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LETTER TO THE EDITOR

Reply: Sperm retrieval rates by micro-TESE versus conventional TESE in men with non-obstructive azoospermia—the assumption of independence in effects sizes might lead to misleading conclusions

Sir,

We thank Esteves and co-authors (2020) for their constructive criticisms of our meta-analysis. They essentially disagree with our conclusions saying that the available evidence did not confirm the superiority of microdissection testicular sperm extraction (micro-TESE) vs. conventional TESE (cTESE) in the achievement of positive sperm retrieval rate (SRR) in patients with non-obstructive azoospermia (NOA). The main problems that Esteves *et al.* (2020) emphasized are the high heterogeneity and the inclusion of all studies with and without direct comparison. In addition, other methodological problems were identified.

First, we want to clarify that observational studies include case-series, case-control, cross-sectional and longitudinal studies where the independent variable is not under the control of the researcher (Higgins & Green, 2008; Guyatt *et al.*, 2011). In particular, it is important to clarify, for the readers of *Human Reproduction Update*, that a specific type of observational study deals with the evaluation of possible effects of a treatment, or of an approach, where the assignment of subjects into a specific group versus a control one is outside the control of the investigator (Higgins & Green, 2008, Guyatt *et al.*, 2011). As already reported in our meta-analysis (Corona *et al.*, 2019), all available studies comparing mTESE vs. cTESE belong to the latter category of the observational trials (Schlegel *et al.*, 1997; Amer *et al.*, 2000; Okada *et al.*, 2002; Tsujimura *et al.*, 2002; Ramasamy *et al.*, 2005; Ghalayini *et al.*, 2011; Salehi *et al.*, 2017) except one, which is a randomised-controlled trial (RCT, Colpi *et al.*, 2009). All types of observational trials, including comparison trials used by Bernie *et al.* (2015) in their meta-analysis, by Deruyver *et al.* (2014) in their systematic review and even in our meta-analysis, present important limitations. The limitations include failure to develop and apply appropriate eligibility criteria, flawed measurement of both exposure and outcome, failure to adequately control confounding factors and incomplete follow-up (Guyatt *et al.*, 2011). For example, due to the lack of blinding assignment, physicians might be more likely to use mTESE in healthier individuals with more favourable clinical characteristics, leading to a higher probability of reaching positive SRR. Accordingly, to better clarify the topic and try to reduce the latter important source of bias, we decided to include in our meta-analysis all observational studies using cTESE or mTESE for SRR as correctly specified in the method section of our study Corona *et al.*, 2019 and in PROSPERO registration (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=92017).

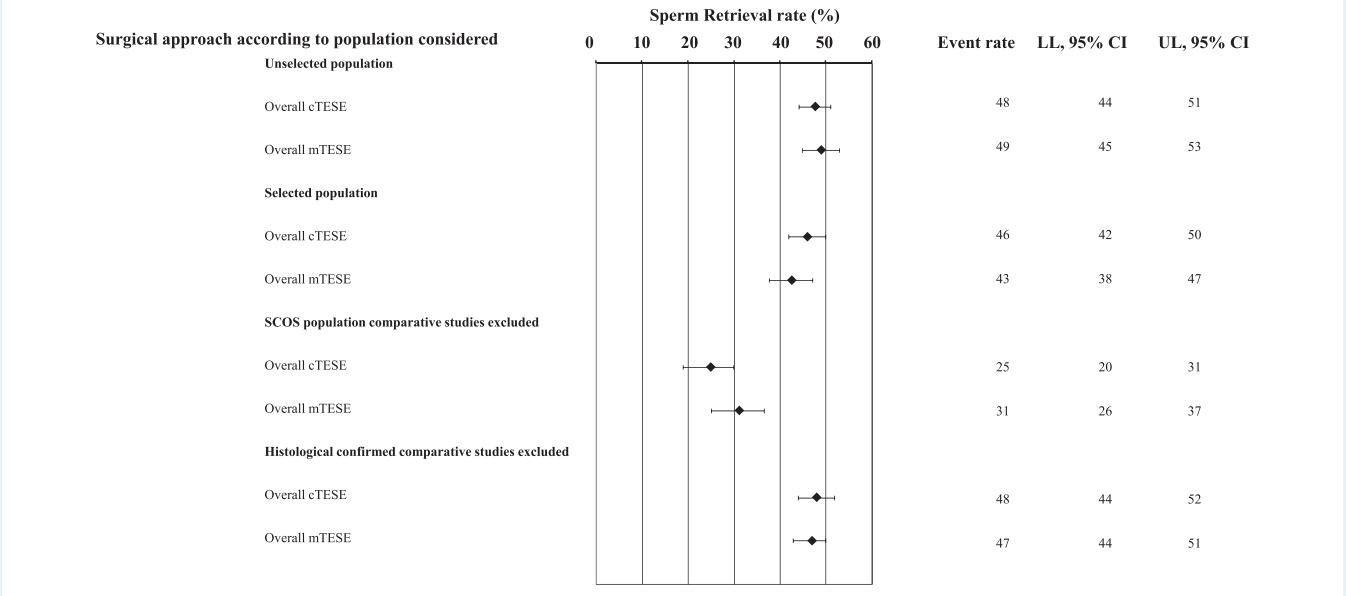

Figure 1 Sperm retrieval rate (SRR) per testicular sperm extraction (TESE) cycle according to the type of surgical approach in different populations. cTESE = conventional TESE; mTESE = microsurgical-TESE. SCOS = Sertoli cell only syndrome; UP = lower limit; UL = upper limit.

