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Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis

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BACKGROUND: Factor affecting sperm retrieval rate (SRR) or pregnancy rates (PR) after testicular sperm extraction (TESE) in patients with non-obstructive azoospermia (NOA) have not been systematically evaluated. In addition, although micro-TESE (mTESE) has been advocated as the gold standard for sperm retrieval in men with NOA, its superiority over conventional TESE (cTESE) remains conflicting.

OBJECTIVE AND RATIONALE: The objective was to perform a meta-analysis of the currently available studies comparing the techniques of sperm retrieval and to identify clinical and biochemical factors predicting SRR in men with NOA. In addition, PRs and live birth rates (LBRs), as derived from subjects with NOA post-ICSI, were also analysed as secondary outcomes.

SEARCH METHODS: An extensive Medline, Embase and Cochrane search was performed. All trials reporting SRR derived from cTESE or mTESE in patients with NOA and their specific determinants were included. Data derived from genetic causes of NOA or testicular sperm aspiration were excluded.

OUTCOMES: Out of 1236 studies, 117 studies met the inclusion criteria for this study, enrolling 21 404 patients with a mean age (± SD) of 35.0 ± 2.7 years. cTESE and mTESE were used in 56 and 43 studies, respectively. In addition, 10 studies used a mixed approach and 8 studies compared cTESE with mTESE approach. Overall, a SRR per TESE procedure of 47[45;49]% (mean percentage [95% CI]) was found. No differences were observed when mTESE was compared to cTESE (46[43;49]% for cTESE versus 46[42;49]% for mTESE). Meta-regression analysis demonstrated that SRR per cycle was independent of age and hormonal parameters at enrolment. However, the SRR increased as a function of testis volume. In particular, by applying ROC curve analysis, a mean testis volume higher than 12.5 ml predicted SRR >60% with an accuracy of 86.2% ± 0.01. In addition, SRR decreased as a function of the number of Klinefelter's syndrome cases included ($S = -0.02[-0.04;-0.01]$; $P < 0.01$. $I = 0.12[-0.05;0.29]$; $P = 0.16$). Information on fertility outcomes after ICSI was available in 42 studies. Overall, a total of 1096 biochemical pregnancies were reported (cumulative PR = 29[25;32]% per ICSI cycle). A similar rate was observed when LBR was analysed (569 live births with a cumulative LBR = 24[20;28]% per ICSI cycle). No influence of male and female age, mean testis volume or hormonal parameters on both PR and LBR per ICSI cycle was observed. Finally, a higher PR per ICSI cycle was observed when the use of fresh sperm was compared to cryopreserved sperm (PR = 35[30;40]%, versus 20[13;29]% respectively): however, this result was not confirmed when cumulative LBR per ICSI cycle was analysed (LBR = 30[20;41]% for fresh versus 20[12;31]% for cryopreserved sperm).

WIDER IMPLICATIONS: This analysis shows that cTESE/mTESE in subjects with NOA results in SRRs of up to 50%, with no differences when cTESE was compared to mTESE. Retrieved sperms resulted in a LBR of up to 28% ICSI cycle. Although no difference between techniques was found, to conclusively clarify if one technique is superior to the other, there is a need for a sufficiently powered and well-designed randomized controlled trial to compare mTESE to cTESE in men with NOA.

Key words: non-obstructive azoospermia / testicular sperm extraction / ART / ICSI / infertility

Introduction

Infertility affects approximately 15% of couples trying to conceive (Eisenberg *et al*., 2013). A male factor is involved in about 50% of cases (Tournaye *et al*., 2017; Pan *et al*., 2018). Azoospermia is the more severe phenotype of male infertility, occurring in 10–15% of males seeking medical care for couple infertility (Tournaye *et al*., 2017; Lotti *et al*., 2014). The vast majority of cases of obstructive azoospermia (OA) are due to congenital or acquired causes (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). Non-obstructive azoospermia (NOA) is the most severe form of male factor infertility accounting for about 5% of infertile couples (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). Whereas OA is usually characterized by normal spermatogenesis, NOA represents a heterogeneous condition, with impaired spermatogenesis ranging from hypospermatogenesis and maturation arrest to Sertoli cell-only syndrome (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). Klinefelter's syndrome (KS) and Y chromosome microdeletions represent the most common congenital causes of NOA (Forti *et al*. 2010; Krausz, 2011; Corona *et al*., 2017). Acquired causes of NOA include torsion, mumps, orchitis, cryptorchidism and iatrogenic problems (chemotherapy and radiotherapy) (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). Historically, OA and NOA were considered untreatable conditions requiring donor spermatozoa for fertilization. The introduction of the technique of ICSI has revolutionized the management of these patients (Van Steirteghem *et al*., 1993). In particular, the combination of conventional (non-magnified) testicular sperm extraction (cTESE) and ICSI has become the first-line treatment for men with azoospermia (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). Testicular sperm aspiration (TESA) using a fine needle represents another option to retrieve sperms in men with azoospermia. In 1999, microdissection TESE (mTESE) was introduced by Schlegel *et al*. (1999). This technique allows magnification, under an operating microscope, of the testis parenchyma allowing selection of the whitish, larger and more opaque tubules, which are more likely to contain sperm (Schlegel *et al*., 1999).

The probability of retrieving sperm is almost 100% in men with OA (Ghanem *et al*., 2005). Conversely, the recovery of spermatozoa in NOA is successful only in approximately 50% of cases, due to partial and heterogeneous preserved focal spermatogenesis (Krausz, 2011; Tournaye *et al*., 2017; Pan *et al*., 2018). In his original work, Schelegel *et al*. (1999) showed that the use of mTESE could improve the sperm retrieval rate (SRR) in men with NOA from 45 to 63%. This finding was thereafter confirmed by other authors (Amer *et al*., 2000; Okada *et al*., 2002; Tsujimura *et al*., 2002; Ramasamy *et al*., 2005; Colpi *et al*., 2009; Ghalayini *et al*., 2011; Salehi *et al*., 2017). It should be mentioned that not all studies find higher SRR by using mTESE and there is a need of great clinical importance to compare mTESE with cTESE. Conversely, TESA has been documented to have limited efficacy in SRR in subjects

with NOA, although it is still practiced as a method of sperm acquisition in IVF centres (Bernie *et al*., 2015a). In a first qualitative analysis of only seven studies, by comparing the SRR achieved using cTESE and mTESE in NOA, Deruyver *et al*. (2014) concluded that mTESE resulted in superior surgical SRR. Similar results were reported in 2015 by Bernie *et al*., (2015a) using a meta-analytic method in the same series of studies previously considered by Deruyver *et al*. (2014). Owing to the limited (approximately 50%) predictive power for successful SRR of the available surgical techniques (i.e. TESA, cTESE or mTESE), the identification of non-invasive parameters (hormonal, molecular, biochemical and cytological, among others) predicting with a high diagnostic accuracy that the positive SRR should be accurately analyzed. In fact, this would reduce not only the surgical risk but also the costs of the NOA diagnostic workup. It is obvious that the establishment of molecular, biochemical, clinical or histopathological parameters that have a role in identifying subpopulations of NOA men positive for foci of advanced spermatogenesis, up to the spermatozoon stage, has great clinical importance. Only limited information is available on this topic. Similarly, data comparing the fertility outcomes between mTESE and cTESE are scant. Furthermore, data reporting on pregnancy rate (PR) and live birth rate (LBR) following m-TESE-ICSI or cTESE-ICSI, an important aspect of patient counselling, are limited.

The aim of this present study was to conduct a meta-analysis of currently available data regarding SRR in subjects with NOA, including all available studies published. The major objective of the current communication was to compare the SRR after mTESE, cTESE and TESA. The contribution of possible predictive factors influencing successful SRR was systematically analysed. In addition, when available, PR and LBR after ICSI are reported.

Methods

This meta-analysis was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline. The protocol of this study (CRD42018092017) was published on the website of the University of York (Centre for Reviews and Dissemination) https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=92017

Search strategy

An extensive Medline, Embase and Cochrane search was performed, including the following words: 'non [All Fields] AND obstructive [All Fields] AND ("azoospermia"[MeSH Terms] OR "azoospermia"[All Fields])'.

The search, which accrued data from 01 January 1969 up to 31 December 2017, was restricted to English-language articles and studies including human participants. The identification of relevant studies was performed independently by 12 of the authors (A.K., C.B., F.F., Z.K., P.V., G.D., A.G., S.M., S.K., M.D., J.R. and N.S.), and conflicts were resolved by the first investigator (G.C.). All the data identified during the first analysis were checked in a second-wave analysis by six of the authors (C.K., F.P., A.P., L.V., M.M., G.C.). Possible further conflicts were discussed and resolved by the first investigator (G.C.). We did not employ search software but hand-searched bibliographies of retrieved papers for additional references. Information was derived from published articles.

Study selection

All prospective and retrospective observational studies reporting SRR after cTESE or mTESE in subjects with NOA without any arbitrary restriction were included (Fig. 1 and Table I). Case reports or trials reporting SRR in OA were excluded from the analysis (Fig. 1). Similarly, due to limited efficacy of the technique (Bernie *et al*., 2015a), data obtained using only TESA were not considered in the final analysis. mTESE was defined according to Schlegel *et al*. (1999) using the operating microscope at 15–20 power.

Outcome and quality assessment

The principal outcome was the analysis of SRR in NOA. Secondary outcomes included the comparison of SRR according to different surgical techniques, including cTESE and mTESE. In addition, when available, PR and LBR after ICSI were also investigated. In particular, when possible PR or LBR either per cycle or cumulative rates, as reported by the authors, was calculated. The quality of trials included was assessed using the Cochrane criteria (Higgins & Green 2008). In particular, we evaluated the following criteria: the weaknesses of the designs that have been used (such as noting their potential to ascertain causality), the execution of the studies through a careful assessment of their risk of bias, especially the potential for selection bias and confounding to which all observational studies are susceptible, and the potential for reporting biases, including selective reporting of outcomes.

