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REVIEW

Q1

Testicular versus ejaculated spermatozoa for ICSI in patients without azoospermia: A systematic review

Q2 Q3

BIOGRAPHY

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Q4 Q5

KEY MESSAGE

Currently, there is limited, low-quality evidence suggesting that a higher probability of pregnancy might be expected with the use of testicular rather than ejaculated spermatozoa, only in men with high DNA fragmentation index and oligozoospermia.

ABSTRACT

The use of testicular spermatozoa in men without azoospermia has been proposed as a means to increase the chances of pregnancy following assisted reproductive treatment. The purpose of this systematic review is to assess whether clinical outcomes are better when testicular rather than ejaculated spermatozoa are used for intracytoplasmic sperm injection in patients with abnormal semen parameters without azoospermia. A literature search identified four eligible studies out of 757 initially found. In a prospective study in men with high DNA fragmentation index (DFI) and oligozoospermia, the probability of live birth was significantly higher with testicular compared to ejaculated spermatozoa (risk ratio [RR]: 1.75, 95% confidence interval [CI]: 1.14–2.70). This was not the case in a retrospective study in men with high DFI only (RR: 2.36, 95% CI: 0.98–5.68). Clinical pregnancy rates were similar in a randomized controlled trial in men with asthenozoospermia with or without teratozoospermia (RR: 2.85, 95% CI: 0.76–10.69) and in a retrospective study in men with isolated asthenozoospermia (RR: 1.09, 95% CI: 0.56–2.14). Currently, there is limited, low-quality evidence suggesting that a higher probability of pregnancy might be expected using testicular rather than ejaculated spermatozoa, only in men with high DFI and oligozoospermia.

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KEYWORDS

Asthenozoospermia
Cryptozoospermia
Dna fragmentation index
Intracytoplasmic sperm injection
Testicular sperm extraction

Q7

INTRODUCTION

Since 1993, intracytoplasmic sperm injection (ICSI) has been the treatment of choice in men with severe male factor infertility, provided that spermatozoa can be identified (*Palermo et al., 1992*) either in the ejaculate or after testicular sperm extraction (TESE) (*Devroey et al., 1995*) or fine needle aspiration (FNA) of the testis (*Cui et al., 2016; Ketabchi, 2016*). The use of testicular spermatozoa is compulsory when no spermatozoa are present in the ejaculate and the couple does not opt for semen donation. In addition, its use has been proposed in patients with high DNA fragmentation index (DFI) (*Arafa et al., 2017; Esteves et al., 2015; Greco et al., 2005; Pabuccu et al., 2016*), asthenozoospermia (*Al-Malki et al., 2017; Kahraman et al., 1996*), teratozoospermia (*Lu et al., 2012; Tsai et al., 2011; Verza and Esteves, 2008; Xu et al., 2009; Yang et al., 2017*) and cryptozoospermia (*Bendikson et al., 2008; Cui et al., 2016*). However, considering the potential complications and burden that TESE is associated with, its beneficial role in the above patients needs to be scrutinized.

The use of testicular as compared to ejaculated spermatozoa in patients with abnormal semen parameters without azoospermia is based on the premise that spermatozoa may suffer oxidative stress and nuclear DNA damage during transit through the male genital tract. This may lead to low ejaculated sperm quality and poor ICSI outcomes (*Greco et al., 2005; Popal and Nagy, 2013*). Moreover, in cases of cryptozoospermia, the repeated centrifugations required may increase the production of reactive oxygen species, which may adversely affect sperm quality by decreasing sperm motility, fertilizing capacity and eliminating the antioxidants that are naturally present in seminal plasma (*Matas et al., 2011; Swanton et al., 2007*).

Two recent meta-analyses compared testicular versus ejaculated spermatozoa for ICSI in patients with high DFI (Esteves et al., 2017) and in patients with cryptozoospermia (*Abhyankar et al., 2016*). However, both meta-analyses are characterized by several limitations, among which are the inclusion of case crossover studies as well as of studies that analysed both fresh and frozen testicular sperm samples. Moreover, an unclear definition of cryptozoospermia was used in the meta-analysis by *Abhyankar et al., (2016)*, limiting the generalizability of the conclusions drawn. Since the publication of these meta-analyses, additional studies have been published in different patient populations such as cryptozoospermia (*Cui et al., 2016*), asthenozoospermia (*Al-Malki et al., 2017*) and high DFI (*Arafa et al., 2017*), offering new relevant information on this topic that could be used to readdress the research question.

The aim of this systematic review was to address whether the use of testicular spermatozoa should be preferred over that of ejaculated spermatozoa for ICSI in patients with abnormal semen parameters without azoospermia by answering the following research question: Is the probability of pregnancy higher when testicular as compared to ejaculated spermatozoa is used for ICSI in patients with abnormal semen parameters without azoospermia?

