

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

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Submitted on January 11, 2019; resubmitted on July 18, 2019; editorial decision on August 2, 2019

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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 ( $\pm$  SD) of  $35.0 \pm 2.7$  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<sub>1</sub>curve analysis, a mean testis volume higher than 12.5 ml predicted SRR >60% with an accuracy of  $86.2\% \pm 0.01$ . 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 revo-

lutionized 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, Schlegel 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](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 SRR 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 1** 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 <sup>a</sup>    | 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 <sup>d</sup> | 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 <sup>a</sup>        | 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/<br>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 1 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 |
|---|---------|----------------|--------------------|--------------------|-----------------|-----|------------------|--------------------------|---------------|-----------|----------|--------------|--------------------|-------------------|--------------------|----|-----|---------------------|
| Bohning <i>et al.</i> , 2002                | 33      | 33             | cTESE              | NR                 | Yes             | 22  | NR               | NA                       | NA            | 16.9      | 5.9      | 13.3         | 20.6               | NR                | NR                 | NR | NR  | CP                  |
| Chiang <i>et al.</i> , 2002                 | 47      | 37             | cTESE              | NR                 | NR              | 5   | NR               | 2.7                      | NA            | NR        | NR       | NR           | NR                 | NA                | NA                 | NA | NA  | NA                  |
| Friedler <i>et al.</i> , 2002               | 83      | 83             | cTESE              | Yes                | Yes             | 32  | 33.7             | NA                       | NA            | 23.5      | NR       | 11.7         | NR                 | 30                | 55                 | 11 | 8   | Mixed               |
| Hauser <i>et al.</i> , 2002                 | 65      | 65             | cTESE              | Yes                | Yes             | 35  | NR               | NA                       | NA            | 18        | 6.8      | 25.3         | 3                  | NR                | NR                 | NR | NR  | Mixed               |
| Mátyás <i>et al.</i> , 2002                 | 75      | 75             | cTESE              | Yes                | Yes             | 52  | 37.9             | NA                       | NA            | NR        | NR       | NR           | NR                 | 30                | NR                 | NR | NR  | Fresh               |
| Okada <i>et al.</i> , 2002                  | 24      | 24             | cTESE              | Yes                | Yes             | 4   | NR               | NA                       | 25            | NR        | NR       | NR           | NR                 | NA                | NA                 | NA | NA  | NA                  |
| Okada <i>et al.</i> , 2002 <sup>a</sup>     | 74      | 74             | mTESE              | NR                 | Yes             | 33  | NR               | NA                       | 14.8          | NR        | NR       | NR           | NR                 | NA                | NA                 | NA | NA  | NA                  |
| Tsujimura <i>et al.</i> , 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 <i>et al.</i> , 2002 <sup>a</sup> | 56      | 56             | mTESE              | Yes                | Yes             | 24  | 33.9             | NA                       | 16.1          | 24        | 8.3      | 17           | 8.6                | NA                | NA                 | NA | NA  | NA                  |
| Vermaeve <i>et al.</i> , 2002               | 185     | 185            | cTESE              | Yes                | Yes             | 92  | 35.6             | NA                       | NA            | NR        | NR       | NR           | NR                 | NA                | NA                 | NA | NA  | NA                  |
| Aydos <i>et al.</i> , 2003                  | 45      | 45             | mTESE              | Yes                | Yes             | 15  | NR               | NA                       | 17.8          | 4.5       | NR       | NR           | 18                 | NA                | NA                 | NA | NA  | NA                  |
| Aydos <i>et al.</i> , 2003 <sup>a</sup>     | 63      | 63             | mTESE              | Yes                | Yes             | 40  | NR               | NA                       | 19.1          | 8.9       | NR       | NR           | 20                 | NA                | NA                 | NA | NA  | NA                  |
| Bailly <i>et al.</i> , 2003                 | 75      | 75             | cTESE              | Yes                | Yes             | 26  | NR               | NA                       | NA            | 21.4      | NR       | NR           | NR                 | 31.8              | 60                 | 11 | NR  | CP                  |
| Mansour <i>et al.</i> , 2003                | 452     | 488            | cTESE              | Yes                | Yes             | 274 | 39.5             | NA                       | NA            | NR        | NR       | NR           | NR                 | 32.1              | 274                | 79 | NR  | Fresh               |
| Mesguier <i>et al.</i> , 2003               | 12      | 12             | cTESE              | Yes                | Yes             | 5   | 34               | NA                       | NA            | 26.1      | NR       | NR           | 16.1               | NR                | 8                  | 1  | 1   | CP                  |
| Samli <i>et al.</i> , 2004                  | 303     | 303            | cTESE              | NR                 | NR              | 107 | NR               | NA                       | NA            | NR        | NR       | NR           | NR                 | NA                | NA                 | NA | NA  | NR                  |
| Tsujimura <i>et al.</i> , 2004 <sup>a</sup> | 60      | 60             | mTESE              | Yes                | Yes             | 22  | 32.4             | 10                       | NA            | 25.4      | 6        | 12.5         | 8.1                | NA                | NA                 | NA | NA  | NR                  |
| Tsujimura <i>et al.</i> , 2004 <sup>b</sup> | 100     | 100            | mTESE              | NR                 | Yes             | 41  | 33.6             | NA                       | NA            | 25.4      | 7        | 12           | 9.9                | NA                | NA                 | NA | NA  | NR                  |
