Medicine®

OPEN

The influence of obesity, smoking, and serum follicular stimulating hormone in azoospermic patients on testicular sperm extraction-intra cytoplasmic sperm injection outcomes
A retrospective cohort study

Guy Shrem, MD[a],[*], Yana Brudner, MD[a], Yuval Atzmon, MD[a], Mediea Michaeli, PhD[a], Adrian Ellenbogen, MD[b], Einat Shalom-Paz, MD[a],[b]

Abstract

To examine the effect of serum follicle-stimulating hormone (sFSH) level, body-mass index (BMI) and smoking on Testicular Sperm Extraction–Intracytoplasmic Sperm Injection (TESE–ICSI), and pregnancy outcomes.

In this retrospective study, data were extracted from files of 52 azoospermic men who underwent TESE and in-vitro fertilization (IVF)-ICSI in our IVF unit. Demographic information, treatment cycle follow-up and pregnancy outcomes were collected.

Fifty-two patients underwent 79 TESE due to azoospermia in 143 IVF cycles. Smoking was found to significantly affect sperm motility in TESE specimens before freezing (45.5% vs 14.8%; $P < .001$); however, this finding did not influence the pregnancy rate. Male FSH was inversely correlated with testicle volume ($r = -0.595$, $P < .0001$). Body weight did not affect semen parameters after TESE or ICSI outcomes.

Among azoospermic patients with extremely poor sperm quality, male BMI, male FSH or smoking did not have an adverse effect sperm parameters or pregnancy and delivery rates.

Abbreviations: ART = assisted reproductive technology, BMI = body-mass index, ICSI = intracytoplasmic sperm injection, IU = international units, IVF = in-vitro fertilization, NOA = non-obstructive azoospermia, TESE = testicullar sperm extraction.

Keywords: follicular-stimulating hormone, intra-cytoplasmic sperm injection, in-vitro fertilization, obesity, smoking, testicular sperm extraction

1. Introduction

Absence of spermatozoa in the ejaculate (azoospermia) is found in 15% of infertile men. Surgical approaches combined with assisted reproductive technology (ART) provide fertility options for those couples. Azoospermia is classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA). These conditions describe differences in the ability of the testis to produce and deliver spermatozoa.

Surgical methods have been developed to retrieve spermatozoa from the testicles in azoospermic men. Testicular sperm extraction (TESE)[1] is the preferred surgical method for sperm retrieval from azoospermic men and is effective for both OA and NOA.[2]

Various lifestyle parameters can influence the sperm profile, including basal FSH levels, body-mass index (BMI), smoking, cause of male infertility, and age among the general population. However, the influence of these parameters on treatment outcomes of TESE–intracytoplasmic sperm injection (ICSI) is not clear.[3,4]

In this retrospective cohort study, we examined the effect of serum FSH levels, BMI, and smoking on TESE–ICSI and pregnancy outcomes.

2. Material and methods

2.1. Study participants

A cohort of 52 azoospermic men underwent TESE and in-vitro fertilization (IVF)–ICSI from 2009 through 2013 at the IVF unit at Hillel Yaffe Medical Center. The study was approved by the Intuitional Ethics Committee.

2.2. Patient evaluation

All patients diagnosed with azoospermia after 2 sperm analyses were evaluated for the etiology. The evaluation included physical examination by a urologist, hormonal profile, testicular ultra-sound, karyotype, and Y-chromosome microdeletion.[5]

2.3. Testicular sperm extraction

TESE was conducted in our center under general anesthesia by a urologist, as described previously.[6] Specimens were processed by an embryologist as follows. Six testicular biopsies were enzymatically digested with collagenase (0.8 mg/mL, Sigma) for 2 hours and centrifuged at 200 g for 10 minutes. The pellet was dissolved in 100 to 200 μL of Sperm Preparation Medium (Medicult, Aarhus, Denmark). Of this suspension, 20 μL was analyzed on a slide divided to 10 to 25 drops and evaluated under the microscope for the appearance of spermatozoa at magnification of 400. The total number of spermatozoa was calculated from the number per microscopic field or was counted per slide. Motility was documented as total motility and as a percentage. All testicular samples were cryopreserved. Ovarian stimulation was initiated only after sperm was retrieved.