MATERIALS AND METHODS

Literature search

A computerized literature search was performed independently by three reviewers (HA, JB, EK) in MEDLINE, Embase, CENTRAL and randomized controlled trials (RCT) registries, covering the period from 1995 to 2017, aiming to identify all available studies evaluating the following research question: Is the probability of pregnancy higher when testicular as compared to ejaculated spermatozoa is used for ICSI in patients with abnormal semen parameters without azoospermia?

For this purpose, the free text search terms ('ICSI', 'microinjection', 'intracytoplasmic sperm injection', 'testicular', 'micro-TESE', 'TESA', 'TESE', 'cryptozoospermia', 'cryptospermia', 'DNA fragmentation index', 'DFI', 'NOT azoospermia') were used. Additionally, the citation lists of all relevant publications and review articles were hand-searched. Meeting proceedings of the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine were also hand-searched to identify relevant studies. No language limitations were applied.

Institutional Board review was not obtained because previously published data were used. The systematic review was registered in the PROSPERO international prospective register of systematic reviews (42,017,059,008).

Study selection and outcome measures

RCT and prospective or retrospective non-randomized observational studies were included if they evaluated ICSI outcomes using fresh spermatozoa, either ejaculated or surgically extracted in patients with abnormal semen parameters without azoospermia undergoing ICSI. Case reports, case crossover studies or studies in which frozen spermatozoa of any origin was used were excluded. Studies comparing ejaculated spermatozoa from a group of patients with abnormal semen parameters without azoospermia to testicular spermatozoa from patients with azoospermia were excluded. Case crossover studies were excluded, because they cannot offer useful information in reproductive medicine regarding the probability of pregnancy (Daya, 1993; *Khan et al., 1996*). Studies in which spermatozoa were extracted by testicular sperm aspiration (TESA) or TESE or both were considered eligible; however, studies in which spermatozoa were extracted by PESA or MESA were not.

Clinical infertility trials should ideally deal with women and not cycles, and in these trials women should only be included once (e.g. one cycle per woman) in the analysis to avoid unit-of-analysis error. This may occur if women are included more than once and analysis is not modified appropriately or if analysis is performed on data per cycle instead of per woman. In these cases, the *P*-values are usually inflated, leading to erroneous conclusions. When such studies were identified, the corresponding authors were contacted to obtain the original data and if authors did not respond the study was excluded.

The main outcome measure in the current systematic review was the achievement of pregnancy per patient expressed as either clinical pregnancy (evidence of intrauterine sac with fetal heart activity at 6–8 weeks of gestation) or live birth. Secondary outcome measures evaluated were sperm DFI, number of two-pronuclear (2PN) oocytes, fertilization rate, proportion of cycles reaching embryo transfer (ET) and abortion rate.

Data extraction

Data extraction was performed independently by three of the authors (HA, JB, EK). The following data were recorded from each of the eligible studies: year of publication, type of population included, study design, number of couples and number of cycles, male age, male FSH concentration, male body mass index (BMI), testicular volume, DFI, female age, female FSH concentration, female BMI, antral follicle count (AFC), anti-Müllerian hormone (AMH) level, endometrial thickness, number of retrieved cumulus–oocyte complexes (COC), number of mature oocytes, fertilization rate, proportion of cycles reaching ET, number of embryos transferred, pregnancy rate, miscarriage rate and live birth rate. Any disagreement between the authors responsible for data extraction was solved unanimously by discussion. In case of missing data, authors were contacted by e-mail to request additional information.

Statistical analysis

The difference in dichotomous data for each of the eligible studies was expressed as risk ratio (RR) with 95% confidence intervals (CI). For this purpose, the Mantel–Haenszel model (*Mantel and Haenszel, 1959*) was used. The difference in continuous data for each of the eligible studies was expressed as weighted mean difference (WMD) with 95% CI. For this purpose, the inverse variance method (*Olkin, 1995*) was used. For all comparisons, a P-value < 0.05 was considered significant. Calculations were performed with the use of STATA v14 (Stata Statistical Software: Release 14; StataCorp LP, College Station, TX, USA).

RESULTS

Literature search

The literature search yielded 757 studies, which, following screening of their titles, resulted in 145 potentially eligible publications. After reading the abstracts of these studies, 114 of them were excluded and the remaining 31 studies were further evaluated by retrieving their full text. The authors of six studies were contacted in an attempt to clarify methodological aspects of the study or to request missing data; however, a reply was received only for the study by *Pabuccu et al., (2016)*. Eventually, four controlled studies, one RCT (*Kahraman et al., 1996*), one prospective (*Esteves et al., 2015*) and two retrospective studies (*Al-Malki et al., 2017; Pabuccu et al., 2016*) were included in the present systematic review (FIGURE 1). Excluded studies in which full articles were retrieved for further evaluation appear in Supplementary Table 1.