| Vermaeve <i>et al.</i> , 2004               | 79      | 79             | cTESE              | Yes                | Yes             | 41  | 34               | NA                       | NA            | 26.4      | NR       | 13.6         | 9.3                | 31.1              | 64                 | 11 | 15  | Mixed               |
| Aydos <i>et al.</i> , 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 1 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 <sup>ae</sup>  | 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 <sup>a</sup>  | 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 <sup>a</sup> | 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 <sup>f</sup>      | 52      | 52             | cTESE              | Yes                | Yes             | 20  | 34.5             | NA                       | NA            | 13.4      | NR       | NR           | 9.3                | NA                | NA                 | NA | NA  | NA                  |
| Vernaev 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-Hagggar 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 1 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 <sup>c</sup>  | 195     | 69             | cTESE              | Yes                | Yes             | 29  | 36               | NA                       | NA            | NR        | NR       | NR           | NR                 | NR                | NR                 | NR | NR  | Fresh               |
| Colpi et al., 2009 <sup>sc</sup> | 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 1 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*    | 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                       | NA            | 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*       | 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 <i>et al.</i> , 2014              | 1127    | 1127           | mTESE              | Yes                | Yes             | 631 | 35               | 3.9                      | 11.6          | 31        | NR       | NR           | 9.1                | 30                | NA                 | NA  | NA  | NA                  |
| Esteves <i>et al.</i> , 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 <i>et al.</i> , 2014             | 86      | 86             | mTESE              | Yes                | Yes             | 45  | 32               | NA                       | NA            | NR        | NR       | NR           | NR                 | 31.9              | NR                 | NR  | NR  | Mixed               |
| Yildirim <i>et al.</i> , 2014            | 131     | 131            | mTESE              | Yes                | Yes             | 69  | 37.7             | NA                       | NA            | 20.6      | NR       | NR           | NR                 | NR                | NR                 | 16  | 16  | NR                  |
| Alrabeeah <i>et al.</i> , 2015           | 81      | 81             | mTESE              | Mixed              | Yes             | 45  | 38               | 7.4                      | 1.2           | 19        | NR       | 12           | 11                 | 32                | 45                 | 21  | NR  | Fresh               |
| Aydin <i>et al.</i> , 2015               | 111     | 111            | mTESE              | NR                 | Yes             | 65  | 31               | NA                       | NA            | 16.4      | 10.2     | 10.4         | NR                 | NR                | 65                 | 29  | NR  | Fresh               |
| Bernie <i>et al.</i> , 2015b             | 211     | 211            | mTESE              | Yes                | Yes             | 110 | 36               | 5.7                      | 0.47          | 22.3      | NR       | NR           | 10                 | 36                | NR                 | NR  | NR  | CP                  |
| Hessel <i>et al.</i> , 2015              | 582     | 582            | cTESE              | No                 | No              | 246 | NR               | NA                       | NA            | NR        | NR       | NR           | NR                 | NR                | 441                | 85  | NR  | NR                  |
| Kalsi <i>et al.</i> , 2015               | 58      | 58             | mTESE              | Yes                | Yes             | 27  | 39               | 1.7                      | 8.6           | 19.4      | NR       | 13.1         | NR                 | NA                | NA                 | NA  | NA  | NA                  |
| Nowroozi <i>et al.</i> , 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 <i>et al.</i> , 2015          | 714     | 714            | Mixed              | Yes                | Yes             | 289 | NR               | NA                       | NA            | NR        | NR       | NR           | NR                 | 31.4              | 437                | 129 | 111 | Mixed               |
| Alrabeeah <i>et al.</i> , 2016           | 16      | 16             | mTESE              | No                 | Yes             | 10  | 36               | 12.5                     | NA            | 17        | NR       | 13           | 12.5               | NR                | 16                 | NR  | NR  | NR                  |
| Cissen <i>et al.</i> , 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 <i>et al.</i> , 2016 <sup>§</sup> | 125     | 125            | cTESE              | Mixed              | Yes             | 50  | 33.2             | 12                       | 1.6           | 16        | 7        | NR           | 11.2               | NA                | NA                 | NA  | NA  | NA                  |
| Heydarian <i>et al.</i> , 2016           | 29      | 29             | cTESE              | Yes                | No              | 12  | 34.1             | NA                       | NA            | 12.2      | 7.3      | 14.3         | NR                 | NA                | NA                 | NA  | NA  | NA                  |
| Ko <i>et al.</i> , 2016                  | 89      | 89             | Mixed              | Yes                | Yes             | 40  | NR               | 11.8                     | 3.37          | NR        | NR       | NR           | NR                 | 37.2              | 40                 | 12  | NR  | Mixed               |
| Saccà <i>et al.</i> , 2016               | 63      | 63             | cTESE              | No                 | Yes             | 30  | 37.3             | NA                       | NA            | 17.8      | 6.5      | 15.5         | NR                 | NA                | NA                 | NA  | NA  | NR                  |
| Takeda <i>et al.</i> , 2017              | 144     | 144            | mTESE              | Yes                | Yes             | 39  | NR               | NA                       | NA            | 26.7      | 6.5      | 15.6         | 9.1                | NA                | NA                 | NA  | NA  | NA                  |
| Alfano <i>et al.</i> , 2017              | 47      | 47             | mTESE              | Yes                | Yes             | 23  | 38               | NA                       | NA            | 18.3      | 7        | 12.7         | 10                 | NR                | NR                 | NR  | NR  | CP                  |

