxiety. Furthermore, a decrease in testicular volume is seen in some men after TESE.

For better counselling of TESE patients on the transient hypogonadism after TESE, more research is necessary to understand whether the decreased total testosterone is accompanied by symptoms and signs of hypogonadism in the short and long term. It is striking that these data are still not available 25 years after the clinical introduction of TESE.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

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Authors' roles

J.E. performed the literature search, data extraction and took the lead in writing the manuscript. J.E. and A.v.P. selected the studies. J.E. and M.v.W. performed risk of bias assessment and M.v.W. provided statistical support and performed the meta-regression analysis. All authors took part in the design of the study and writing and revising several drafts of the article.

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Conflict of interest

The authors report no financial or other conflict of interest relevant to the subject of this article.

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