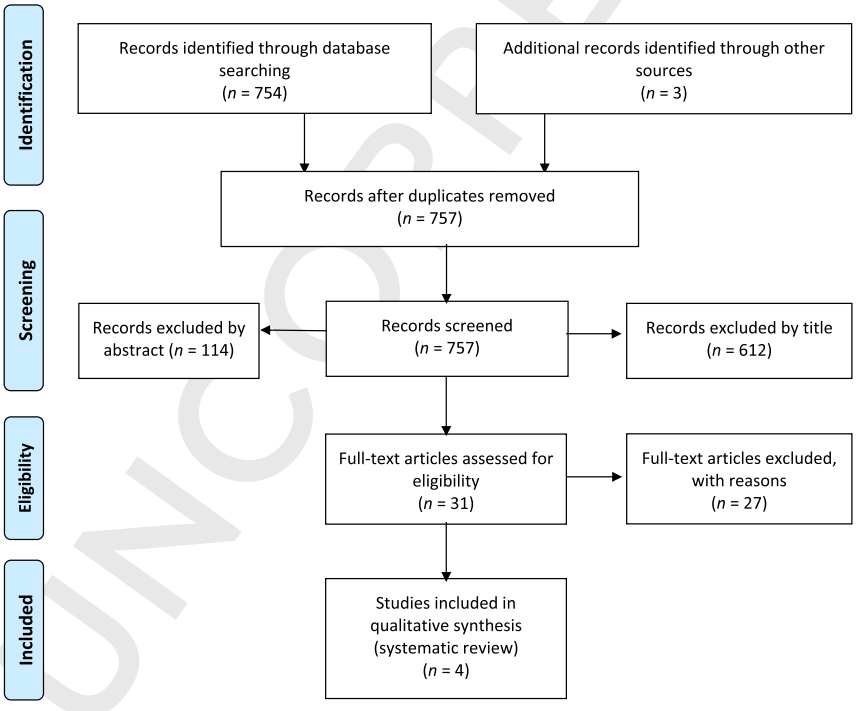

FIGURE. 1 Study flow diagram.

Eligible studies

Characteristics of the eligible studies appear in TABLE 1. Eligible studies were published between 1996 and 2017 and comparison of testicular versus ejaculated spermatozoa was performed in four different patient groups: high DFI and oligozoospermia (Esteves et al., 2016), high DFI only (*Pabuccu et al., 2016*), asthenozoospermia with or without teratozoospermia (*Kahraman et al., 1996*) and isolated asthenozoospermia (*Al-Malki et al., 2017*). The method used to assess DFI was different in the two relevant studies (TABLE 1).

Assessment of study quality

The quality of eligible studies was evaluated using the modified Newcastle–Ottawa Scale in the three cohort studies (Supplementary Table 2). The Cochrane Risk of Bias Tool was used in the single RCT (Supplementary Table 3).

Ovarian stimulation in the eligible studies

Different gonadotrophin-releasing hormones (GnRH) analogues and protocols were used, while different types of FSH were administered for ovarian stimulation. The type of triggering signal for inducing final oocyte maturation, as well as the criteria used for this purpose, varied between studies (TABLE 2).

Synthesis of data

Due to the obvious heterogeneity of the populations studied, as well as the limited number of patients and studies available, a meta-analysis to answer the research question by synthesizing the available evidence was not clinically meaningful and thus the current study is confined to a systematic review.

Patient and cycle characteristics in the eligible studies

High DFI only. In the retrospective study by *Pabuccu et al., (2016)*, no significant differences were observed between testicular and ejaculated spermatozoa groups regarding male age, DFI, female age, female BMI, AFC, AMH level, endometrial thickness, number of oocytes retrieved and number of metaphase II (MII) oocytes (Supplementary Tables 4 and 5). On the other hand, female FSH concentration was significantly lower (WMD: –1.50, 95% CI: –2.83 to –0.16) (Supplementary Table 4) and the number of embryos transferred was significantly higher (WMD: 0.30, 95% CI: 0.02 to 0.58) in

TABLE 1 CHARACTERISTICS OF THE ELIGIBLE STUDIES.

Study population, study, country of origin, journal	Study period	Patients/cycles	Study type	Power analysis	Company sponsored	Method of DFI assessment Cut-off level[a]
High DFI only, *Pabuccu et al., (2016)*, Turkey, Andrologia	2014–2015	71/71	Retrospective	Not reported	Not reported	TUNEL > 30%
High DFI and oligozoospermia, *Esteves et al., (2015)*, Brazil, Fertil Steril	2011–2013	172/172	Prospective	Not reported	No	SCD > 30%
Isolated asthenozoospermia, *Al-Malki et al., (2017)*, Canada, Andrology	2010–2015	57/57	Retrospective	Not reported	Not reported	Not reported
Asthenozoospermia with or without teratozoospermia, *Kahraman et al., (1996)*, Turkey, Hum Reprod	Not reported	24/24	Prospective	Not reported	Not reported	Not reported

DFI = DNA fragmentation index; SCD = sperm chromatin dispersion; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labelling.

[a] Cut-off level used to define normal DFI.

TABLE 2 OVARIAN STIMULATION IN THE ELIGIBLE STUDIES.