Table 1 Continued.

| Study                    | No. pts | No. procedures | Surgical procedure | Bilateral approach | Multiple biopsies | 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                  |
| Chehrizi, 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/<br>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.

<sup>a</sup>Different series within the same work.

<sup>b</sup>Different series.

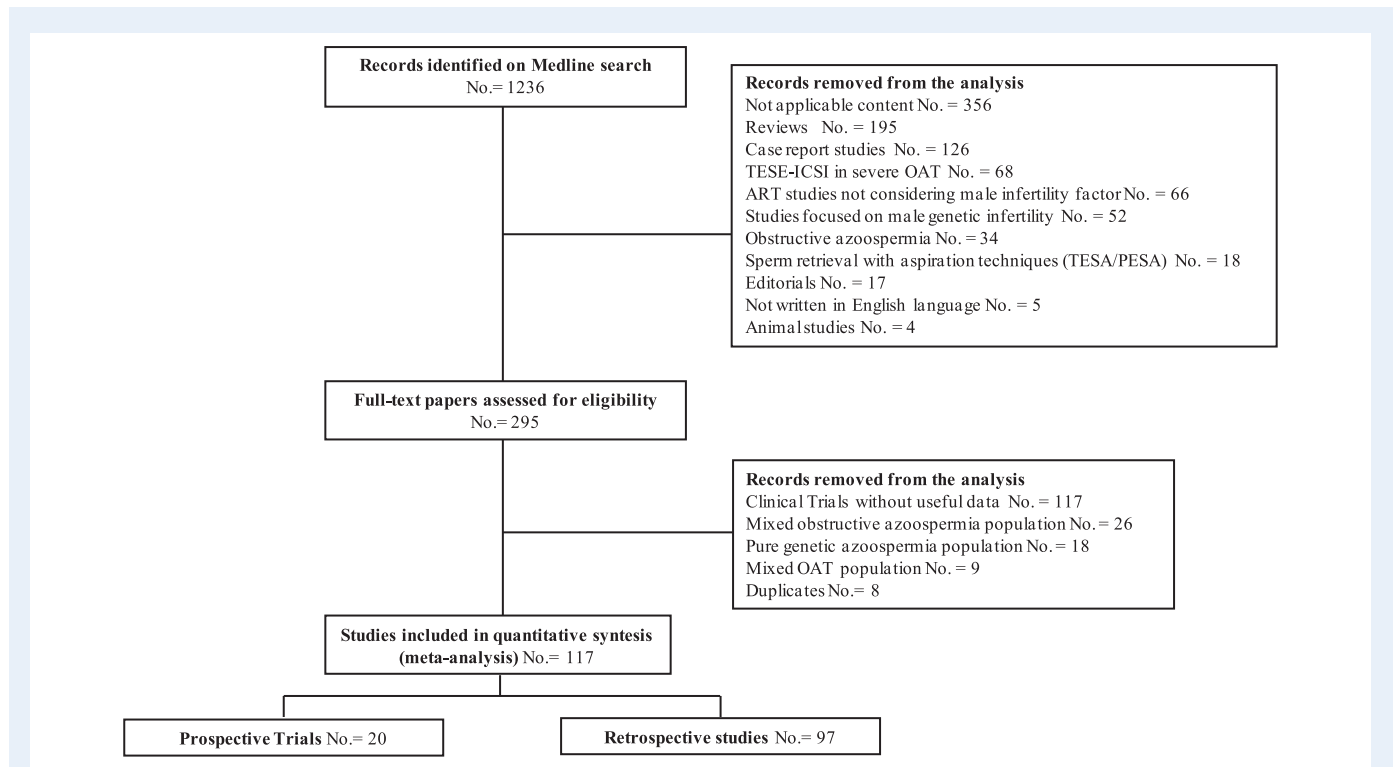
<sup>c</sup>Randomized controlled trial.

<sup>d</sup>Loupe magnification not otherwise specified

<sup>e</sup>Loupe magnification ( $\times 3.5$ );

<sup>f</sup>Loupe magnification ( $\times 10$ );

<sup>g</sup>Loupe magnification ( $\times 5$ ).



**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 ( $\pm$  SD) age of  $35.0 \pm 2.7$  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  $\tau$ : 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  $86.2 \pm 0.01\%$  ( $P < 0.0001$ ) 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<br>Single-centre | Moderate        | Low           |
| Friedler et al. (1997)  | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Mansour et al. (1997)   | Moderate       | Retrospective<br>Single-centre | Strong          | Low           |
| Ezeh et al. (1998)      | Weak           | Prospective<br>Single-centre   | Strong          | Strong        |
| Rosenlund et al. (1998) | Moderate       | Retrospective<br>Single-centre | Low             | Low           |
| Amer et al. (1999)      | Moderate       | Retrospective<br>Single-centre | Low             | Moderate      |
| Ben-Yosef et al. (1999) | Moderate       | Retrospective<br>Single-centre | moderate        | Low           |
| Ezeh et al. (1999)      | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Palermo et al. (1999)   | Moderate       | Retrospective<br>Single-centre | Low             | Moderate      |