2.4. Fertilization and embryo development

The ICSI procedure followed the TESE according to previously reported technique[7] at 200 g at 10 minutes, and the pellet was suspended in 100 to 200 μL of sterile medium (Medicult). Two μL of this suspension was added to 3 μL drops of medium containing 0 to 3 μL of polyvinyl pyrrolidone in a sterile Petri dish covered in mineral oil. After the cells settled, each drop was examined for the presence of elongated spermatids by screening each microscopic field at 400X. A single spermatozoon was taken up into an ICSI pipette (Humagen, Charlottesville, SC) and transferred to a collecting drop. Motile and morphologically normal sperm were preferred, but when only immotile or abnormal sperm were found, these were used for standard ICSI. Maximum collection time did not exceed 4 hours. The selected sperm was found by embryologist at usual magnification of 200X to 400X. Conventional ICSI was performed using an inverted microscope. The injected oocyte was transferred to a 4-well dish containing either ISM1 medium (Origio) or Cleavage medium (Sage) pre-equilibrated culture media, overlaid with oil from the same company. The culture dish was incubated at 37°C to 37.2°C with 5.3% to 5.5% CO_2.

Sixteen to 18 hours after ICSI, fertilization was assessed as previously described.[8] Further embryonic development was evaluated daily. Embryo quality was assessed on day 2 or 3. Top-quality embryos on day 2 were defined as presenting with 4 equal size blastomeres and no more than 10% fragmentation. Top-quality embryos on day 3 were those with at least 7 equal blastomeres and no more than 10% fragmentation.

Pregnancy was determined by positive beta Human Chorionic Gonadotropin (βHCG) test 14 days after embryo transfer. Clinical pregnancy was defined as the presence of intrauterine gestational sac with fetal heart activity.

2.5. Data collection

Demographic data recorded included age, infertility diagnosis, and basal hormone levels for male and female partners. Treatment follow-up data included protocols, gonadotropins used, number of follicles and endometrial thickness during treatment, number of mature and immature oocytes retrieved, fertilization, and cleavage rate. The numbers of transferred embryos, implantation, and pregnancy rates, as well as pregnancy outcome, were also recorded. All viable intrauterine pregnancies were followed until delivery. Pregnancy and neonatal data were recorded, including gestational age at delivery, and any abnormalities.

Table 1

Characteristics of the male patients.

Characteristic (N = 52)	Value
Average age, range, yr	31 [29–36]
Ethnicity (n = 51)	
Arab	35 (67.3%)
Jewish	17 (32.7%)
BMI, kg/m²	26.6 [24.2–29.5]
Comorbidity before TESE (n = 51)	24 (47%)
Previous testicular intervention (surgery, trauma) (n = 51)	17 (33.4%)
Chronic medication used (n = 51)	9 (17.6%)
Smoking (n = 51)	34 (66.7%)
FSH, IU	9.0 (3.9–19.5)
LH, IU	5.6 (4–10)
Testosterone, ng/dL	16.5 (10.0–22.8)

Data are presented as median (25%–75%) and percentage.
BMI = body mass index, FSH = follicular stimulating hormone, LH = luteinizing hormone, TESE = testicular sperm extraction.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software package (SPSS Inc., Chicago, IL). We used Shapiro–Wilks test to evaluate the distribution of the data. Comparisons were analyzed using Student t test or Mann–Whitney U test, each when appropriate. Proportions were compared using Chi-square or Fisher exact test. P values less than .05 were considered significant. We used a multivariate logistic regression analysis model to rule out any other confounders that might influence the clinical results.