Study population, study (year)	GnRH analogue /dose	Type of analogue protocol	Gonadotrophin type/ starting dose	Signal for triggering final oocyte maturation	Criteria for HCG administration	OPU
High DFI only, *Pabuccu et al., (2016)*	Cetrotide/0.25 mg	Fixed antagonist protocol	Rec FSH + HMG/150–300 IU/day	Rec HCG/250 mg Ovitrelle	Three follicles ≥ 18 mm	NR
High DFI and oligozoospermia, *Esteves et al., (2015)*	Cetrorelix/0.25 mg	Antagonist protocol	Rec FSH/112.5–300 IU/day	Rec HCG/250 mg Ovitrelle	Two follicles ≥ 17 mm	36 h later
Isolated asthenozoospermia, *Al-Malki et al., (2017)*	NR	NR	NR	NR	NR	NR
Asthenozoospermia with or without teratozoospermia, *Kahraman et al., (1996)*	Suprefact/NR	Long agonist protocol	FSH + HMG/NR	HCG/10,000 IU	NR	36 h later

DFI = DNA fragmentation index; GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin hormone; OPU = ovum pick-up; NR = not reported; Rec = recombinant.

the testicular compared to the ejaculated spermatozoa group (Supplementary Table 5). No data were reported regarding male FSH concentration and testicular volume in the above study (Supplementary Table 4). Fertilization was attempted by ICSI; no data were reported regarding the day of ET and type of luteal phase support.

High DFI and oligozoospermia. In the prospective study by Esteves et al. (2016), no significant differences were observed between the testicular and the ejaculated spermatozoa groups regarding male age, sperm DFI, female FSH concentration, number of oocytes retrieved, number of MII oocytes and number of embryos transferred (Supplementary Tables 4 and 5). On the other hand, female age was significantly higher (WMD: 1.50, 95% CI: 0.28 to 2.71) with the use of testicular as compared to the ejaculated spermatozoa group. No data were reported in the above study regarding male FSH concentration, testicular volume, female BMI, AFC, AMH level and endometrial thickness (Supplementary Tables 4 and 5). Fertilization was attempted by ICSI; embryos were transferred on day 3 and daily vaginal progesterone gel was given as luteal phase support.

Isolated asthenozoospermia. In the retrospective study by *Al-Malki et al., (2017)*, no significant differences were observed between the testicular and the ejaculated spermatozoa groups regarding female age and number of MII oocytes (Supplementary Tables 4 and 5). However, male age (WMD: –3.00, 95% CI: –5.89 to –0.10) and male FSH concentration (WMD: –6.00, 95% CI: –8.58 to –3.41) were significantly lower in the testicular as compared to the ejaculated spermatozoa group (Supplementary Table 4). In addition, testicular volume was significantly higher (WMD: 2.00, 95% CI: 0.09 to 3.90) in the testicular as compared to the ejaculated spermatozoa group (Supplementary Table 4). No data were reported regarding female FSH concentration, BMI, AFC, AMH level, endometrial thickness, number of oocytes retrieved, number of embryos transferred and sperm DFI in the above study (Supplementary Tables 4 and 5). Fertilization was attempted by ICSI; no data were reported regarding the day of ET and type of luteal phase support.

Asthenozoospermia with or without teratozoospermia. In the RCT by *Kahraman et al., (1996)*, no baseline characteristics or IVF stimulation outcomes were reported between the two groups compared (Supplementary Tables 4 and 5). Fertilization was attempted by ICSI and no data were reported regarding the day of ET and type of luteal phase support.

Primary outcome measure

No difference in the probability of pregnancy, expressed as either

TABLE 3 CLINICAL PREGNANCY AND LIVE BIRTH IN THE TESTICULAR AND EJACULATED SPERMATOZOA GROUPS.

	High DFI only *Pabuccu et al., (2016)*	High DFI and oligozoospermia *Esteves et al., (2015)*	Isolated asthenozoospermia *Al-Malki et al., (2017)*	Asthenozoospermia with or without teratozoospermia *Kahraman et al., (1996)*
Clinical pregnancy	NR	NR	RR: 1.09, 95% CI: 0.56 to 2.14	RR: 2.85, 95% CI: 0.76 to 10.69
Live birth	RR: 2.36, 95% CI: 0.98 to 5.68	RR: 1.75, 95% CI: 1.14 to 2.70	NR	NR

CI = confidence interval; DFI = DNA fragmentation index; NR = not reported; RR = relative risk.

TABLE 4 SECONDARY OUTCOME MEASURES IN THE TESTICULAR AND EJACULATED SPERMATOZOA GROUPS.