Statistical analysis

Heterogeneity in SRR was assessed using I^2 statistics. Even when low heterogeneity was detected, a random-effect model was applied because the validity of tests of heterogeneity can be limited with a small number of component studies. We used funnel plots and the Begg adjusted rank correlation test to estimate possible publication or disclosure bias (Begg *et al*., 1994); however, undetected bias may still be present because these tests have low statistical power when the number of trials is small. SRRs are expressed as mean percentage (95% CI).

An iterative ROC analysis, weighting each study for the number of subjects enrolled, was used to determine the lowest proper testis volume for the detection of SRR > 60%, and the accuracy, sensitivity and specificity at that threshold were calculated. In particular, since the highest 95% CI of SRR was close to 50% (see below), the SRR of 50% was arbitrarily used as a binary study classifier to select the best lower testis volume. The arbitrarily selected SRR was then increased by 5%, and the analysis described above was repeated iteratively, until when the further increment reduced substantially the accuracy, sensitivity and specificity of the test.

In addition, a meta-regression analysis was performed to test the effect of different parameters on SRR, PR and LBR. Finally, a linear regression analysis model, weighting each study for the number of subjects enrolled, was performed to verify the independent effect of specific parameters on SRR after the adjustment for confounders. Thereafter, potential predictors of SSR were included as continuous variables: age, geographical areas, hormone levels (total testosterone, LH and FSH), testicular volume and percentage of men with KS. All data were calculated using Comprehensive Meta-Analysis Version 2 (Biostat, Englewood, NJ, USA). Logistic multivariate analysis was performed on the Statistical Package for the Social Sciences, for Windows 20.1 (IBM: Chicago, IL, USA).

Table I Characteristics of the clinical studies in men with non-obstructive azoospermia included in the meta-analysis.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Fahmy *et al.*, 1997	30	NR	cTESE	NR	NR	NR	38.8	NA	NA	NR	NR	NR	NR	33	30	5	NR	Fresh
Friedler *et al.*, 1997	37	37	cTESE	Yes	Yes	16	33	NA	NA	20.1	NR	NR	NR	NR	16	4	NR	Fresh
Mansour *et al.*, 1997	103	NR	cTESE	NR	NR	NR	39.6	NA	NA	NR	NR	NR	NR	32.5	106	12	NR	Mixed
Ezeh *et al.*, 1998	35	35	Mixed	No	Yes	22	NR	5.7	5.7	18.5	NR	NR	15.2	NR	NR	NR	NR	CP
Rosenlund *et al.*, 1998	12	16	cTESE	Yes	NR	8	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Amer *et al.*, 1999	216	216	cTESE	Yes	No	37	32	NA	NA	17	NR	NR	12.4	NA	NA	NA	NA	NA
Amer *et al.*, 1999[a]	100	100	cTESE	Yes	Yes	49	36	NA	NA	16.3	NR	NR	12.7	NA	NA	NA	NA	NA
Ben-Yosef *et al.*, 1999	55	55	cTESE	Yes	Yes	33	NR	NA	NA	17.3	NR	NR	NR	NR	57	13	10	Mixed
Ezeh *et al.*, 1999	40	40	cTESE	Yes	No	28	34	NA	NA	18	NR	NR	16	NA	NA	NA	NA	NA
Palermo *et al.*, 1999[d]	83	83	cTESE	No	Yes	53	NR	NA	10.8	NR	NR	NR	NR	NR	53	26	NR	Mixed
Schlegel 1999	27	27	mTESE	Yes	Yes	17	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Schlegel 1999[a]	22	22	cTESE	Yes	Yes	10	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Amer *et al.*, 2000	100	100	cTESE/ mTESE	Yes	Yes	56	33.5	NA	4	15	NR	NR	NR	NA	NA	NA	NA	NA
Ballescà *et al.*, 2000	17	17	cTESE	Yes	Yes	10	32	NA	NA	12.7	NR	NR	NR	NR	NR	NR	NR	Mixed
Mercan *et al.*, 2000	452	452	Mixed	Yes	Yes	291	NR	NA	NA	NR	NR	NR	NR	NR	291	97	73	Fresh
Amer *et al.*, 2001	100	100	cTESE	Yes	Yes	49	36	NA	NA	16.2	NR	NR	12.7	NA	NA	NA	NA	NA
Battaglia *et al.*, 2001	13	9	cTESE	Yes	No	12	39.8	NA	NA	16.3	6.6	10.4	9.9	NA	NA	NA	NA	NA
Chan *et al.*, 2001	17	20	cTESE	Yes	Yes	9	37.4	NA	NA	21.8	7.6	11.2	11.7	33.5	20	3	2	Fresh
Eytan *et al.*, 2001	7	7	cTESE	No	Yes	4	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Kahrman *et al.*, 2001	363	363	cTESE	NR	Yes	106	35.8	NA	NA	15.5	NR	NR	NR	NR	NR	NR	NR	CP

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Bohring *et al.*, 2002	33	33	cTESE	NR	Yes	22	NR	NA	NA	16.9	5.9	13.3	20.6	NR	NR	NR	NR	CP
Chiang *et al.*, 2002	47	37	cTESE	NR	NR	5	NR	2.7	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Friedler *et al.*, 2002	83	83	cTESE	Yes	Yes	32	33.7	NA	NA	23.5	NR	11.7	NR	30	55	11	8	Mixed
Hauser *et al.*, 2002	65	65	cTESE	Yes	Yes	35	NR	NA	NA	18	6.8	25.3	3	NR	NR	NR	NR	Mixed
Mátyás *et al.*, 2002	75	75	cTESE	Yes	Yes	52	37.9	NA	NA	NR	NR	NR	NR	30	NR	NR	NR	Fresh
Okada *et al.*, 2002	24	24	cTESE	Yes	Yes	4	NR	NA	25	NR	NR	NR	NR	NA	NA	NA	NA	NA
Okada *et al.*, 2002[a]	74	74	mTESE	NR	Yes	33	NR	NA	14.8	NR	NR	NR	NR	NA	NA	NA	NA	NA
Tsujimura *et al.*, 2002	37	37	cTESE	Yes	Yes	13	32.4	NA	16.2	22.6	11.3	15.1	7.2	NA	NA	NA	NA	NA
Tsujimura *et al.*, 2002[a]	56	56	mTESE	Yes	Yes	24	33.9	NA	16.1	24	8.3	17	8.6	NA	NA	NA	NA	NA
Vernaeve *et al.*, 2002	185	185	cTESE	Yes	Yes	92	35.6	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Aydos *et al.*, 2003	45	45	mTESE	Yes	Yes	15	NR	NA	17.8	4.5	NR	NR	18	NA	NA	NA	NA	NA
Aydos *et al.*, 2003[a]	63	63	mTESE	Yes	Yes	40	NR	NA	19.1	8.9	NR	NR	20	NA	NA	NA	NA	NA
Bailly *et al.*, 2003	75	75	cTESE	Yes	Yes	26	NR	NA	NA	21.4	NR	NR	NR	31.8	60	11	NR	CP
Mansour *et al.*, 2003	452	488	cTESE	Yes	Yes	274	39.5	NA	NA	NR	NR	NR	NR	32.1	274	79	NR	Fresh
Meseguer *et al.*, 2003	12	12	cTESE	Yes	Yes	5	34	NA	NA	26.1	NR	NR	16.1	NR	8	1	1	CP
Samli *et al.*, 2004	303	303	cTESE	NR	NR	107	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NR
Tsujimura *et al.*, 2004a[a]	60	60	mTESE	Yes	Yes	22	32.4	10	NA	25.4	6	12.5	8.1	NA	NA	NA	NA	NR
Tsujimura *et al.*, 2004b[b]	100	100	mTESE	NR	Yes	41	33.6	NA	NA	25.4	7	12	9.9	NA	NA	NA	NA	NR
Vernaeve *et al.*, 2004	79	79	cTESE	Yes	Yes	41	34	NA	NA	26.4	NR	13.6	9.3	31.1	64	11	15	Mixed
Aydos *et al.*, 2005	177	177	mTESE	Yes	Yes	102	34	NA	3.4	15.4	7.1	13.7	10.6	NR	93	34	NR	Fresh

