Accepted: 25 July 2017

DOI: 10.1111/and.12889

ORIGINAL ARTICLE

WILEY | First International Journal of Andrology | ANDROLOGIA

Association between body mass index and sperm quality and sperm DNA integrity. A large population study

J. B. A. Oliveira[1,2] | C. G. Petersen[1,2] | A. L. Mauri[1,2] | L. D. Vagnini[2] | A. Renzi[2] | B. Petersen[2] | M. Mattila[1] | F. Dieamant[1,2] | R. L. R. Baruffi[1,2] | J. G. Franco Jr[1,2]

[1]Center for Human Reproduction Prof. Franco Jr, Ribeirão Preto, SP, Brazil

[2]Paulista Center for Diagnosis Research and Training, Ribeirão Preto, SP, Brazil

Correspondence
Jose G. Franco Jr, Center for Human Reproduction Prof. Franco Jr, Ribeirão Preto, SP, Brazil.
Email: crh@crh.com.br

Summary

This study aimed to analyse whether the functional quality of spermatozoa is associated with body mass index (BMI). Semen samples were obtained from 1824 men undergoing fertility evaluation/treatment. Semen analysis was performed using World Health Organization (WHO) criteria, and morphology was evaluated with the motile sperm organelle morphology examination (MSOME). The percentages of sperm DNA fragmentation (using TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling (TUNEL) assays), sperm chromatin packaging/underprotamination (using chromomycin A3/CMA_3), mitochondrial damage (using MitoTracker Green) and apoptosis (using annexin V) were also assessed. At least 200 spermatozoa were examined in each evaluation. The following BMI values were used as cut-off points: $\leq$24.9 kg/m^2, 25–29.9 kg/m^2 (overweight) and $\geq$30 kg/m^2 (obese). High BMI negatively affects sperm concentration, vitality, motility and morphology ($p < .05$). Conversely, high BMI does not seem to be associated with impaired sperm DNA integrity, as assessed by DNA fragmentation, sperm protamination and sperm apoptosis ($p > .05$). However, increased BMI is associated with increased mitochondrial damage in spermatozoa ($p < .05$). In conclusion, given the adverse consequences of obesity and the possible effect of male BMI on assisted reproduction technology (ART) outcomes, the benefits of weight reduction should be discussed when counselling couples interested in fertility treatment.

KEYWORDS

body mass index, mitochondria, obesity, spermatozoon

1 | INTRODUCTION

Due to changes towards more sedentary lifestyle, as well as dietary changes, obesity is currently a cause of concern worldwide and has even reached epidemic proportions in several countries. Individuals with obesity or overweight currently represent more than two-thirds of the population of developed (EASO, 2014; GHO, 2016; Ogden, Carroll, Kit, & Flegal, 2014) and developing (EASO, 2014; GHO, 2016) countries. According to the World Health Organization (WHO), most of the world's population live in countries where overweight and obesity kill more people than underweight (WHO, 2016). Obesity is associated with multiple inter-related disorders, such as insulin resistance/diabetes, arterial hypertension, dyslipidaemia and sleep apnoea, which together contribute to the development of metabolic syndrome and are associated with a greater reduction in life expectancy (Flegal, Graubard, Williamson, & Gail, 2007; Flegal, Kit, Orpana, & Graubard, 2013; Kyrou, Randeva, & Weickert, 2014; Yoon, Bastian, Anderson, Collins, & Jaffe, 2014). In addition, obesity might also affect reproductive function.

Presented at the 32nd Annual Meeting of ESHRE, Helsinki, Finland, 2016.