	High DFI only *Pabuccu et al., (2016)*	High DFI and oligozoospermia *Esteves et al., (2015)*	Isolated asthenozoospermia *Al-Malki et al., (2017)*	Asthenozoospermia with or without teratozoospermia *Kahraman et al., (1996)*
Sperm DFI	NR	WMD: –32.60, 95% CI: –34.99 to –30.20	NR	NR
Number of 2PN oocytes	NR	NR	WMD: –0.20, 95% CI: –2.13 to 1.73	NR
Fertilization rate	WMD: 3.00, 95% CI: –8.07 to 14.07	WMD: –13.30, 95% CI: –18.08 to –8.52	WMD: –1.70, 95% CI: –3.39 to –0.01	NR
Proportion of cycles reaching ET	RR: 1.10, 95% CI: 0.96 to 1.26	RR: 0.99, 95% CI: 0.93 to 1.06	RR: 1.30, 95% CI: 0.92 to 1.83	RR: 0.94, 95% CI: 0.76 to 1.16
Abortion rate	RR: 0.08, 95% CI: 0.01 to 0.58	RR: 0.29, 95% CI: 0.10 to 0.82	NR	RR: 0.33, 95% CI: 0.10 to 1.07

2PN = two-pronuclear; CI = confidence interval; DFI = DNA fragmentation index; ET = embryo transfer; NR = not reported; RR = relative risk; WMD = weighted mean difference.

live birth or clinical pregnancy, was present in three of the four eligible studies (*Al-Malki et al., 2017; Kahraman et al., 1996; Pabuccu et al., 2016*). A higher probability of live birth was observed only in men with high DFI and oligozoospermia (RR: 1.75, 95% CI: 1.14 to 2.70) (Esteves et al., 2016) (TABLE 3).

Secondary outcome measures

High DFI only. In the retrospective study by *Pabuccu et al., (2016)*, fertilization rate and proportion of patients reaching ET were no different when testicular as compared to the ejaculated spermatozoa were used for ICSI, while abortion rate was significantly lower (RR: 0.08, 95% CI: 0.01 to 0.58) in the testicular group. On the other hand, no data were reported regarding sperm DFI and number of 2PN oocytes (TABLE 4).

High DFI and oligozoospermia. In the prospective study by Esteves et al. (2016), sperm DFI (WMD: –32.60, 95% CI: –34.99 to –30.20), fertilization rate (WMD: –13.30, 95% CI: –18.08 to –8.52) and abortion rate (RR: 0.29, 95% CI: 0.10 to 0.82) were significantly lower, when testicular compared to ejaculated spermatozoa were used for ICSI, while there was no difference in the proportion of patients reaching ET. On the other hand, no data were reported regarding number of 2PN oocytes (TABLE 4).

Isolated asthenozoospermia. In the retrospective study by *Al-Malki et al., (2017)*, fertilization rate was significantly lower (WMD: –1.70, 95% CI: –3.39 to –0.01) when testicular as compared to ejaculated spermatozoa were used for ICSI, while no difference was observed in the number of 2PN oocytes and proportion of patients reaching ET. No data were available for sperm DFI and abortion rate (TABLE 4).

Asthenozoospermia with or without teratozoospermia. In the RCT by *Kahraman et al., (1996)*, the proportion of patients reaching ET as well as abortion rate were no different when testicular as compared to ejaculated spermatozoa were used for ICSI. No data were available for sperm DFI, number of 2PN oocytes and fertilization rate (TABLE 4).

DISCUSSION

The current systematic review firstly highlights a conspicuous lack of well-designed prospective studies that compare the outcome of treatment with ejaculated versus testicular spermatozoa in cases without azoospermia, such as patients with asthenozoospermia with or without teratozoospermia, patients with isolated asthenozoospermia as well as in patients with high DFI only. There is only limited low-quality evidence to suggest that the probability of live birth is significantly higher with the use of testicular as compared to ejaculated spermatozoa in men with high DFI and oligozoospermia (RR: 1.75, 95% CI: 1.14 to 2.70).

This conclusion contradicts the data presented in a recent meta-analysis of seven studies, evaluating the use of testicular as compared to ejaculated spermatozoa in patients with high DFI or in patients with high DFI and oligozoospermia (Esteves et al., 2017). In that meta-analysis, a higher probability of clinical pregnancy (OR: 2.42, 95% CI: 1.57–3.73) and live birth (OR: 2.58, 95% CI: 1.54–4.35) was observed with the use of testicular as compared to ejaculated spermatozoa. However, by examining the eligible studies in that systematic review and meta-analysis, several limitations became apparent. The conclusion of a higher probability of live birth with the use of testicular as compared to ejaculated spermatozoa was based on the meta-analysis of two studies evaluating patients with high

DFI and oligozoospermia. However, in one of these studies, data used in the meta-analysis came from both fresh and frozen spermatozoa samples (Bradley et al., 2016), while no information was provided in the manuscript regarding the actual number of patients in whom insemination was performed with cryopreserved or with fresh spermatozoa. Moreover, analysis of these patients was not reported separately, which limits the study's generalizability.