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Bettella *et al.*, 2005	125	125	cTESE	Yes	Yes	74	37.6	NA	NA	20.2	4.8	13.8	11.1	NR	NR	NR	NR	CP
Giorgetti *et al.*, 2005	118	118	cTESE	Yes	No	51	NR	NA	NA	NR	NR	NR	NR	31.2	99	35	29	CP
Koscinski *et al.*, 2005	37	37	cTESE	Yes	No	18	32.9	NA	NA	22.9	NR	NR	8.1	NA	NA	NA	NA	NA
Mitchell *et al.*, 2005	34	NR	cTESE	No	No	NR	NR	NA	NA	10.4	NR	NR	NR	NR	53	10	10	Mixed
Mulhall *et al.*, 2005	92	44	cTESE	Yes	Yes	20	26	9	14	NR	NR	NR	NR	NA	NA	NA	NA	NA
Mulhall *et al.*, 2005[ae]	92	48	cTESE	Yes	Yes	24	29	13	9	NR	NR	NR	NR	NA	NA	NA	NA	NA
Nagata *et al.*, 2005	62	62	cTESE	Yes	Yes	17	35	NA	NA	26.1	NR	NR	9.6	NA	NA	NA	NA	NA
Ramasamy *et al.*, 2005	83	83	cTESE	Yes	Yes	27	38	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Ramasamy *et al.*, 2005[a]	435	460	mTESE	Yes	Yes	267	36	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Wu *et al.*, 2005	30	30	cTESE	NR	NR	23	NR	NA	NA	NR	NR	NR	NR	NR	30	17	18	Mixed
Everaert *et al.*, 2006	48	48	mTESE	Yes	Yes	17	NR	NA	NA	NR	NR	NR	NR	31	28	8	4	Mixed
Hauser *et al.*, 2006	87	87	cTESE	Yes	Yes	50	NR	9.6	NA	NR	NR	NR	NR	NR	NR	NR	NR	Fresh
Tsujimura *et al.*, 2006	46	46	mTESE	Yes	Yes	21	34.8	NA	8.7	27.9	8.5	11	9.1	NA	NA	NA	NA	NA
Tsujimura *et al.*, 2006[a]	134	134	mTESE	Yes	Yes	59	34.1	NA	18.7	28.1	9.7	11.3	8.3	NA	NA	NA	NA	NA
Tunc *et al.*, 2006[f]	52	52	cTESE	Yes	Yes	20	34.5	NA	NA	13.4	NR	NR	9.3	NA	NA	NA	NA	NA
Vernaeve *et al.*, 2006	628	784	cTESE	Yes	Yes	384	NR	4.1	10.3	NR	NR	NR	NR	NR	NR	NR	NR	Mixed
Zitzmann *et al.*, 2006	179	179	cTESE	Yes	Yes	95	NR	2.79	1.12	11.4	NR	NR	NR	33	NR	NR	NR	CP
El-Haggar *et al.*, 2008	100	100	mTESE	Yes	Yes	52	30.4	NA	NA	18.7	NR	NR	9.9	NA	NA	NA	NA	NA
Hibi *et al.*, 2007	5	7	mTESE	Yes	Yes	3	34.6	NA	NA	24.3	9.3	12.5	7.9	NR	7	2	3	CP
Mitchell *et al.*, 2007	23	NR	cTESE	Yes	Yes	NR	NR	NA	NA	18.4	NR	NR	NR	NR	47	20	18	Fresh

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Mostafa *et al.*, 2007	40	40	cTESE	NR	NR	21	38.1	NA	NA	15.1	NR	NR	11.3	NA	NA	NA	NA	NA
Amer *et al.*, 2008	264	264	mTESE	Yes	Yes	105	37	NA	3.4	18.1	10.5	NR	10	NA	NA	NA	NA	NA
Houwen *et al.*, 2008	199	199	cTESE	Yes	Yes	82	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NR
Kanto *et al.*, 2008	40	40	mTESE	NR	NR	17	NR	NA	NA	NR	NR	NR	NR	34.2	17	9	NR	Fresh
Madbouly *et al.*, 2008	100	100	mTESE	Yes	Yes	33	36.4	NA	NA	19.1	NR	12.6	NR	NA	NA	NA	NA	NA
Ravizzini *et al.*, 2008	56	56	mTESE	Yes	Yes	32	37.3	1.78	1.78	15.1	9.4	14.5	9	32.6	32	13	3	Mixed
Colpi *et al.*, 2009[c]	195	69	cTESE	Yes	Yes	29	36	NA	NA	NR	NR	NR	NR	NR	NR	NR	NR	Fresh
Colpi *et al.*, 2009[ac]	195	69	mTESE	Yes	Yes	36	36	NA	NA	NR	NR	NR	NR	NR	NR	NR	NR	Fresh
Hallak *et al.*, 2009	5	5	mTESE	No	Yes	4	35.8	NA	NA	9.6	NR	15.2	14.8	NR	NR	NR	NR	Fresh
Haimov-Kochman *et al.*, 2009	146	149	cTESE	No	No	79	NR	4.69	NA	20.3	NR	NR	NR	NR	164	63	NR	Mixed
Haraguchi *et al.*, 2009	47	47	mTESE	NR	Yes	15	NR	NA	NA	16.5	4.8	16.7	NR	NR	NR	NR	NR	Mixed
Inci *et al.*, 2009	96	96	mTESE	Yes	Yes	44	35.4	NA	NA	13.3	NR	NR	17.8	31.2	44	13	11	NR
Ishikawa, 2009	140	140	mTESE	Yes	Yes	46	33.8	NA	NA	NR	NR	NR	NR	33.4	75	21	18	CP
Ramasamy *et al.*, 2009	792	792	mTESE	Yes	Yes	475	35.8	NA	NA	23.5	NR	NR	9.4	31.8	NA	NA	NA	NA
Wiser *et al.*, 2009	42	42	cTESE	Yes	Yes	25	33.4	NA	NA	23.3	11	14.4	14	30.6	25	9	7	Fresh
Yarali *et al.*, 2009	113	130	mTESE	NR	Yes	57	34.3	NA	NA	NR	NR	NR	NR	20.9	57	15	12	Fresh
Zohdy *et al.*, 2009	20	20	mTESE	Yes	Yes	13	36.9	5	10	13.6	7	11.4	7.3	NA	NA	NA	NA	NA
Ishikawa *et al.*, 2010	150	150	mTESE	Yes	Yes	62	34.7	NA	14	20.5	7.4	16.4	11.2	NA	NA	NA	NA	NA
Mitchell *et al.*, 2010	139	139	cTESE	No	No	60	33.4	2.9	5	21.4	NR	NR	17.6	NR	NR	NR	NR	Fresh
Turunc *et al.*, 2010	335	335	Mixed	Yes	Yes	147	35.2	5.6	23.2	17.9	NR	NR	12.9	30	129	65	43	NR

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Boitrelle *et al.*, 2011	280	280	cTESE	Yes	Yes	149	33.2	NA	NA	21.8	NR	NR	16.5	31.7	169	38	33	CP
Cavallini *et al.*, 2011	149	149	cTESE	Yes	Yes	79	43.6	7.6	NA	12.3	NR	NR	18.2	35.8	184	14	13	CP
Ghalayini *et al.* 2011	133	68	cTESE	Yes	Yes	26	35.4	NA	NA	16.7	11.1	13.4	11.9	NA	NA	NA	NA	NR
Ghalayini *et al.* 2011[a]	133	65	mTESE	Yes	Yes	37	34.8	NA	NA	19.7	11	14.7	11.8	NA	NA	NA	NA	NR
Hauser *et al.*, 2011	13	16	cTESE	Yes	Yes	16	36.7	7.69	NA	19.1	NR	NR	NR	NR	59	12	9	Mixed
Hsiao *et al.*, 2011	73	84	mTESE	Yes	Yes	36	34.5	NA	NA	21.9	7.1	12.3	9.1	31.8	36	18	20	Fresh
Ma *et al.*, 2011	280	280	cTESE	Yes	Yes	110	32.9	NA	NA	15.1	6.3	21.2	12.9	NA	NA	NA	NA	NA
Ando *et al.*, 2012	52	52	mTESE	Yes	Yes	23	34	NA	NA	18.6	6.9	13.9	12.6	NA	NA	NA	NA	NA
Huang *et al.*, 2012	305	305	cTESE	No	No	137	29	NA	NA	13.7	6	10.9	10.9	NR	NR	NR	NR	Fresh
Nowroozi *et al.*, 2012	385	385	cTESE	Yes	Yes	196	33	NA	NA	21.7	12.7	15.4	14.8	NA	NA	NA	NA	NA
Ashraf *et al.*, 2013	14	14	mTESE	Yes	Yes	7	35	NA	7.1	19	6.9	12	11.9	30.6	7	2	NR	Fresh
Dadkhah *et al.*, 2013	741	741	cTESE	Yes	Yes	330	NR	NA	NA	NR	NR	NR	NR	NA	NA	NA	NA	NA
Freour *et al.*, 2013	40	40	mTESE	Yes	No	20	33.1	2.5	17.5	25.4	NR	14.4	5	NR	NR	NR	NR	Mixed
Karacan *et al.*, 2013	406	406	mTESE	Yes	Yes	223	37.9	NA	NA	8.5	NR	NR	NR	NR	209	57	52	Mixed
Modarresi *et al.*, 2013	150	150	Mixed	Yes	Yes	36	33.3	7.3	15.4	19.9	6.7	13.6	3.7	NA	NA	NA	NA	NA
Abdel Raheem *et al.*, 2013	276	276	Mixed	Yes	Yes	219	36	1.1	1.8	NR	NR	NR	NR	NA	NA	NA	NA	NA
Schwarzer *et al.*, 2013	220	220	Mixed	Yes	Yes	128	NR	NA	NA	NR	NR	NR	NR	NR	NR	NR	NR	CP
Arafa *et al.*, 2015	22	22	mTESE	NR	NR	2	33	NA	NA	9.2	6	15.8	15.1	NR	5	1	NR	NR
Arafa *et al.*, 2015[a]	97	97	mTESE	NR	NR	44	35	NA	NA	14.2	7.7	15.8	15.8	NR	44	9	NR	Mixed
Berookhim *et al.*, 2014	640	640	mTESE	No	Yes	285	34	4	13	25.2	NR	NR	8.3	31	NA	NA	NA	NA