3. Results

Fifty-two patients underwent 79 TESE due to azoospermia in 143 IVF cycles. The demographic and clinical characteristics of the male patients are presented in Table 1 and for female partners in Table 2. Table 3 summarizes the ICSI cycles. There was a median of 7 oocytes per cycle, which yielded a median of 3 normal fertilizations (2PN), no top-quality embryos developed. The cumulative chemical pregnancy rate per TESE was 59.6%, the cumulative clinical pregnancy rate per TESE was 57.7%, with cumulative delivery rate of 44%. The pregnancy rate per IVF–ICSI treatment was 38.7% and clinical pregnancy rate per transfer was 32.4% with a 25.2% delivery rate.

We divided the cohort into nonsmokers and smokers and found that the only significant difference was reflected in sperm motility after TESE before freezing (45.5% vs 14.8%; P < .001;

Table 2

Characteristics of the female patients.

Characteristic (N = 52)	Value
Age, range, yr	27 [23.8–32.3]
BMI, kg/m²	27 [23.7–31.3]
FSH, IU	6.0 [5–7]
LH, IU	5 [4–8]
Estradiol, ng/dL	143 [104–218]
Serum prolactin level, ng/dL	357 [248.8–508.3]
Serum TSH level, mIU/mL	2.0 [1.38–2.92]
Follicular phase length, days	11 [9–14]
Total gonadotropins used for stimulation, IU	1350 [984–1800]
Estradiol level on day of hCG administration, pg/mL	1378 [943.1–2044.3]

Data are presented as median (25%–75%) and percentage.
BMI = body mass index, FSH = follicular stimulating hormone, LH = luteinizing hormone, TESE = testicular sperm extraction.

Table 3

IVF-ICSI outcomes.

Outcomes	Per IVF-ICSI (N=111)	Cumulative per TESE (N=52)
Number of cycles per couple	3 [2–4]	
Number of injected oocytes	7 [4–11]	
Number of 2PN	3 [1–5]	
Number of cleaved embryos	3 [1–5.75]	
Number of Top quality embryos per cycle		
0	94 (72%)	
1 or more	36 (28%)	
Number of embryos transferred, mean±sd	2 [1–2]	
Cycles with frozen embryos, %	15/131 (11%)	
Chemical pregnancy	43 (38.7%)	31 (59.6%)
Clinical pregnancy rate per transfer	36 (32.4%)	30 (57.7%)
Early pregnancy failure rate	7 (6.3%)	
Fetal anomaly rate	7 (6.3%)	
Pre-term delivery rate (before 37 weeks)	11 (9.9%)	
Delivery rate	28 (25.2%)	23 (44%)
Newborn malformation rate	1 (0.9%)	

Data are presented as median (25%–75%) and percentage.
2PN = 2 pronuclei, ICSI = intracytoplasmic sperm injection, IVF = in-vitro fertilization, TESE = testicular sperm extraction.

respectively) (Table 4). However, smoking did not have a significant effect on pregnancy outcomes.

Evaluating the influence of male-FSH on pregnancy outcomes per TESE cycles (total of 52 cycles), we found that lower FSH levels were correlated with positive pregnancy test and delivery rate (Table 5). A univariate analysis found that median male FSH 5 international units (IU) compared to median of 13.5 IU were significantly correlated with positive pregnancy rate and delivery rate (P=.018 for chemical pregnancy; P=.008 for clinical pregnancy) (Table 6).

Univariate analysis of all IVF–ICSI cycles conducted for those couples we found that age less than 37 was significantly correlated with positive pregnancy outcome: chemical pregnancy 41% versus 15%, P=.039 and clinical pregnancy 35.6% versus 10%, P=.032.

Multivariable logistic regression model was used to assess the association between several independent parameters and pregnancy outcomes, including smoking, male FSH, male age, presence of motile sperm before freezing, and BMI. Motile sperm before freezing was significantly correlated with an increase in chemical pregnancy (OR=4.26; P=.018; 95% CI 1.27–14.09). Advanced paternal age had a significant adverse correlation with live birth (OR=0.86; P=.027; 95% CI 0.76–0.98).