| Schlegel (1999)         | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Amer et al. (2000)      | Weak           | Prospective<br>Multi-centre    | Low             | Moderate      |
| Ballescà et al., 2000   | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Mercan et al. (2000)    | Moderate       | Retrospective<br>Single-centre | Moderate        | Low           |
| Amer et al. (2001)      | Moderate       | Retrospective<br>Single-centre | Moderate        | Low           |
| Battaglia et al. (2001) | Weak           | Prospective<br>Single-centre   | Strong          | Moderate      |
| Chan et al. (2001)      | Moderate       | Retrospective<br>Single-centre | Moderate        | Moderate      |
| Eytan et al. (2001)     | Weak           | Prospective<br>Single-centre   | Low             | Low           |
| Kahrman et al. (2001)   | Moderate       | Retrospective<br>Single-centre | Low             | Moderate      |
| Bohring et al. (2002)   | Moderate       | Retrospective<br>Multi-centre  | Low             | Moderate      |
| Chiang et al. (2002)    | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Friedler et al. (2002)  | Moderate       | Retrospective<br>Single-centre | Moderate        | Moderate      |
| Hauser et al. (2002)    | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Mátyás et al. (2002)    | Moderate       | Retrospective<br>Single-centre | Moderate        | Low           |
| Okada et al. (2002)     | Moderate       | Retrospective<br>Single-centre | Strong          | Moderate      |
| Tsujimura et al. (2002) | Moderate       | Retrospective<br>Multi-center  | Moderate        | Moderate      |
| Vernaev et al. (2002)   | Moderate       | Retrospective<br>Single-centre | Low             | Moderate      |