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Bryson *et al.*, 2014	1127	1127	mTESE	Yes	Yes	631	35	3.9	11.6	31	NR	NR	9.1	30	NA	NA	NA	NA
Esteves *et al.*, 2014	365	365	mTESE	Yes	Yes	151	42	NA	2.2	16.7	7.9	14.1	14.3	32.7	151	42	30	Mixed
Karacan *et al.*, 2014	86	86	mTESE	Yes	Yes	45	32	NA	NA	NR	NR	NR	NR	31.9	NR	NR	NR	Mixed
Yildirim *et al.*, 2014	131	131	mTESE	Yes	Yes	69	37.7	NA	NA	20.6	NR	NR	NR	NR	NR	16	16	NR
Alrabeeah *et al.*, 2015	81	81	mTESE	Mixed	Yes	45	38	7.4	1.2	19	NR	12	11	32	45	21	NR	Fresh
Aydin *et al.*, 2015	111	111	mTESE	NR	Yes	65	31	NA	NA	16.4	10.2	10.4	NR	NR	65	29	NR	Fresh
Bernie *et al.*, 2015b	211	211	mTESE	Yes	Yes	110	36	5.7	0.47	22.3	NR	NR	10	36	NR	NR	NR	CP
Hessel *et al.*, 2015	582	582	cTESE	No	No	246	NR	NA	NA	NR	NR	NR	NR	NR	441	85	NR	NR
Kalsi *et al.*, 2015	58	58	mTESE	Yes	Yes	27	39	1.7	8.6	19.4	NR	13.1	NR	NA	NA	NA	NA	NA
Nowroozi *et al.*, 2015	74	74	Mixed	Yes	Yes	45	31.3	NA	NA	11.6	5.1	14.1	12.5	NA	NA	NA	NA	NA
Thornhill 2015	56	56	cTESE	Mixed	Yes	18	NR	NA	NA	NR	NR	NR	NR	NR	31	4	NR	CP
Vloeberghs *et al.*, 2015	714	714	Mixed	Yes	Yes	289	NR	NA	NA	NR	NR	NR	NR	31.4	437	129	111	Mixed
Alrabeeah *et al.*, 2016	16	16	mTESE	No	Yes	10	36	12.5	NA	17	NR	13	12.5	NR	16	NR	NR	NR
Cissen *et al.*, 2016	1371	1371	cTESE	Yes	Yes	599	34.3	4.59	6.19	22.1	9	14	12.5	NR	NR	NR	NR	CP
Güneri *et al.*, 2016[g]	125	125	cTESE	Mixed	Yes	50	33.2	12	1.6	16	7	NR	11.2	NA	NA	NA	NA	NA
Heydarian *et al.*, 2016	29	29	cTESE	Yes	No	12	34.1	NA	NA	12.2	7.3	14.3	NR	NA	NA	NA	NA	NA
Ko *et al.*, 2016	89	89	Mixed	Yes	Yes	40	NR	11.8	3.37	NR	NR	NR	NR	37.2	40	12	NR	Mixed
Saccà *et al.*, 2016	63	63	cTESE	No	Yes	30	37.3	NA	NA	17.8	6.5	15.5	NR	NA	NA	NA	NA	NR
Takeda *et al.*, 2017	144	144	mTESE	Yes	Yes	39	NR	NA	NA	26.7	6.5	15.6	9.1	NA	NA	NA	NA	NA
Alfano *et al.*, 2017	47	47	mTESE	Yes	Yes	23	38	NA	NA	18.3	7	12.7	10	NR	NR	NR	NR	CP

Table I Continued.

Study	No. pts	No. procedures	Surgical procedure	Bilateral approach	Multiple biopsy	SR	Mean age (years)	% pts with AZF deletions	% pts with KS	FSH (U/l)	LH (U/l)	Total T (nM)	Testis volume (ml)	Women age (years)	No. of ICSI cycles	CP	LBC	Sperm used for ICSI
Althakafi *et al*., 2017	421	421	mTESE	NR	NR	166	36.3	NA	3.09	17.5	10.5	11.6	9.5	NA	NA	NA	NA	NA
Binsaleh 2017	255	255	mTESE	Yes	Yes	112	35.8	NA	4.3	19.7	8.7	12.6	13	NA	NA	NA	NA	NA
Caroppo *et al*., 2017	356	356	cTESE	NR	Yes	158	36.8	NA	NA	19.6	NR	NR	7.9	NA	NA	NA	NA	NA
Chehrazi, *et al*., 2017	537	537	mTESE	Yes	Yes	119	34.1	NA	NA	22.6	8.8	14	NR	NA	NA	NA	NA	NA
Iwatsuki *et al*., 2017	172	172	mTESE	Yes	Yes	45	NR	NA	NA	27.3	6.9	15.4	10.2	NA	NA	NA	NA	NA
Huang *et al*., 2018	156	156	Mixed	Yes	NR	132	NR	NA	NA	25.4	11.6	11.6	17.5	NA	NA	NA	NA	NA
Salehi 2017	170	170	C/TESE/ mTESE	Yes	Yes	83	NR	NA	10	NR	8.9	NR	NR	NA	NA	NA	NA	NA
Eelaminejad *et al*., 2018	50	50	mTESE	Yes	Yes	22	31.9	NA	NA	12.4	6.3	15.4	12.5	NA	NA	NA	NA	NA

cTESE, conventional testicular sperm extraction; mTESE, microsurgical testicular sperm extraction; SR = sperm retrieved; CP = clinical pregnancies; LBC = live birth children; azoospermia factor (AZF) region; PR = pregnancy NR, not reported; NA, not applicable/available; CP, cryopreserved.

[a]Different series within the same work.

[b]Different series.

[c]Randomized controlled trial.

[d]Loupe magnification not otherwise specified

[e]Loupe magnification (×3.5);

[f]Loupe magnification (×10);

[g]Loupe magnification (×5).

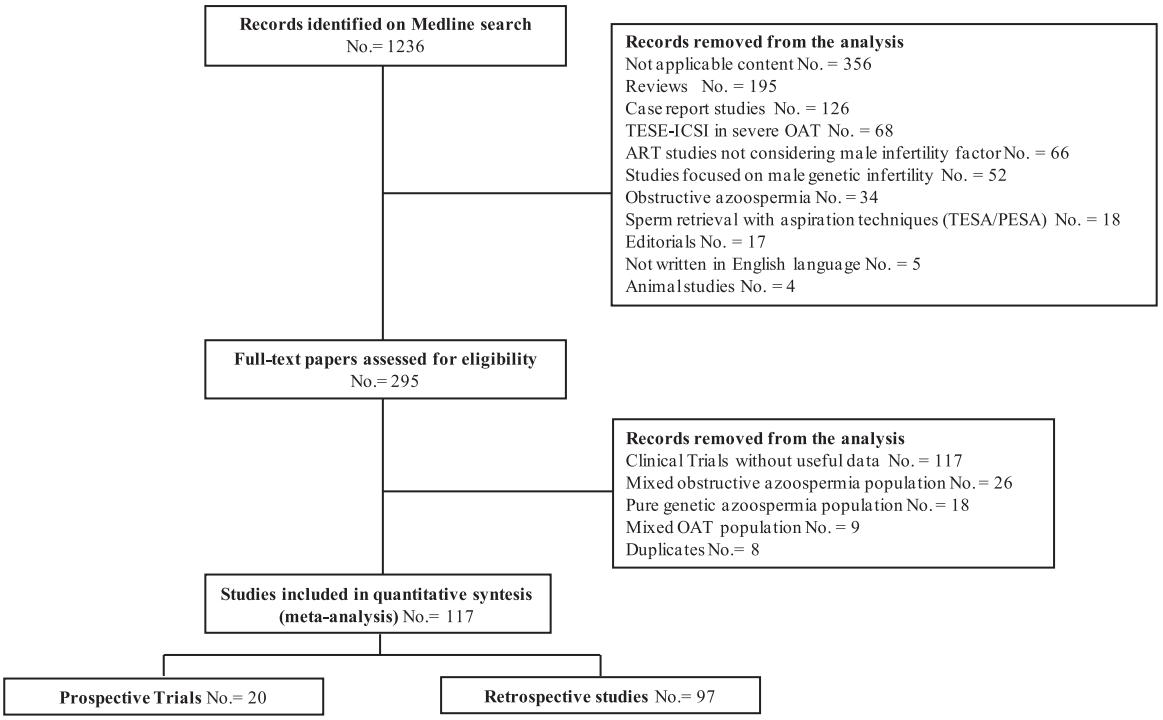

Figure 1 Trial flow diagram for a systematic review and meta-analysis of SRR in men with non-obstructive azoospermia. SRR: sperm retrieval rate, TESE = testicular sperm extraction TESA = testicular sperm aspiration; PESA = percutaneous epididymal sperm aspiration; OAT = oligoasthenoteratozoospermia.

Results

Sperm retrieval outcome

Out of 1236 retrieved articles, 117 were included in the study (Table I). Among them, only one RCT was available (Table I). The study flow is summarized in Fig. 1. cTESE and mTESE were used in 56 and 43 studies, respectively. In addition, 10 studies used a mixed approach and 8 studies compared cTESE with mTESE. Surgical approaches included a bilateral procedure in 85 and unilateral method in 12 studies (Table I). The latter information was not available in 16 cases, and in three studies a mixed approach was reported. Finally, one study, which compared cTESE with mTESE, reported only data (bilateral procedure) for cTESE but not for mTESE. In addition, multiple biopsies were performed in 94 cases whereas 11 studies used a single biopsy (Table I): information related to the number of biopsies performed was not available in 11 cases. Finally, one study compared single to multiple biopsies. The characteristics of the retrieved trials (including parameters on trial quality) are reported in Tables I and II. Retrieved trials included 21 404 patients with a mean (± SD) age of 35.0 ± 2.7 years. The inclusion of subjects with NOA due to genetic problems, including azoospermia factor (AZF) region Y-chromosome microdeletions and KS, were reported in 27 and 39 studies, respectively. Finally, 55 studies were performed in Europe, with 15 in North America, 3 in Southern America, 20 in Asia, 10 in Africa and 14 in the Arabian Peninsula or Iran.