Testicular volume in this model was not correlated with TESE or pregnancy outcomes.

There was 1 neonate with anomalies (5.5%).

4. Discussion

The present study evaluated the outcomes of azoospermic couples who underwent TESE–ICSI cycles. We focused on male age, smoking status, BMI, and FSH, predictive factors known to influence IVF outcome that is known to affect fertility outcomes among patients with very poor prognoses. To the best of our knowledge, this is the first study to evaluate the influence of these parameters in relation to TESE due to azoospermia.

It is well-established that fertility of both partners is adversely affected by smoking and smoking is associated with decreased IVF–ICSI success rates.[9,10] Some studies identified smoking as reducing semen parameters.[9–11] Lotti et al found that smokers had lower ejaculate and seminal vesicle volume as compared to non-smokers.[12] Other studies reported that cigarette smoking has an overall negative effect on semen parameters, primarily sperm count and motility.[13,14] Waylen et al reported significant evidence on the negative effect of cigarette smoking on ART clinical outcomes with 0.53 odds ratio for live birth per cycle and significantly higher odds (2.65) of spontaneous abortions.[15]

No previous study evaluated the impact of smoking on TESE results. The present study found significantly fewer motile sperm in the smokers compared to the non-smokers. However, this did not affect the pregnancy and delivery rates in our study. In non-TESE cycles, smoking was suggested to cause genetic damage due to tobacco and reactive oxygen species.[16] The assumption is that

Table 4

Characteristics and cycle outcome compared between smokers male and non-smokers.

Characteristic	Smokers N=34	Non-smokers N=17	P value
Age, yr	31.5 [28–36]	31 [29–35]	.49
Ethnicity (%, Jews)	8 (23.5%)	9 (52.9%)	.058
BMI, kg/m²	28.4 [23.3–30.2]	25.9 [24.2–28.0]	.18
Morbidity	15 (44.1%)	9 (52.9%)	.57
FSH, IU	12 [4–20.5]	6.3 [2.2–19]	.27
LH, IU	6.4 [4.1–10]	4.9 [4–10]	.36
Sperm motility TESE, %	12 (14.8%)	20 (45.5%)	<.001
Testosterone, ng/dL	17 [9.1–23.8]	15.5 [10.9–19.8]	.62
Presence of sperm in specimen	30 (90.9%)	14 (82.4%)	.36
Right testicular volume, mL	12 [8.8–14]	12 [8.4–17.5]	.39
Left testicular volume, mL	12 [6–14]	11.3 [7–17.5]	.53
Normal karyotype, %	21/24 (87.5%)	13/13 (100%)	.53
Chemical pregnancy per transfer	31/73 (42.5%)	12/38 (31.6%)	.31
Clinical pregnancy per transfer	26/73 (35.6%)	10/38 (26.3%)	.39
Early pregnancy loss	6/73 (8.2%)	1/38 (2.6%)	.42
Pre-term delivery	8/73 (11.0%)	3/38 (7.9%)	.75
Number of newborns	21/73 (28.8%)	7/38 (18.4%)	.26
Newborn malformation	1/73 (1.4%)	0	

Data is presenting by median (25%–75%) and percentage.
TESE = testicular sperm extraction; FSH = follicular stimulating hormone; LH = luteinizing hormone

Table 5

Pregnancy outcomes of smokers and non-smokers azoospermic patients.