The same study has been used in the calculation of the probability of clinical pregnancy with the use of testicular as compared to ejaculated spermatozoa (Bradley et al., 2016). For that calculation, a case crossover study was also used (*Greco et al., 2005*). These types of studies have been considered inappropriate for evaluating outcomes in reproductive medicine (Daya, 1993; *Khan et al., 1996*). Thus, it appears that the authors' conclusion 'infertile couples may benefit from Testi-ICSI if male partners have confirmed high SDF in the ejaculate' (Esteves et al., 2017) appears to be ill supported and premature, while it may mislead clinicians into routinely applying TESE in patients without azoospermia and high DFI or high DFI and oligozoospermia.

No studies evaluating the research question were identified in patients with cryptozoospermia, although a seemingly relevant meta-analysis has already been published (*Abhyankar et al., 2016*). This meta-analysis, however, was excluded from the current systematic review, because by examining the eligible studies it became apparent that they were not evaluating the research question in patients with cryptozoospermia. In fact, in three out of the five eligible studies included, TESE was performed only if there were no spermatozoa in the ejaculate on the day of oocyte retrieval (*Amirjannati et al., 2012*; *Bendikson et al., 2008; Hauser et al., 2011*) and thus the diagnosis of included patients in these studies was, in fact, virtual azoospermia and not cryptozoospermia. Obviously, the clinically important dilemma of using testicular versus ejaculated spermatozoa only exists in the presence of spermatozoa in the ejaculate. In a different case, the use of testicular spermatozoa is mandatory if the couple do not wish to proceed to semen donation. In addition, in that meta-analysis data on the probability of pregnancy were pooled from a case crossover study (*Hauser et al., 2011*), from studies that analysed both fresh and frozen testicular sperm samples (*Ben-Ami et al., 2013; Hauser et al., 2011*) as well as from case reports (*Weissman et al., 2008*).

No studies evaluating the research question in men with teratozoospermia were identified in the current systematic review. The only existing, comparative, retrospective studies (*Lu et al., 2012; Tsai et al., 2011; Verza and Esteves, 2008*) evaluated the use of ejaculated spermatozoa from patients with teratozoospermia with that of testicular spermatozoa from patients with azoospermia and thus were not answering the research question asked in the current systematic review.

Studies including both fresh and frozen samples might be relevant, but only if cryopreservation is performed in both groups of the study (testicular and ejaculated). This is due to the fact that an adverse effect of freezing on semen parameters might be associated with an impaired outcome and thus may confound the results obtained. This is the case with the studies by *Ben-Ami et al., (2013)* and Bradley et al. (2016). In these studies, baseline and IVF stimulation characteristics referred to both fresh and frozen samples together, not allowing a subgroup analysis for fresh and frozen TESE samples.

Any conclusions drawn from the current systematic review are based on single studies comparing the use of testicular with that of ejaculated spermatozoa in different patient populations and as such, they are of limited value. It is generally agreed that caution should be exercised prior to application of any intervention in clinical practice, the value of which has not been shown convincingly in relevant high-quality trials. This is particularly true for surgical interventions, such as TESE in patients without azoospermia, considering its short-term complications, such as testicular haematoma, fibrosis and testicular atrophy (Donoso et al., 2007), as well as its long-term complications such as hypogonadism (*Ramasamy et al., 2005*). Moreover, it might be argued that due to the frequently present genetic basis of male infertility in these patients, the source of spermatozoa might not be a crucial factor for determining success (*Wosnitzer et al., 2014*).

The primary motivation of the clinician offering TESE to a subfertile male without azoospermia should be the increase in the probability of live birth by averting potential damage to sperm DNA during transit through the male reproductive tract. However, there is currently insufficient evidence to convincingly guide such a clinical decision. This should be clearly explained to patients, especially those who have already experienced pregnancy failure with ejaculated spermatozoa and might be keen to undergo TESE. If this assumption is true, TESE might enhance the probability of pregnancy, however there is currently no relevant hard evidence supporting this hypothesis.

In order to answer the research question convincingly, future studies should use a randomized parallel arm design, avoiding crossover and non-randomized designs that are prone to bias. They should also use strict and clear definition criteria for selecting patients (cryptozoospermia, teratozoospermia, asthenozoospermia or high DFI). Regarding the use of testicular spermatozoa in men with high DFI, there is an apparent need to adopt universal criteria for classifying patients into those with high or normal DFI. Given the fact that the prognostic value of sperm DFI on ART outcome is confounded by oocyte quality and its ability to repair DNA damage (*Meseguer et al., 2011*), future studies should control for maternal age and ovarian reserve, factors that are inherently related to oocyte quality. Moreover, baseline and IVF stimulation characteristics of female partners should be clearly presented to ensure comparability of the studied groups, while ovarian stimulation should be performed with the same protocol in all female partners of the couples evaluated. In addition, future studies should evaluate as primary outcome live birth, after fertilization with either fresh or frozen spermatozoa, and use techniques for DFI assessment which are shown to be highly accurate in the investigation of male infertility (Cui et al., 2015).