The I^2 in trials assessing overall SRR per TESE cycle was 87.69 ($P < 0.0001$). Mean SRR per TESE cycle was 47[45;49]% (Fig. 2 and Supplementary Fig. S1). A funnel plot and Begg adjusted rank correlation test (Kendall's τ: 0.06; $P = 0.36$) suggested no publication bias. In addition, similar results were observed when mTESE was compared to cTESE (Fig. 2; $Q = 0.02$, $P = 0.88$). Similar results were observed when studies using cTESE along with loop magnification were excluded from the analysis ($Q = 0.06$, $P = 0.81$). No differences were observed when SRR per patient was considered (SRR of 46[44;48]%). Similar results were observed in a sensitivity analysis performed by excluding those studies enrolling subjects with genetic problems 47[44;50]% or by considering only high-quality studies 50[47;54]%. When the analysis was limited to only those studies directly comparing mTESE and cTESE, the former resulted in a significantly higher SRR of 57[47–59]% versus 39[25;45]%; $Q = 9.17$, $P = 0.002$. However, the results were not confirmed when the only randomized controlled trial (RCT) available was considered ($Q = 1.42$, $P = 0.23$).

Meta-regression analysis showed that SRR per cycle was independent of age and hormonal parameters at enrolment (Fig. 3A, B, C and D). However, the SRR increased as a function of testis volume (Fig. 3E).

In particular, by applying ROC curve analysis, we found that a mean volume higher than 12.5 ml predicted a SRR >60%, with an accuracy of 86.2 ± 0.01% ($P < 0.0001$) and a specificity and sensitivity of 73 and 74%, respectively (Supplementary Fig. S2). In addition, when only studies declaring the prevalence of patients with KS were considered ($n = 35$), SRR decreased a function of the number of KS cases included (Fig. 3F). The latter was confirmed even after adjusting for testis volume (adjusted $r = -0.024$; $P = 0.048$). Finally, no differ-

Table II Quality assessment of the clinical studies included in the meta-analysis.

Study	Selection bias	Study design	Data collection	Global rating
Fahmy et al. (1997)	Moderate	Retrospective Single-centre	Moderate	Low
Friedler et al. (1997)	Moderate	Retrospective Single-centre	Strong	Moderate
Mansour et al. (1997)	Moderate	Retrospective Single-centre	Strong	Low
Ezeh et al. (1998)	Weak	Prospective Single-centre	Strong	Strong
Rosenlund et al. (1998)	Moderate	Retrospective Single-centre	Low	Low
Amer et al. (1999)	Moderate	Retrospective Single-centre	Low	Moderate
Ben-Yosef et al. (1999)	Moderate	Retrospective Single-centre	moderate	Low
Ezeh et al. (1999)	Moderate	Retrospective Single-centre	Strong	Moderate
Palermo et al. (1999)	Moderate	Retrospective Single-centre	Low	Moderate
Schlegel (1999)	Moderate	Retrospective Single-centre	Strong	Moderate
Amer et al. (2000)	Weak	Prospective Multi-centre	Low	Moderate
Ballescà et al., 2000	Moderate	Retrospective Single-centre	Strong	Moderate
Mercan et al. (2000)	Moderate	Retrospective Single-centre	Moderate	Low
Amer et al. (2001)	Moderate	Retrospective Single-centre	Moderate	Low
Battaglia et al. (2001)	Weak	Prospective Single-centre	Strong	Moderate
Chan et al. (2001)	Moderate	Retrospective Single-centre	Moderate	Moderate
Eytan et al. (2001)	Weak	Prospective Single-centre	Low	Low
Kahrman et al. (2001)	Moderate	Retrospective Single-centre	Low	Moderate
Bohring et al. (2002)	Moderate	Retrospective Multi-centre	Low	Moderate
Chiang et al. (2002)	Moderate	Retrospective Single-centre	Strong	Moderate
Friedler et al. (2002)	Moderate	Retrospective Single-centre	Moderate	Moderate
Hauser et al. (2002)	Moderate	Retrospective Single-centre	Strong	Moderate
Mátyás et al. (2002)	Moderate	Retrospective Single-centre	Moderate	Low
Okada et al. (2002)	Moderate	Retrospective Single-centre	Strong	Moderate
Tsujimura et al. (2002)	Moderate	Retrospective Multi-center	Moderate	Moderate
Vernaeve et al. (2002)	Moderate	Retrospective Single-centre	Low	Moderate

Table II Continued.

Study	Selection bias	Study design	Data collection	Global rating
Aydos *et al.* (2003)	Weak	Prospective Single-centre	Strong	Moderate
Bailly *et al.* (2003)	Moderate	Retrospective Single-centre	Moderate	Moderate
Mansour *et al.* (2003)	Moderate	Retrospective Single-centre	Moderate	Low
Meseguer *et al.* (2003)	Moderate	Retrospective Single-centre	Moderate	Moderate
Samli *et al.* (2004)	Moderate	Retrospective Single-centre	Moderate	Low
Tsujimura *et al.* (2004a)	Moderate	Retrospective Multi-centre	Moderate	Moderate
Tsujimura *et al.* (2004b)	Moderate	Retrospective Multi-centre	Moderate	Moderate
Vernaeve *et al.*, (2004)	Moderate	Retrospective Single-centre	Moderate	Low
Aydos *et al.* (2005)	Weak	Prospective Single-centre	Strong	Moderate
Bettella *et al.* (2005)	Moderate	Retrospective Single-centre	Moderate	Strong
Giorgetti *et al.* (2005)	Weak	Prospective Single-centre	Strong	Low
Koscinski *et al.* (2005)	Moderate	Retrospective Single-centre	Moderate	Moderate
Mitchell *et al.* (2005)	Moderate	Retrospective Single-centre	Low	Low
Mulhall *et al.* (2005)	Moderate	Retrospective Single-centre	Moderate	Strong
Nagata *et al.* (2005)	Moderate	Retrospective Single-centre	Moderate	Moderate
Ramasamy *et al.* (2005)	Moderate	Retrospective Single-centre	Strong	Low
Wu *et al.* (2005)	Moderate	Retrospective Single-centre	Low	Low
Everaert *et al.* (2006)	Moderate	Retrospective Single-centre	Moderate	Low
Hauser *et al.* (2006)	Weak	Prospective Single-centre	Strong	Strong
Tsujimura *et al.* (2006)	Moderate	Retrospective Single-centre	moderate	Low
Tunc *et al.* (2006)	Moderate	Retrospective Single-centre	moderate	Moderate
Vernaeve *et al.* (2006)	Moderate	Retrospective Single-centre	moderate	Strong
Zitzmann *et al.* (2006)	Moderate	Retrospective Single-centre	moderate	Strong
El-Haggar *et al.* (2008)	Weak	Prospective Single-centre	Strong	Moderate
Hibi *et al.* (2007)	Moderate	Retrospective Single-centre	Low	Moderate
Mitchell *et al.* (2007)	Moderate	Retrospective Single-centre	Low	Low
Mostafa *et al.* (2007)	Moderate	Retrospective Multi-centre	Strong	Strong

Table II Continued.

Study	Selection bias	Study design	Data collection	Global rating
Amer et al. (2008)	Weak	Prospective Single-centre	Strong	Moderate
Houwen et al. (2008)	Moderate	Retrospective Single-centre	Moderate	Low
Kanto et al. (2008)	Moderate	Retrospective Single-centre	Low	Low
Madbouly et al. (2008)	Weak	Prospective Single-centre	Strong	Moderate
Ravizzini et al. (2008)	Moderate	Retrospective Single-centre	Moderate	Strong
Colpi et al. (2009)	Weak	Prospective RCT Single-centre	Strong	Low
Hallak et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Moderate
Haimov-Kochman et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Strong
Haraguchi et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Moderate
Inci et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Moderate
Ishikawa et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Low
Ramasamy et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Moderate
Wiser et al. (2009)	Moderate	Retrospective Single-centre	Moderate	Strong
Yarali et al. (2009)	Weak	Prospective Single-centre	Moderate	Low
Zohdy et al. (2009)	Weak	Prospective Single-centre	Moderate	Strong
Ishikawa et al. (2010)	Moderate	Retrospective Single-centre	Moderate	Low
Mitchell et al. (2010)	Weak	Prospective Single-centre	Moderate	Strong
Turunc et al. (2010)	Weak	Prospective Single-centre	Moderate	Strong
Boitrelle et al. (2011)	Moderate	Retrospective Single-centre	Moderate	Moderate
Cavallini et al. (2011)	Moderate	Retrospective Single-centre	Moderate	Moderate
Ghalayini et al. (2011)	Moderate	Retrospective Single-centre	Strong	Moderate
Hauser et al. (2011)	Moderate	Retrospective Single-centre	Moderate	Strong
Hsiao et al. (2011)	Moderate	Retrospective Single-centre	Moderate	Moderate
Ma et al. (2011)	Moderate	Retrospective Single-centre	Moderate	Moderate
Ando et al. (2012)	Moderate	Retrospective Single-centre	Moderate	Moderate
Huang et al. (2012)	Moderate	Retrospective Single-centre	Moderate	Strong
Nowroozi et al. (2012)	Weak	Prospective Single-centre	Moderate	Moderate

Table II Continued.