Characteristic	Smokers N=73 (1)			Non-smokers N=38 (2)		
	No pregnancy	Pregnancy	P value	No pregnancy	Pregnancy	P value
Male age, yr	35 [30–36]	35 [30–36]	.66	34.5 [30.8–48]	30 [29–32.5]	.024
Ethnicity (%, Jews)	7 (16.7%)	11 (35.5%)	.099	16 (61.5%)	8 (66.7%)	1.00
BMI, kg/m²	27.7 [21.8–28.8]	28.4 [24.2–29.8]	.25	27.2 [24.8–29.1]	25.1 [23.4–26.4]	.014
Morbidity	14 (33.3%)	12 (38.7%)	.80	12 (46.2%)	5 (41.7%)	1.00
Serum FSH level, IU	12 [4–22]	12 [4–22]	.82	8 [4.9–19.5]	5.6 [2.0–6.7]	.023
Serum LH level, IU	6 [4–9.9]	6.4 [4.1–10.8]	083	4.9 [3.9–12.0]	6.0 [4–7]	.55
Sperm motility TESE, %	8 (21.6%)	3 (11.1%)	.33	12 (50.0%)	5 (50.0%)	1.00
Testosterone, ng/dL	13 [10.4–19]	15 [9.6–21]	.93	17 [14.5–24.5]	15 [4.7–17.8]	.028
Presence of sperm in specimen	39 (95.1%)	28 (96.6%)	1.00	22 (84.6%)	12 (100%)	.28
Right testicular volume, mL	12 [10–13.6]	13.6 [12–14]	.77	10 [9.9–16]	10 [6–20]	.55
Left testicular volume, mL	12 [9.8–14]	12.5 [12–14]	.86	10 [7–13]	8.3 [6–20]	.50
Normal karyotype, %	32/35 (91.4%)	18/20 (90%)	1.00	19 (73.1%)	10 (83.3%)	.69
Early pregnancy loss	NA	6 (19.4%)		NA	1 (8.3%)	
Pre-term delivery	NA	8 (25.8%)		NA	3 (25.0%)	
Number of newborns	NA	21/31		NA	7	
Number of newborns	NA	26/31		NA	8	
Newborn malformation	NA	1 (3.2%)		NA	0	

Data are presented as median (25%–75%) and percentage.
BMI=body mass index, FSH=follicular stimulating hormone, LH=luteinizing hormone, NA=not applicable.

toxins originating from cigarettes damage sperm mitochondrial activity and damage the chromatin structure in human sperm, therefore, leading to impaired fertilization capacity was not evaluated among azoospermic patients with very poor semen quality.[17,18]

BMI was also evaluated for its influence on male fertility. Evidence varies regarding male obesity and subfertility. Increased DNA damage to sperm, decreased mitochondrial activity, increased oxidative stress in testes, and poorer ART outcomes were suggested to be s influenced by obesity. Conflicting evidence is presented in studies discussing the influence of BMI on sperm quality but it was related to decreased ejaculate volume, lower sperm concentration, and increased DNA damage.[4,19,20] Other studies reported no significant correlation between BMI and semen parameters.[21] A meta-analysis by Le et al demonstrated that overweight or obesity in males had no negative influence on clinical pregnancy and live birth rates in ART.[22] Our study did

not demonstrate any effect of overweight on semen parameters after TESE or on ICSI outcomes.

Paternal age might have an effect on pregnancy outcomes in ART. Conflicting data have been presented regarding an association between increased paternal age and lower fertilization rates, decreased blastocyst formation, and pregnancy rates.[23–26] Univariate regression revealed that paternal age above 37 years was significantly adversely correlated with chemical pregnancy (P=.032) and clinical pregnancy (P=.039). The sensitivity was 92.5%, but the specificity was 24.3% and this was not seen in multivariate regression.

Paternal FSH and testosterone in our study did not correlate with TESE outcomes. Other studies found that an FSH cutoff of 20 IU predicted pregnancy.[27] Cissen et al developed a predictive model for obtaining spermatozoa with TESE In a retrospective, multicenter study, with data from 1371 TESE procedures they found that older male age, higher levels of serum testosterone and

Table 6

Comparison between pregnant to non-pregnant groups with sub-analyzing to smoking.