**Table II Continued.**

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

**Table II Continued.**

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

**Table II Continued.**

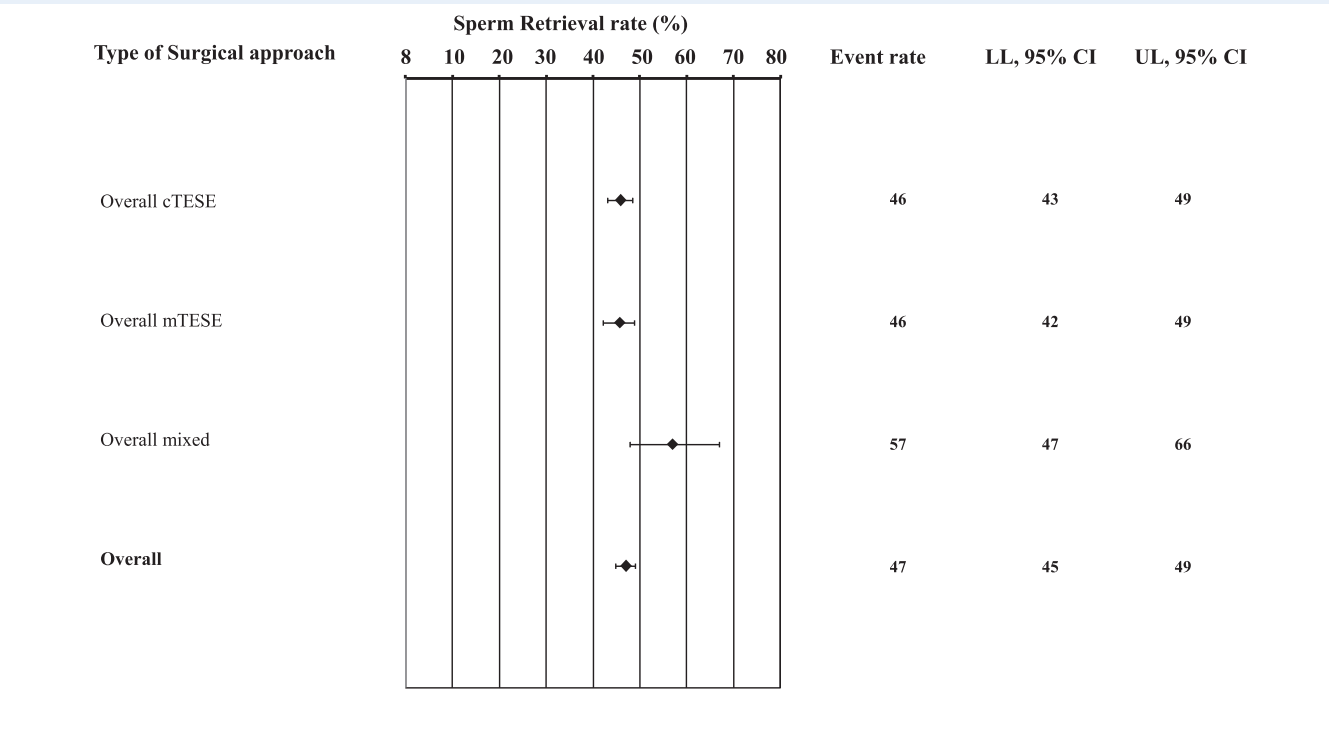
| Study                             | Selection bias | Study design                   | Data collection | Global rating |
|-----------------------------------|----------------|--------------------------------|-----------------|---------------|
| <i>Ashraf et al. (2013)</i>       | Weak           | Prospective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