Until then, research may focus on developing strategies for improving DFI, including intake of oral antioxidant or selection of spermatozoa with low DFI using discontinuous density gradient technique for semen preparation (*Said et al., 2005*), intracytoplasmic morphologically selected sperm injection (IMSI) (*Garolla et al., 2014*), Petri Dish®

for Sperm Selection (PICSI) (*Parmegiani et al., 2010*) or a magnetic cell sorting technique (*Said et al., 2005*), methods for which the existing evidence is scant.

The value of the current systematic review is that it clearly shows that there is currently no basis for routine clinical application of TESE in men without azoospermia and, as such, this intervention, which also increases the overall cost of treatment, should be confined to a research setting. In a different case and currently only for patients with high DFI and oligozoospermia, who have already failed trials with ejaculated spermatozoa, a detailed discussion with the patient explaining the problems with the existing literature is imperative prior to signing an informed consent and undergoing the procedure.

In conclusion, there is currently limited low-quality evidence suggesting that a higher probability of pregnancy might be expected with the use of testicular as compared to ejaculated spermatozoa only in men with high DFI and oligozoospermia.

UNCITED REFERENCES

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Harbord et al., 2006, Higgins et al., 2003, DerSimonian and Laird, 1986.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2018.08.017.

REFERENCES

Abhyankar, N., Kathrins, M., Niederberger, C. **Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis.** Fertil Steril 2016; 105: 1469–1475

Al-Malki, A.H., Alrabeeah, K., Mondou, E., Brochu-Lafontaine, V., Phillips, S., Zini, A. **Testicular sperm aspiration (TESA) for infertile couples with severe or complete asthenozoospermia.** Andrology 2017; 5: 226–231

Amirjannati, N., Heidari-Vala, H., Akhondi, M.A., Hosseini Jadda, S.H., Kamali, K., Sadeghi, M.R. **Comparison of intracytoplasmic sperm injection outcomes between spermatozoa retrieved from testicular biopsy and from ejaculation in cryptozoospermic men.** Andrologia 2012; 44: 704–709

Arafa, M., AlMalki, A., AlBadr, M., Burjaq, H., Majzoub, A., AlSaid, S., Elbardisi, H. **ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa.** Andrologia 2017

Ben-Ami, I., Raziel, A., Strassburger, D., Komarovsky, D., Ron-El, R., Friedler, S. **Intracytoplasmic sperm injection outcome of ejaculated versus extracted testicular spermatozoa in cryptozoospermic men.** Fertil Steril 2013; 99: 1867–1871

Bendikson, K.A., Neri, Q.V., Takeuchi, T., Toschi, M., Schlegel, P.N., Rosenwaks, Z., Palermo, G.D. **The outcome of intracytoplasmic sperm injection using occasional spermatozoa in the ejaculate of men with spermatogenic failure.** J Urol 2008; 180: 1060–1064

Cui, X., Ding, P., Gao, G., Zhang, Y. **Comparison of the Clinical Outcomes of Intracytoplasmic Sperm Injection Between Spermatozoa Retrieved From Testicular Biopsy and From Ejaculate in Cryptozoospermia Patients.** Urology 2016

DerSimonian, R., Laird, N. **Meta-analysis in clinical trials.** Control Clin Trials 1986; 7: 177–188

Devroey, P., Liu, J., Nagy, Z., Goossens, A., Tournaye, H., Camus, M., Van Steirteghem, A., Silber, S. **Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia.** Hum Reprod 1995; 10: 1457–1460

Esteves, S.C., Sanchez-Martin, F., Sanchez-Martin, P., Schneider, D.T., Gosalvez, J. **Comparison of reproductive outcome in oligozoospermic men with high sperm DNA fragmentation undergoing intracytoplasmic sperm injection with ejaculated and testicular sperm.** Fertil Steri 2015; 104: 1398–1405

Garolla, A., Cosci, I., Menegazzo, M., De Palo, R., Ambrosini, G., Sartini, B., Pizzol, D., Foresta, C. **Sperm selected by both birefringence and motile sperm organelle morphology examination have reduced deoxyribonucleic acid fragmentation.** Fertil Steril 2014; 101: 647–652

Greco, E., Scarselli, F., Iacobelli, M., Rienzi, L., Ubaldi, F., Ferrero, S., Franco, G., Anniballo, N., Mendoza, C., Tesarik, J. **Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa.** Hum Reprod 2005; 20: 226–230

Harbord, R.M., Egger, M., Sterne, J.A. **A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints.** Stat Med 2006; 25: 3443–3457

Hauser, R., Bibi, G., Yogev, L.., Carmon, A., Azem, F., Botchan, A., Yavetz, H., Klieman, S.E., Lehavi, O., Amit, A., Ben-Yosef, D. **Virtual azoospermia and cryptozoospermia–fresh/frozen testicular or ejaculate sperm for better IVF outcome?.** J Androl 2011; 32: 484–490

Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G. **Measuring inconsistency in meta-analyses.** BMJ 2003; 327: 557–560