	No pregnancy (n=68) (Group 1)			Pregnancy (n=43) (Group 2)		
	Non-smokers	Smokers	P value	Non-smokers	Smokers	P value
Age, yr	34.5 [30.8–48.0]	35 [30–36]	.26	30 [29–32.5]	35 [30–36.0]	.015
BMI, kg/m²	27.2 [24.8–29.]	27.7 [21.8–28.8]	.13	25.1 [23.4–26.4]	28.4 [24.2–29.8]	.13
Morbidity, %	12 (46.2%)	14 (33.3%)	1.00	5 (41.7%)	12 (38.7%)	.64
FSH, IU/L	8 [4.9–19.5]	12 [4–22]	.82	5.6 [2–6.7]	12 [4–22]	NS
LH, IU/L	4.9 [3.9–12.0]	6.0 [4–9.9]	.39	6 [4–7]	6.4 [4.1–10.8]	.12
Sperm motility in TESE, %	12 (50%)	8 (21.6%)	.02	5 (50.0%)	3 (11.3%)	.028
Testosterone, ng/dL	17 [14.5–24.]	13 [10.4–19.0]	.24	15 [47–17.8]	15 [9.6–21.0]	.065
Presence of sperm in specimen	22 (84.6%)	39 (95.1%)	1.00	0	1 (3.4%)	.19
Right testicular volume, mL³	10 [9.9–16.0]	12 [10–13.6]	.78	10 [6–20]	13.6 [12–14]	.84
Left testicular volume, mL³	10 [7–13]	12 [9.8–14]	.71	8.3 [6–20]	12.5 [12–14]	.52
Early pregnancy loss	—			1 (8.3%)	6 (19.4%)	.65
Pre-term delivery	—			9 (25.0%)	8 (25.8%)	1.00
Number of newborns	—			7 (58.3%)	21 (67.7%)	.72

Data are presented as median (25%–75%) and percentage.
BMI=body mass index, FSH=follicular stimulating hormone, LH=luteinizing hormone, TESE=testicular sperm extraction

lower FSH and luteinizing hormone (LH) levels were predictive of successful sperm retrieval. They did not evaluate fertilization and pregnancy rates.[28]

This study had a few limitations, including the relatively small sample size and retrospective design. We included all azoospermic patients who underwent TESE, without reference to the etiology of azoospermia. Moreover, potential confounders include patient selection, and clinical and laboratory techniques used to find spermatozoa.

The strengths of this study include evaluation of the female partner and fertilization rates. The long term follow-up included pregnancy and delivery rates and neonatal outcomes.

In conclusion, this study evaluated the influence of different parameters on TESE–ICSI cycles. In those studied patients, as sperm quality is extremely low, no adverse influence on sperm parameters was seen. However, we advise our patients to maintain a healthy lifestyle, including normal weight and avoiding smoking, to prevent future health problems.

Author contributions

All authors contributed substantially to this work. The authors collectively developed the original concept of this study. GS wrote the manuscript. ESP revised it critically. Data collection was performed by YB, GS, and YA, statistical analysis by ESP. All authors contributed to critical discussion and reviewed and approved the final version of the manuscript for submission.

Conceptualization: Guy Shrem, Adrian Ellenbogen, Einat Shalom-Paz.

Data curation: Guy Shrem, Yana Brudner, Einat Shalom-Paz.

Formal analysis: Guy Shrem, Einat Shalom-Paz.

Investigation: Guy Shrem, Mediea Michaeli, Einat Shalom-Paz.

Methodology: Guy Shrem, Einat Shalom-Paz.

Project administration: Guy Shrem, Einat Shalom-Paz.

Resources: Guy Shrem.

Supervision: Einat Shalom-Paz.

Visualization: Yuval Azmon, Einat Shalom-Paz.

Writing – original draft: Guy Shrem.

Writing – review & editing: Guy Shrem, Einat Shalom-Paz.

Guy Shrem orcid: 0000-0002-3825-3965.

References

[1] Silber SJ, Van Steirteghem AC, Liu J, et al. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. Hum Reprod 1995;10:148–52.

[2] Tournaye H, Verheyen G, Nagy P, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients. Hum Reprod 1997;12:80–6.

[3] Practice Committee of the American Society for Reproductive Medicine. "Smoking and infertility: a committee opinion." Fertil Steril. 2012;98: 1400–1406.