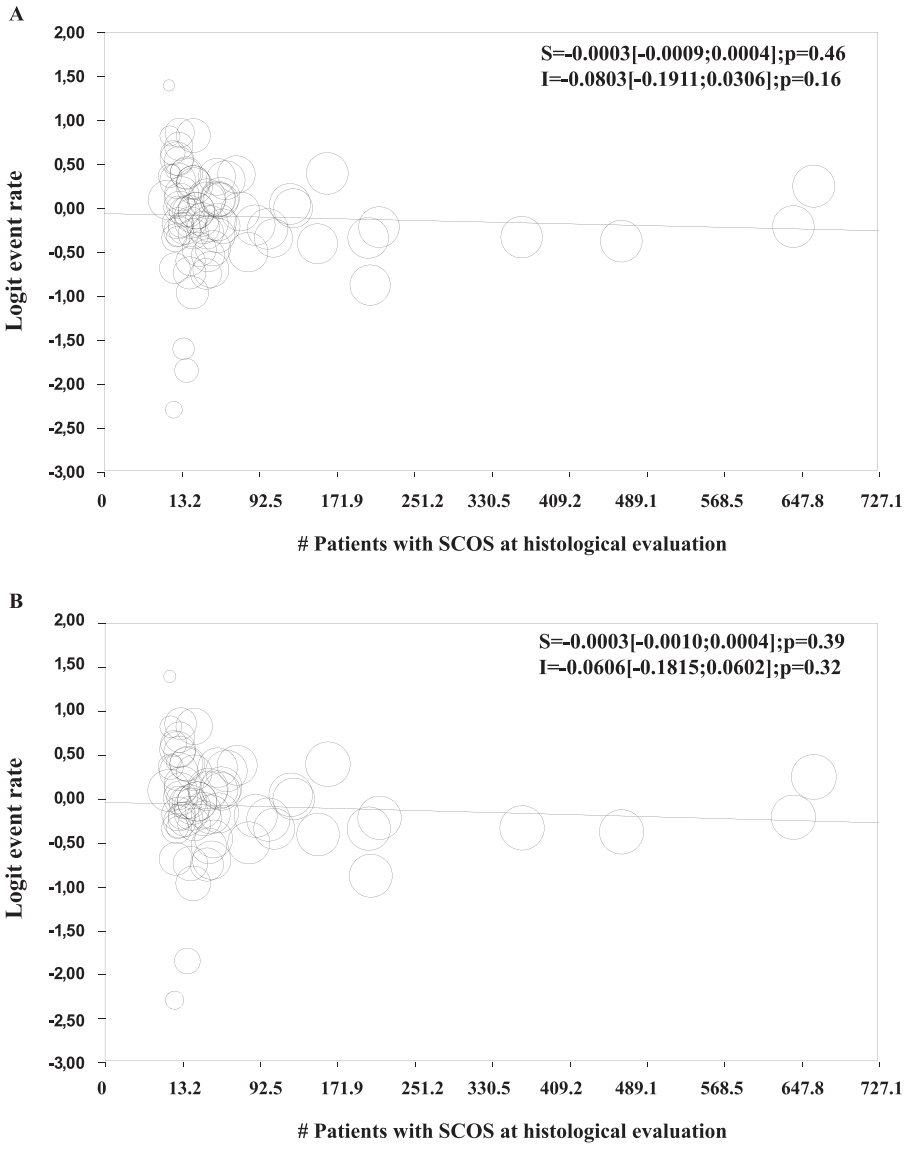

Figure 2 Influence of the number of patients with Sertoli cell only syndrome (SCOS) in the whole sample. (**A**) and when comparative studies were excluded (**B**). Data refer to studies reporting histological evaluation for the whole sample. The size of the circles indicates sample dimension.

Regarding the methodological problems emphasized by Esteves *et al.* (2020), we want to clarify that in order to reduce the risk of problems related to data imputing, all data were blindly reviewed by four of the authors (G.C., A.P., C.B. and N.S.) already involved in our work and by one (W.V.) not previously involved in the analysis. Some differences, when compared to the Esteves *et al.* (2020) analysis, were detected (see Supplementary Table S1).

In addition, the following points need to be better elucidated.