Study	Selection bias	Study design	Data collection	Global rating
Ashraf *et al.* (2013)	Weak	Prospective Single-centre	Strong	Strong
Dadkhah *et al.* (2013)	Moderate	Retrospective Single-centre	Low	Low
Freour *et al.* (2013)	Moderate	Retrospective Single-centre	Moderate	Strong
Karacan *et al.* (2013)	Moderate	Retrospective Single-centre	Moderate	Moderate
Modarresi *et al.* (2013)	Weak	Prospective Single-centre	Low	Strong
Abdel Raheem *et al.* (2013)	Moderate	Retrospective Single-centre	Moderate	Strong
Schwarzer *et al.* (2013)	Moderate	Retrospective Single-centre	Strong	Low
Arafa *et al.*, 2015)	Moderate	Retrospective Single-centre	Moderate	Moderate
Berookhim *et al.* (2014)	Moderate	Retrospective Single-centre	Strong	Strong
Bryson *et al.* (2014)	Moderate	Retrospective Single-centre	Moderate	Strong
Esteves *et al.* (2014)	Moderate	Retrospective Single-centre	Strong	Strong
Karacan *et al.* (2014)	Moderate	Retrospective Single-centre	Low	Moderate
Yildirim *et al.* (2014)	Moderate	Retrospective Single-centre	Moderate	Moderate
Alrabeeah *et al.* (2015)	Moderate	Retrospective Single-centre	Strong	Strong
Aydin *et al.* (2015)	Moderate	Retrospective Single-centre	Moderate	Moderate
Bernie *et al.* (2015b)	Moderate	Retrospective Single-centre	Moderate	Strong
Hessel *et al.* (2015)	Moderate	Retrospective Single-centre	Strong	Low
Kalsi *et al.* (2015)	Moderate	Retrospective Single-centre	Moderate	Strong
Nowroozi *et al.* (2015)	Moderate	Retrospective Single-centre	Strong	Moderate
Thornhill *et al.* (2015)	Moderate	Retrospective Single-centre	Strong	Low
Vloeberghs *et al.* (2015)	Moderate	Retrospective Single-centre	Moderate	Low
Alrabeeah *et al.* (2016)	Moderate	Retrospective Single-centre	Moderate	Moderate
Cissen *et al.* (2016)	Moderate	Retrospective Multi-centre	Strong	Strong
Güneri *et al.*, (2016)	Moderate	Retrospective Single-centre	Moderate	Strong
Heydarian *et al.* (2016)	Moderate	Retrospective Single-centre	Low	Moderate
Ko *et al.* (2016)	Moderate	Retrospective Single-centre	Moderate	Strong
Saccà *et al.* (2016)	Weak	Prospective Single-centre	Strong	Moderate

Table II Continued.

Study	Selection bias	Study design	Data collection	Global rating
Takeda et al. (2017)	Moderate	Retrospective Multi-centre	Moderate	Moderate
Alfano et al. (2017)	Moderate	Retrospective Multi-centre	Strong	Moderate
Althakafi et al. (2017)	Moderate	Retrospective Single-centre	Low	Moderate
Binsalehet al. (2017)	Moderate	Retrospective Single-centre	Moderate	Moderate
Caroppo et al. (2017)	Moderate	Retrospective Single-centre	Moderate	Moderate
Chehrazi, et al. (2017)	Moderate	Retrospective Single center	Moderate	Moderate
Iwatsuki et al. (2017)	Moderate	Retrospective Single center	Moderate	Moderate
Huang et al. (2018)	Moderate	Retrospective Single-centre	Moderate	Moderate
Salehi et al. (2017)	Moderate	Retrospective Single-centre	Moderate	Moderate
Eelaminejad et al. (2018)	Moderate	Retrospective Single-centre	Moderate	Moderate

The quality of trials was assessed using the Cochrane criteria (Higgins & Green 2008).
RCT = randomized controlled trial.
*different series

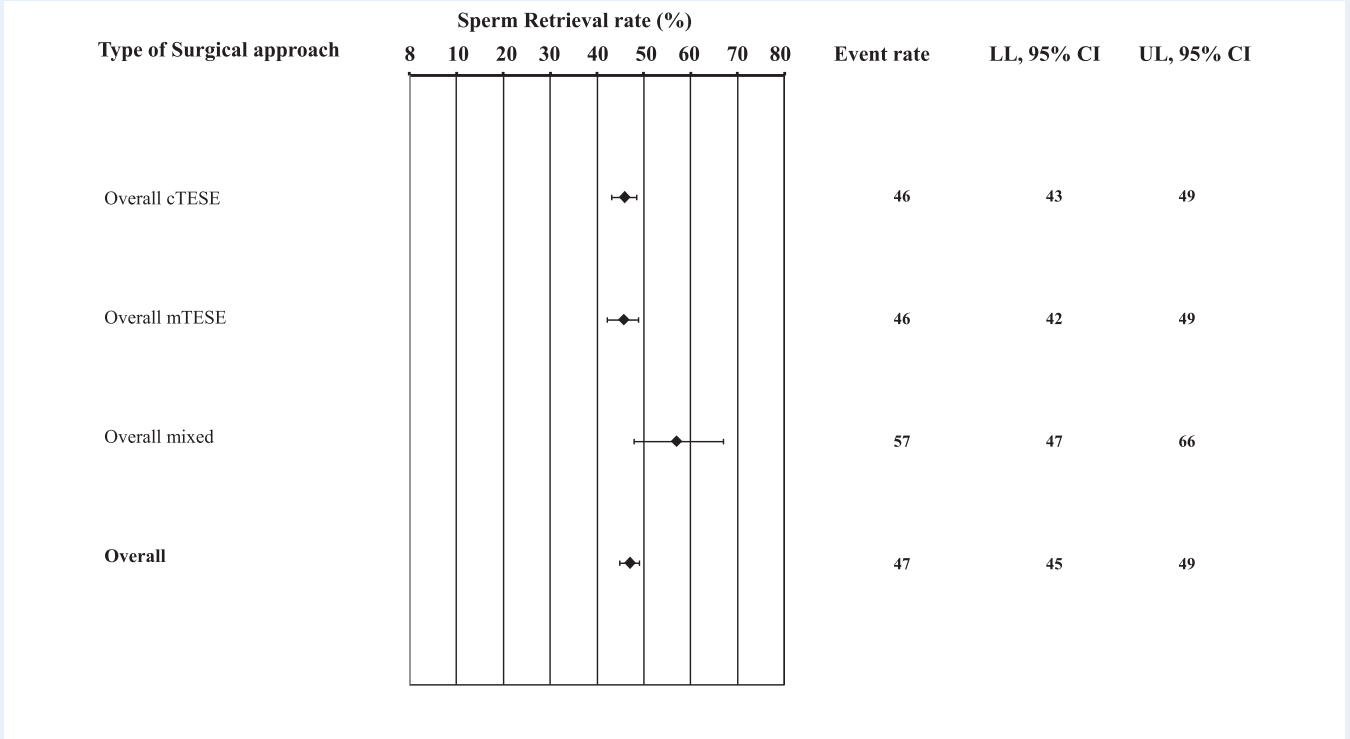

Figure 2 SRR per TESE cycle according to the type of surgical approach. cTESE = conventional TESE; mTESE = microsurgical TESE, LL: lower limit, UL: upper limit.

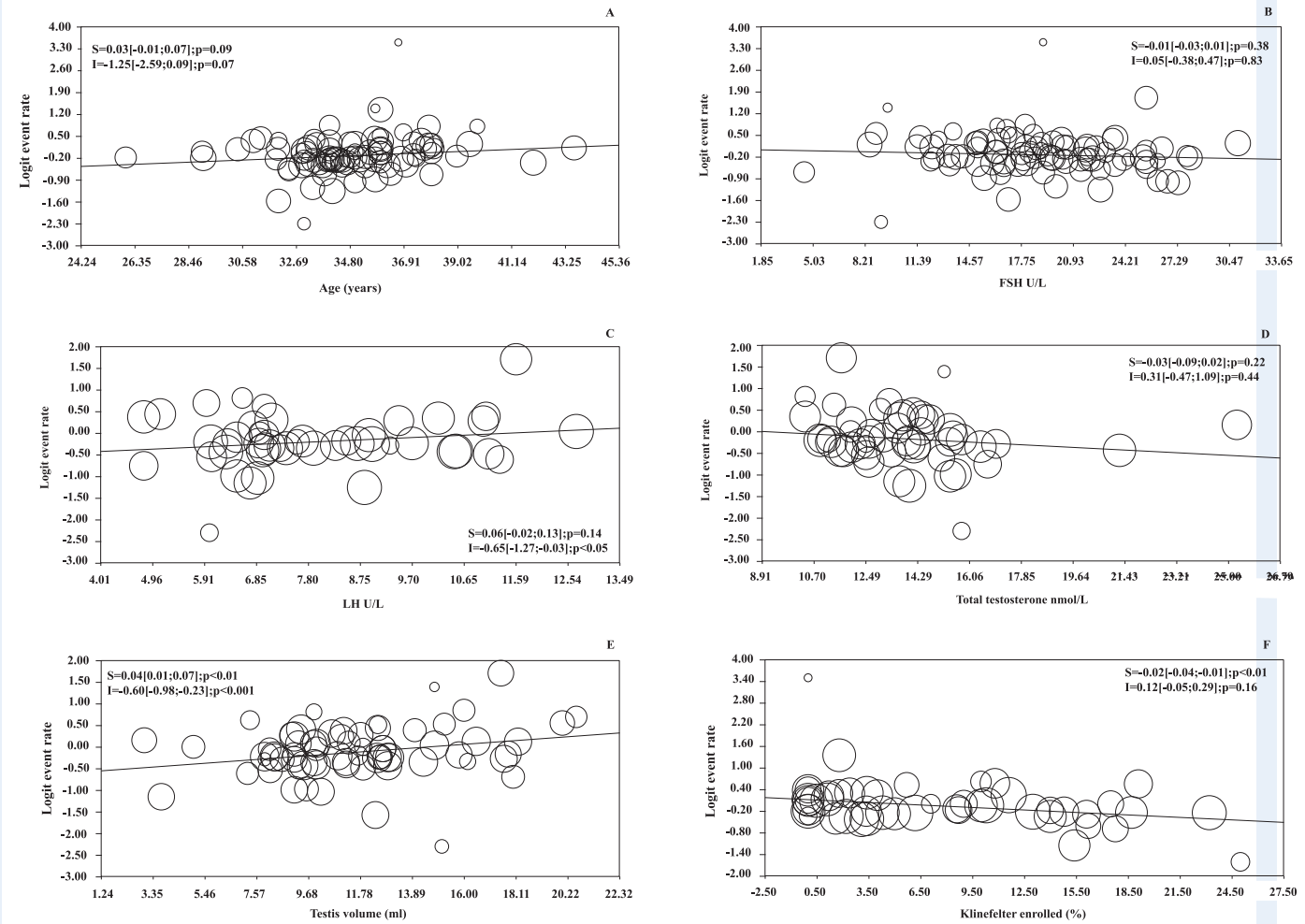

Figure 3 Influence of hormonal and other factors at enrolment on SRR. (**A**) age, (**B**) FSH, (**C**) LH, (**D**) total testosterone, (**E**) testis volume, (**F**) Klinefelter syndrome. The size of the circles indicates sample size.