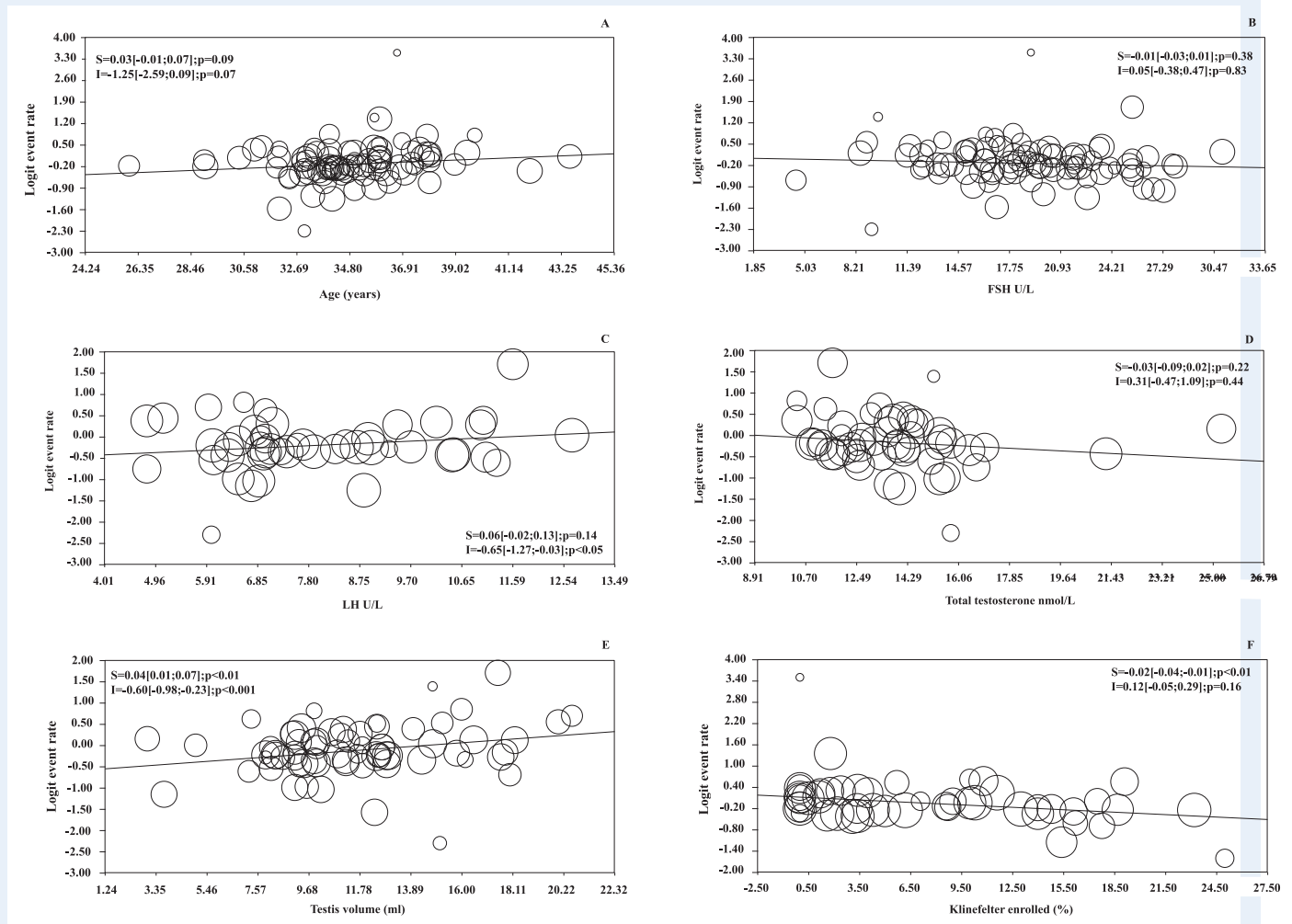
**Table II Continued.**

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

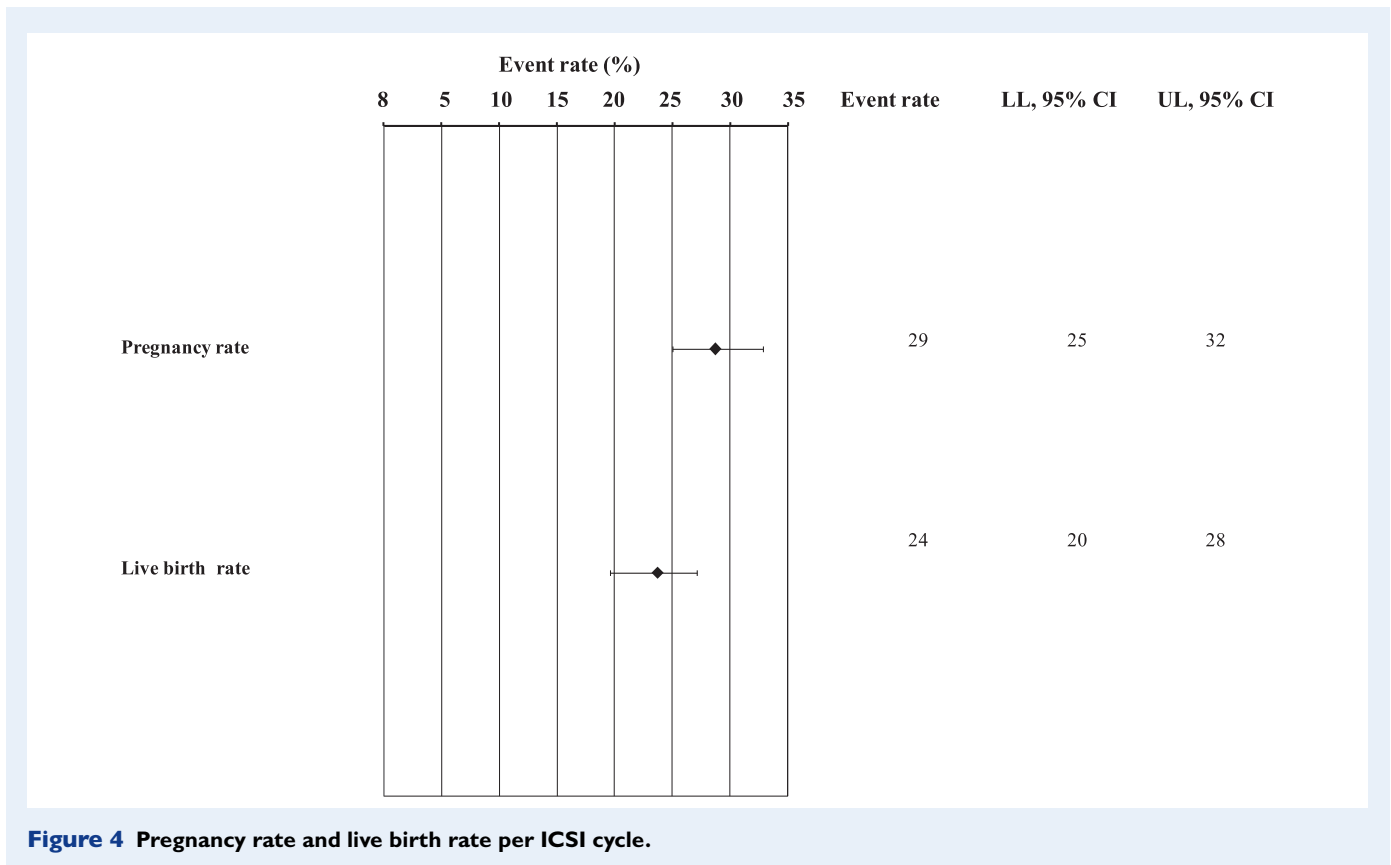
ence in SRR was observed according to year of study publication or the number of subjects with AZF-c Y region microdeletions (not shown).

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 ( $\pm$  SD) age of the female was  $31.8 \pm 2.7$  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  $\tau$ :  $-0.09$ ;  $P = 0.40$ ) 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 observa-

tion 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; Ezech *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 (Nicopoulos *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 Kadioglu: 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.

## Funding

This research project did not receive any funding.

## Conflict of interest

The authors declare that they have no conflict of interest.

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