Firstly, Esteves *et al.* (2020) pointed out the problem related to a substantial evidence of bias observed in our meta-analysis since up to 43% of included mTESE studies involved selected patient populations with an overall unfavourable prognosis, and by contrast, only 7.5% of the cTESE studies included the so-called 'unfavourable' patients, thus possibly over-estimating the SRR. In response to Esteves *et al.*'s (2020) indication, we performed a sensitivity analysis by comparing SRR in cTESE and mTESE in selected and unselected studies (see Supplementary Table S1). Similar to what was observed in our overall analysis, we did not observe any statistical differences between the two techniques (SRR = 48[44;51]% vs. 49[45;53]%, $Q = 0.44$, $P = 0.51$ and 46[42;50]% vs.43[38;47]%, $Q = 1.14$; $P = 0.28$ for cTESE and mTESE in unselected and selected studies, respectively; see also Fig. 1).

Secondly, Esteves *et al.* (2014), in their original study, found that mTESE was able to detect higher SRR when compared to cTESE when patients with Sertoli only syndrome (SCOS) or tubular atrophy were considered. Hence, they reassessed the SRR in non-comparative studies, included in our meta-analysis, showing that, when the analysis was limited to patients with SCOS, mTESE resulted in a significantly higher SRR (Esteves *et al.*, 2020). First of all, we wonder how it is

possible to support one technique for a surgical approach taking into account an outcome obtained only after a histological diagnosis, hence at the end of the procedure. In addition, it is important to recognize that SCOS is a specific histological diagnosis of testis specimens which can result from different pathological conditions either congenital (pure or primitive) or acquired (partial or secondary) (Anniballo *et al*., 2000; Dohle *et al*., 2012). In the pure and complete form, the detection of sperm is theoretically impossible whereas in secondary or partial SCOS it may sometimes be possible. Hence, the correct testis histological classification represents an essential and crucial point. However, several classification systems have been described (Anniballo *et al*., 2000; Dohle *et al*., 2012). Although Johnsen score represents the most widely quoted and quantitative histological grading system, the use of different classifications has resulted in a wide variation of diagnosis by different pathologists, limiting the diagnostic and prognostic value of the testicular biopsy (Anniballo *et al*., 2000; Dohle *et al*., 2012). This is the reason why we decided not to use the histological classification as a possible confounder in our meta-analysis (Corona *et al*., 2019). In fact, it is our opinion that it adds only a further source of bias. However, in order to address Esteves *et al*.'s (2020) request, we carefully collected all the studies reporting data on SCOS (Supplementary Table S1). In addition, it is important to emphasize that several studies reported histological classification only for a limited number of patients and not for the whole population studied (see Supplementary Table S1). This represents a further source of bias. By considering only studies which provided a histological classification for all patients evaluated and by considering only SOCS diagnosis as reported by the original authors (Supplementary Table S1), we were unable to detect any prognostic value of SCOS when a meta-regression analysis was performed (Fig. 2A). Similar findings were observed when only non-comparative studies were considered (Fig. 2B). The latter result confirms our hypothesis regarding the limitations related to the use of different histological testis classifications (Anniballo *et al*., 2000; Dohle *et al*., 2012). In line with this finding, by applying the aforementioned criteria, when only non-comparative studies were considered, we were not able to find any difference when cTESE was compared to mTESE (SRR = 25[20;31] vs. 31[26;37], $Q = 2.28$; $P = 0.13$, for cTESE and mTESE, respectively; see also Fig. 1).

Thirdly, Esteves *et al*. (2020) documented that when the analysis was limited only to non-comparative studies providing confirmatory diagnostic histopathology details, mTESE resulted in significantly higher SRR when compared to cTESE. Similar to what was reported for SCOS diagnosis, when the analysis was limited to only studies reporting histological data in the whole sample and only non-comparative studies were considered, we did not find any difference when cTESE was compared to mTESE (SRR = 48[44;52] vs. 47[44;51], $Q = 0.12$; $P = 0.73$ for cTESE and mTESE, respectively; see also Fig. 1).

As correctly indicated by Esteves *et al*. (2020) and already recognized in our study limitations (Corona *et al*., 2019), high heterogeneity was detected. An iterative analysis was performed by excluding outliers in order to reduce the heterogeneity (<50%) of the study (Higgins & Green, 2008). By performing a sensitivity analysis with a study heterogeneity <50% ($I^2 = 49.2$), we confirmed the absence of difference between cTESE and mTESE in SRR (SRR = 46[44;48] vs. 47[45;49], $Q = 0.83$; $P = 0.36$ for cTESE and mTESE, respectively).

In conclusion, even after the new analysis of the data, according to Esteves *et al*.'s (2020) suggestions, we here confirm that available evidence cannot support the superiority of mTESE vs. cTESE in SRR for patients with NOA. We agree with Esteves *et al*. (2020) that further high-quality RCTs are strongly advisable to better clarify this important topic.

Supplementary data

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

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