ence in SRR was observed according to year of study publication or the number of subjects with AZF-c Y region microdeletions (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 48[45;50] versus 49[45;53]%, $Q = 0.21$, $P = 0.65$).

Finally, when the geographical area of the subjects was taken into account, SRR was not different between studies performed in Europe and North America (49[47;52] versus. 53[49;57]; $Q = 2.1$; $P = 0.15$); however, both SRRs were higher when compared to those in Asia or the Arabian peninsula (39[34;45]; 42[36;48]; all $< P < 0.05$). Insufficient data were available to compare other geographical areas. Similarly, insufficient data were available to evaluate the effect of previous infertility treatments before the surgical approach on SRR. Finally, due to an insufficient number of studies applying enzymatic, or a combination of mechanical and enzymatic, procedures for sperm isolation after surgical procedure, no comparison with the use of only the mechanical approach was possible.

Fertility outcome

Among the studies included in the SRR analysis, information on fertility outcome after ICSI was available for 42 trials (Table I). In these trials, the mean (± SD) age of the female was 31.8 ± 2.7 years. In addition, the ICSI procedure was performed either with cryopreserved or fresh sperm in eight and 14 trials, respectively (Table I). Sixteen studies applied a mixed approach using both cryopreserved and fresh sperm whereas this information was not available in four cases (Table I). I^2 in trials assessing overall PR was 78.39 ($P < 0.001$). Overall, a total of 1096 biochemical pregnancies were observed (cumulative PR = 29[25;32]% per ICSI cycle; Fig. 4 and Supplementary Fig. S3A). A funnel plot and Begg adjusted rank correlation test (Kendall's τ: −0.09; $P = 0.40$) suggested the absence of publication bias. Similar results were observed when LBR per ICSI cycle was analyzed: 569 live births (cumulative LBR = 24[20;28]%per ICSI cycle; Fig. 4, Supplementary Fig. S3B). Similar to observations for SRR, there was no influence of male age, mean testis volume and hormonal parameters on both PR and LBR per ICSI cycle (not shown). Similarly, no influence of female age on both PR and LBR was observed (Supplementary Fig. S4).

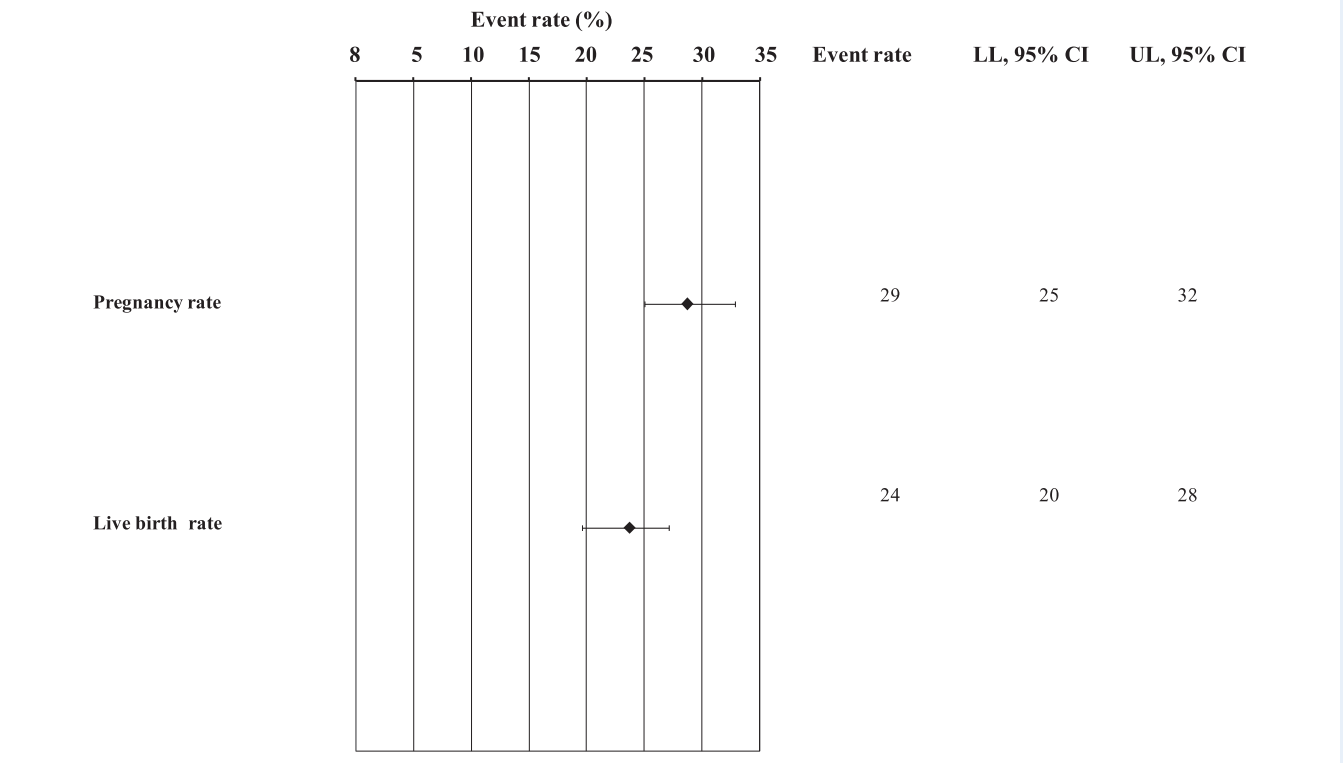

Figure 4 **Pregnancy rate and live birth rate per ICSI cycle.**

When sensitivity analysis was performed according to the type of sperm used for ICSI procedure, a higher PR per ICSI cycle was observed when fresh sperm was compared to cryopreserved sperm (PR = 35[30;40]%, versus 20[13;29]%, respectively; $Q = 7.85$; $P = 0.005$). However, this result was not confirmed when cumulative LBR per ICSI cycle was analyzed (LBR = 30[20;41]% versus 20[12;31]%, respectively; $Q = 1.90$, $P = 0.17$).

Finally, when cumulative LBR was calculated according to the number of biochemical pregnancies obtained, an abortion rate of (19[14;25]%) was detected.

Discussion

In this study, we conducted a systematic review and meta-analysis, for the first time, of all available information regarding SRR and fertility outcomes in subjects with NOA. Our results show an overall successful SRR of about 47%, with no differences when cTESE was compared to mTESE. Testis volume is the only significant predictive factor of successful SRR, among several clinical and biochemical parameters investigated. In particular, a mean testis volume greater than 12 ml predicts successful SRR >60% with an accuracy of 86%. In addition, after ICSI performed using the retrieved sperms, a LBR of up to 28% was achieved, leading to a final cumulative LBR per ICSI cycle of about 10% for the couples who initiated ART.

The absence of differences in final successful SRR when cTESE was compared to mTESE warrants further discussion. All available trials performing a direct comparison between cTESE and mTESE reported better outcomes with the latter technique. This observation was confirmed here using a meta-analytic approach. However, the better outcome with mTESE was not confirmed when the only RCT comparing the two techniques was considered. In addition, it is important to recognize that among the trials which directly compared cTESE and mTESE (Schlegel *et al.*, 1999; Amer *et al.*, 2000; Okada *et al.*, 2002; Tsujimura *et al.*, 2002; Ramasamy *et al.*, 2005; Colpi *et al.*, 2009; Ghalayini *et al.*, 2011; Salehi *et al.*, 2017), only one (Colpi *et al.*, 2009) was a RCT. Conversely, the majority of the studies comparing the two technique outcomes were not RCTs. It is well known that non-RCTs suffer from several methodological problems (Loke *et al.*, 2011). In particular, residual confounding factors may be a source of selection bias due to the non-random assignment. Accordingly, physicians might prefer to select the larger testis for mTESE. In addition, data derived from observational studies present other important limitations, including inadequate or incomplete information regarding clinical and biochemical parameters of the subjects participating in the studies. Accordingly, this information was present only in a minority of studies evaluated in the Bernie *et al.* (2015a) meta-analysis, suggesting the superiority of mTESE. By comparing the largest number of studies published so far, our results did not confirm the superiority of mTESE in comparison to cTESE in successful sperm retrieval in subjects with NOA.