[4] Practice Committee of the American Society for Reproductive Medicine. "Obesity and reproduction: a committee opinion."Fertil Steril. 2015; 104:1116–1126.

[5] American Society for Reproductive Medicine. Evaluation of the azoospermic male. Fertil Steril 2008;90:S74–S77.

[6] Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod 2000;15:2278–84.

[7] Van Steirteghem AC, Liu J, Joris H, et al. Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report

of a second series of 300 consecutive treatment cycles. Hum Reprod 1993;8:1055–60.

[8] Meseguer M, Herrero J, Tejera A, et al. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26:2658–71.

[9] National Collaborating Centre for Women's and Children's Health (UK. Fertility: assessment and treatment for people with fertility problems. RCOG press; 2004.

[10] National Institute for Health and Care Excellence. Fertility Problems: Assessment and Treatment. London: NICE; 2013. Available at: https://www.nice.org.uk/guidance/cg156/resources/fertility-problems-assessment-and-treatment-35109634660549 [accessed date October 14, 2016].

[11] Li Y, Lin H, Li Y, et al. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril 2011;95:116–23.

[12] Lotti F, Corona G, Vitale P, et al. Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples. Hum Reprod 2015;30: 590–602.

[13] Sharma R, Harlev A, Agarwal A, et al. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur Urol 2016;70:635–45.

[14] Künzle R, Mueller MD, Hänggi W, et al. Semen quality of male smokers and nonsmokers in infertile couples. Fertil Steril 2003;79:287–91.

[15] Waylen AL, Metwally M, Jones GL, et al. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis. Hum Reprod Update 2009;15:31–44.

[16] Fraga CG, Motchnik PA, Shigenaga MK, et al. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc Natl Acad Sci U S A 1991;88:11003–6.

[17] Sharma R, Biedenharn KR, Fedor JM, et al. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol 2013;11:66.

[18] Calogero A, Polosa R, Perdichizzi A, et al. Cigarette smoke extract immobilizes human spermatozoa and induces sperm apoptosis. Reprod Biomed Online 2009;19:564–71.

[19] Chavarro JE, Toth TL, Wright DL, et al. Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. Fertil Steril 2010; 93:2222–31.

[20] Eisenberg ML, Kim S, Chen Z, et al. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. Hum Reprod 2014;29:193–200.

[21] Macdonald AA, Stewart AW, Farquhar CM. Body mass index in relation to semen quality and reproductive hormones in New Zealand men: a cross-sectional study in fertility clinics. Hum Reprod 2013;28:3178–87.

[22] Le W, Su S-H, Shi L-H, et al. Effect of male body mass index on clinical outcomes following assisted reproductive technology: a meta-analysis. Andrologia 2016;48:406–24.

[23] Frattarelli JL, Miller KA, Miller BT, et al. Male age negatively impacts embryo development and reproductive outcome in donor oocyte assisted reproductive technology cycles. Fertil Steril 2008;90:97–103.

[24] Watanabe Y, Cornet D, Merviel P, et al. Influence of husband's age on outcome of a shared oocyte donation program. Fertil Steril 2000;74: S78–9.

[25] Klonoff-Cohen HS, Natarajan L. The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or gamete intrafallopian transfer. Am J Obstet Gynecol 2004;191: 507–14.

[26] Aboulghar M, Mansour R, Al-Inany H, et al. Paternal age and outcome of intracytoplasmic sperm injection. Reprod Biomed Online 2007; 14:588–92.

[27] Zitzmann M, Nordhoff V, von Schönfeld V, et al. Elevated follicle-stimulating hormone levels and the chances for azoospermic men to become fathers after retrieval of elongated spermatids from cryopreserved testicular tissue. Fertil Steril 2006;86:339–47.

[28] Cissen M, Meijerink AM, D'Hauwers KW, et al. Prediction model for obtaining spermatozoa with testicular sperm extraction in men with non-obstructive azoospermia. Hum Reprod 2016;31:1934–41.