Kahraman, S., Tasdemir, M., Tasdemir, I., Vicdan, K., Ozgur, S., Polat, G., Islk, A.Z., Biberoglu, K., Vanderzwalmen, P., Nijs, M., Schoysman, R. **Pregnancies achieved with testicular and ejaculated spermatozoa in combination with intracytoplasmic sperm injection in men with totally or initially immotile spermatozoa in the ejaculate.** Hum Reprod (Oxford, England) 1996; 11: 1343–1346

Ketabchi, A.A. **Intracytoplasmic Sperm Injection Outcomes with Freshly Ejaculated Sperms and Testicular or Epididymal Sperm Extraction in Patients with Idiopathic Cryptozoospermia.** Nephrourol Mon 2016; 8: e41375

Khan, K.S., Daya, S., Collins, J.A., Walter, S.D. **Empirical evidence of bias in infertility research: overestimation of treatment effect in crossover trials using pregnancy as the outcome measure.** Fertility and sterility 1996; 65: 939–945

Lu, Y.H., Gao, H.J., Li, B.J., Zheng, Y.M., Ye, Y.H., Qian, Y.L., Xu, C.M., Huang, H.F., Jin, F. **Different sperm sources and parameters can influence intracytoplasmic sperm injection outcomes before embryo implantation.** Journal of Zhejiang University. Science. B 2012; 13: 1–10

Mantel, N., Haenszel, W. **Statistical aspects of the analysis of data from retrospective studies of disease.** J Natl Cancer Inst 1959; 22: 719–748

Matas, C., Vieira, L., Garcia-Vazquez, F.A., Aviles-Lopez, K., Lopez-Ubeda, R., Carvajal, J.A., Gadea, J. **Effects of centrifugation through three different discontinuous Percoll gradients on boar sperm function.** Anim Reprod Sci 2011; 127: 62–72

Meseguer, M., Santiso, R., Garrido, N., Garcia-Herrero, S., Remohi, J., Fernandez, J.L. **Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality.** Fertil Steril 2011; 95: 124–128

Olkin, I. **Statistical and theoretical considerations in meta-analysis.** J Clin Epidemiol 1995; 48: 133–146

Pabuccu, E.G., Caglar, G.S., Tangal, S., Haliloglu, A.H., Pabuccu, R. **Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high sperm DNA fragmentation and previous ART failures.** Andrologia 2016

Palermo, G., Joris, H., Devroey, P., Van Steirteghem, A.C. **Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte.** Lancet 1992; 340: 17–18

Parmegiani, L., Cognigni, G.E., Bernardi, S., Troilo, E., Ciampaglia, W., Filicori, M. **'Physiologic ICSI': hyaluronic acid (HA) favours selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality.** Fertil Steril 2010; 93: 598–604

Popal, W., Nagy, Z.P. **Laboratory processing and intracytoplasmic sperm injection using epididymal and testicular spermatozoa: what**

can be done to improve outcomes?. Clinics (Sao Paulo) 2013; 68: 125–130
Ramasamy, R., Yagan, N., Schlegel, P.N. **Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction.** Urology 2005; 65: 1190–1194
Said, T.M., Grunewald, S., Paasch, U., Glander, H.J., Baumann, T., Kriegel, C., Li, L., Agarwal, A. **Advantage of combining magnetic cell separation with sperm preparation techniques.** Reprod Biomed Online 2005; 10: 740–746
Swanton, A., Itani, A., McVeigh, E., Child, T. **Azoospermia: is sample centrifugation indicated? A national survey of practice and the Oxford experience.** Fertil Steril 2007; 88: 374–378
Tsai, C.C., Huang, F.J., Wang, L.J., Lin, Y.J., Kung, F.T., Hsieh, C.H., Lan, K.C. **Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study.** Fertil Steril 2011; 96: 567–571
Verza, S.Jr., Esteves, S.C. **Sperm defect severity rather than sperm Source is associated with lower fertilization rates after intracytoplasmic sperm injection.** International braz j urol: official journal of the Brazilian Society of Urology 2008; 34: 49–56
Weissman, A., Horowitz, E., Ravhon, A., Nahum, H., Golan, A., Levran, D. **Pregnancies and live births following ICSI with testicular spermatozoa after repeated implantation failure using ejaculated spermatozoa.** Reprod Biomed Online 2008; 17: 605–609
Wosnitzer, M., Zhao, F.J., Li, P.S. **[Update of genetic evaluation for male infertility].** Zhonghua nan ke xue–Nat J Androl 2014; 20: 99–105
Xu, Z.P., Sun, H.X., Zhang, N.Y., Wang, B., Xu, Z.P. **[Efficient treatment of severe teratozoospermia by ICSI with testicular spermatozoa: a report of 5 cases].** Zhonghua nan ke xue = National journal of andrology 2009; 15: 218–222
Yang, S., Gao, L., Wang, W., Ding, J., Xu, Y., Li, H. **Successful intracytoplasmic sperm injection with testicular spermatozoa from a man with multiple morphological abnormalities of the sperm flagella: a case report.** J Assist Reprod Genet 2017

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