The identification of specific prognostic clinical or biochemical parameters may contribute to reducing the costs of the surgical procedures. Our results show that the successful SRR increases as a function of testis volume. In particular, a mean testis volume higher than 12 ml leads to a successful SRR greater than 60% with an accuracy of higher than 80%. However, the possibility to retrieve sperm is still

present even in patients with a testis volume lower than 8 ml. Hence, the presence of a reduced testis volume should not be considered as a crucial limitation for advocating TESE in patients with NOA. The inverse relationship between successful SRR and testis volume is not surprising since spermatogenesis and Sertoli cells account for more than 80% of the total testis volume. The specific mechanisms underlying impairment of spermatogenesis in subjects with NOA are still not completely understood. NOA represents a heterogeneous condition in which both congenital and acquired factors mutually interact in impairing sperm production. The working hypothesis is that the final damage is usually not homogenous, allowing the preservation of tubules with normal residual activity (Okada *et al*., 2002; Flannigan *et al*., 2017). The latter possibility has been documented in subjects with KS (Franik *et al*., 2016; Geis *et al*., 2016), as well as in other forms of NOA, such as Sertoli cell-only syndrome (Silber *et al*., 1995) and NOA occurring post-cryptorchidism or post-chemotherapy (Silber *et al*., 1996). A recent meta-analysis of 21 studies reporting on histopathological findings suggests that testis volume has limited predictive value in SRR when only mTESE technique is considered (Li *et al*., 2018). Our results performed in a larger number of studies did not confirm this hypothesis.

Genetic background might profoundly influence the SRR in patients with NOA. Accordingly, it has been reported that subjects with KS present with progressive hyalinization of seminiferous tubules, preventing recovery of spermatozoa (Forti *et al*. 2010). In line with this hypothesis, our study shows that successful SRR decreased as a function of the number of KS subjects included in the population of NOA. Interestingly, however, a recent meta-analysis, including all available studies evaluating SRR in patients with KS, reported a similar overall successful SRR to that observed in the present study including all subjects with NOA. No specific study directly comparing successful SRR in patients with KS and in subjects with NOA without genetic problems is available. However, it has been reported that testis fibrosis, which is characteristic in KS testes after puberty, is not ubiquitous and it is possible to observe tubules with normal residual activity (Franik *et al*., 2016; Geis *et al*., 2016). This observation can explain, at least partially, the similar results for successful SRR observed in KS and in patients with NOA overall. On the other hand, the lower male age of the subjects included in the meta-analysis performed on KS (30.9 years) versus that reported in this study (35.0 years) can be considered a possible confounding factor in comparing the two studies.

Besides testis volume, other factors including age, the type of technique used for sperm separation after surgery and hormone pattern have been advocated as possible prognostic indicators for successful sperm retrieval in NOA (Ramasamy *et al*., 2011; Ishikawa *et al*., 2012). Aging is a clear factor that might impact on spermatogenetic function (Grunewald *et al*., 2013). A previous meta-analysis performed in only 11 studies, including 1350 patients, showed that age might influence the predictive value of FSH in SRR (Yang *et al*., 2015). Our data, performed in a 10-times higher number of studies, did not confirm these results. In fact, age did not represent a limiting factor for undergoing TESE in NOA. However, it should be recognized that only a limited number of studies were performed in patients aged younger than 30 years or older than 40 years.

The most frequently used method for obtaining sperm from testicular tissue after surgery is by the mechanical approach, performed by mincing and shredding the whole tissue obtained (Schlegel *et al*., 1997). However, enzymatic digestion using DNAse and collagenase has also been proposed by others (Crabbe *et al*., 1997). In addition, it has also been reported that the use of the enzymatic approach might improve sperm retrieval in subjects where no spermatozoa were detected after the mechanical approach (Ramasamy *et al*., 2011). The present results seem not to confirm this hypothesis, since no difference in successful SRR was observed when the mechanical approach was compared to a mixed mechanical–enzymatic sperm separation. However, it is important to recognize that only a limited number of studies applied the combination approach. In addition, embryologists' experience has a significant effect, which was not evaluated in the present study.

Serum FSH has been proposed to predict positive SRR after cTESE (Ishikawa *et al*., 2012); however, these results were not confirmed by other authors (Jezek *et al*., 1998; Ezeh *et al*., 1999). Silber *et al*. (1996) reported that serum FSH levels inversely correlated with the number of germ cells in the testis but not with more advanced stages of spermatogenesis. Our data are in line with this finding since meta-regression analysis documented that FSH did not predict SRR. Similar results were reported by Li *et al*. (2018) in a meta-analysis of studies using only mTESE, and the same study documented that FSH had a better predictive value in patients from East Asia. We also report that SRR was lower in studies performed in men from East Asia and the Arabian Peninsula when compared to Europe and North America. It is therefore possible to speculate that ethnicity may influence SRR in NOA. Otherwise, differences in surgical facilities and techniques could be considered as another factor for explaining this difference.

Another strength of this study is that we conducted a meta-analysis, for the first time, of fertility outcomes after ICSI derived from patients with NOA. The results of the present meta-analysis show that live births could be obtained in about 10% of subjects who underwent the TESE approach. Interestingly, the LBR data in the present study are lower than recently reported when only NOA linked to KS (16%) was considered (Corona *et al*., 2017). However, it is important to recognize that the mean female age in the present meta-analysis is almost 3 years higher than that reported in the meta-analysis of Corona *et al*. (2017), when only KS was considered. Although no comparative study is available, the female age factor can explain the lower LBR and higher miscarriage rate observed in the present study. In addition, similar to what was observed for successful SRR, and in line with what has been reported in KS (Corona *et al*., 2017), no clinical and biochemical factors influenced the final pregnancy outcome. Finally, although the use of fresh sperm was associated with a higher PR, this was not the case when LBR was considered. The latter finding is not surprising and in line with what has been reported in RCTs from oligo-astheno-teratospermic men (Kuczynski *et al*., 2001) or when data from OA has been considered (Nicopoullos *et al*., 2004).

Several limitations of our study should be acknowledged. First of all, it should be recognized that heterogeneity exists in men with NOA. This can be partially explained by differences in surgical techniques, such as time spent during mTESE, experience of the embryologist and time spent by the embryologist looking for sperm. In particular, the overall skill level of the embryology laboratory, including quality control, experience of embryologist, type of microscope used, time dedicated by the embryologist for sperm detection and time dedicated by the surgeon for dilated seminiferous tubule identification

for sperm identification, represents a crucial point. Second, only limited information was available regarding causative factors for NOA. Depending on specific causes, SRR can be widely different. Meta-analyses deal with the synthetic reports of average results obtained in each study, without access to patient-level data. For this reason, some of the original data in each study are lost in meta-analyses. Moreover, we cannot exclude that some selection bias derived from retrospective studies is included in this meta-analysis. On the other hand, meta-analyses can improve the statistical power to identify differences and might reduce the risk of missing a true effect, but they cannot allow correction for any bias within the individual studies or consider the effects of confounding factors. Hence, great caution is required in the interpretation of results, which should be confirmed in large-scale observational studies. It has been reported that the use of clomiphene citrate, hCG and hMG administration, leading to an increased level of FSH and total testosterone, might improve SRR in patients with NOA (Hussein *et al*., 2013). Due to the limited available information, the present study cannot better clarify this issue.

In conclusion, the present data show that in men with NOA, a positive SRR can be obtained in almost 50% of cases independent of the surgical approach applied. Testicular volume is the only parameter that can predict a higher SRR. It has been reported that mTESE may be associated with a reduction in short- and long-term complications when compared to cTESE with respect to the endocrine and exocrine function of the testis (Okada *et al*., 2002; Flannigan *et al*., 2017). In particular, a lower rate of haematoma and testicular fibrosis, a decreased testicular volume (>2 ml) and a decrease in serum testosterone levels have been reported following mTESE when compared to cTESE (Deruyver *et al*., 2014). However, available data seem to suggest minimal clinical impact of these differences, often not reaching statistical significance between groups (Deruyver *et al*., 2014). The latter point remains crucial, when considering the comparable results in terms of SRR between mTESE and cTESE, as suggested in the present meta-analysis, as the technique with the lower incidence of adverse events should be preferred. The information on adverse events was available only in a limited number of studies, preventing adequate statistical analysis. Well-designed RCTs which are sufficiently powered, including short- and long-term complications as secondary measures, should be conducted to determine if mTESE is superior to cTESE in men with NOA.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

All the authors adequately contributed to the analysis of the paper and reviewed the final vision before the submission. Giovanni Corona: study conception and design, acquisition, analysis and interpretation of data, drafting the article, critical revision of the article and final approval. Suks Minhas: acquisition of data, critical revision of the article and final approval. Aleksander Giwercman: acquisition of data, critical revision of the article and final approval. Carlo Bettocchi: acquisition of data and final approval. Marij Dinkelman-Smit: acquisition of data and final approval. Gert Dohle: acquisition of data and final approval. Ferdinando Fusco: acquisition of data and final approval. Ates Kadioglou: acquisition of data and final approval. Sabine Kliesch: acquisition of data, critical revision of the article and final approval. Zsolt Kopa: acquisition of data and final approval. Csilla Krausz: critical revision of the article and final approval. Fiore Pelliccione: acquisition and interpretation of data, drafting and critical revision of the article and final approval. Alessandro Pizzocaro: acquisition and interpretation of data, drafting and critical revision of the article and final approval. Jens Rassweiler: acquisition of data and final approval. Paolo Verze: acquisition of data and final approval. Linda Vignozzi: critical revision of the article and final approval. Wolfgang Weidner: critical revision of the article and final approval. Mario Maggi: study conception and design, analysis and interpretation of data, critical revision of the article and final approval. Nikolaos Sofikitis: study conception and design, acquisition of data, critical revision of the article and final approval.

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Conflict of interest

The authors declare that they have no conflict of interest.

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