© 2017 Blackwell Verlag GmbH

Concerning men, a decline in semen quality was reported in parallel to the obesity epidemic. Nevertheless, the studies addressing specific relationships between semen parameters and obesity have yielded contradictory results. Although some recent data suggest a correlation between the increase in obesity and a reduction in sperm concentration, motility and/or morphology (Alshahrani, Ahmed, Gabr, Abalhassan, & Ahmad, 2016; Belloc et al., 2014; Bieniek et al., 2016; Dupont et al., 2013; Eisenberg et al., 2014; Luque et al., 2015; MacDonald, Stewart, & Farquhar, 2013; Taha et al., 2016; Umul et al., 2015), other studies did not detect statistically significant adverse effects of obesity on semen quality (Bandel et al., 2015; Thomsen, Humaidan, Bungum, & Bungum, 2014). Although three meta-analyses were published, the contradictions remain. MacDonald, Herbison, Showell, and Farquhar (2010) did not find evidence of an association between increased obesity and semen parameters. The main limitation of this review is that data from most studies could not be aggregated for meta-analysis. Later, Sermondade et al. (2013) observed that overweight and obesity were associated with an increased prevalence of azoospermia or oligozoospermia. The main limitation of this report is that the studied populations varied, with men recruited from both the general population and infertile couples. Subsequently, in the meta-analysis of Campbell, Lane, Owens, and Bakos (2015), a clinically significant association was not found for conventional semen (except abnormal morphology), despite the demonstration that paternal obesity negatively affects male fertility. However, many studies could not be aggregated, and some analyses found significant heterogeneity between studies. Nonconventional parameters of semen quality were also assessed and were mainly related to DNA damage; however, the number of such studies is still small, or their results are contradictory.

Given the discrepancies among study results and the belief that an understanding of the relationship between obesity and male fertility will allow better counselling of infertile couples, this study aimed to analyse whether sperm quality (volume, pH, concentration, motility, morphology and vitality) or sperm DNA integrity (DNA fragmentation, apoptosis, underprotamination and mitochondrial damage) are associated with obesity assessed based on the body mass index (BMI).

2 | MATERIALS AND METHODS

2.1 | Population

Semen samples (one per subject) were obtained from 1824 men from a random group of couples undergoing infertility investigation and treatment from January 2011 to December 2015 at the Centre for Human Reproduction Prof. Franco Jr. No men received an antioxidant treatment beforehand. Exclusion criteria were azoospermia, any known reproductive tract pathology in the last six months, any hormonal therapy in the last six months, chronic medical disorders, congenital genital tract abnormalities or previous treatment that can alter fertility (cancer treatment). Written informed consent was obtained from all participants, and this study was approved by the institutional review board and its local ethics committees.

2.2 | BMI determination

BMI, calculated by dividing body mass by the square of height [BMI = weight(kg)/ height^2(m)], is the method most widely used to estimate body fat (WHO – World Health organization, 2016). On the same day of semen collection, the weight (measured using a digital scale with a 180 kg platform 40 × 50—Filizola® Brazil) and height of each man included in this study was measured, and BMI was calculated. The scale used was periodically calibrated according to the manufacturer's instructions.

2.3 | Sample collection

Semen samples were collected in sterile containers by masturbation after a sexual abstinence period of 2–5 days. A portion of each semen sample was used for analysis according to the WHO guidelines (WHO, 2010). The other portion of each semen sample was immediately processed for morphological analysis by motile sperm organelle morphology examination (MSOME). The remainder of the semen samples was immediately processed for sperm DNA fragmentation analysis using the TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling (TUNEL) assay, sperm apoptosis analysis using the annexin V assay, sperm chromatin packing/protamination using chromomycin A3 (CMA_3) staining and sperm mitochondrial membrane potential (MMP) using MitoTracker Green FM.

2.4 | Determination of morphology by MSOME

MSOME procedures were performed as described previously (Oliveira et al., 2014; Silva et al., 2012). At least 200 motile spermatozoa per sample were evaluated, and the percentages of normal spermatozoa were determined.

2.5 | Determination of sperm DNA fragmentation

DNA fragmentation in spermatozoa was measured using the TUNEL assay, which was performed using an in situ cell death detection kit with tetramethylrhodamine red-labelled dUTP (Roche), as described previously (Oliveira et al., 2014; Vagnini et al., 2007). The final evaluation was performed using a fluorescence microscope (Olympus BX 50), and the percentage of TUNEL-positive spermatozoa was determined. At least 200 spermatozoa were evaluated for each slide, with the appropriate filter.

2.6 | Determination of sperm chromatin packaging/protamination

Sperm protamine deficiency (underprotamination)/chromatin packaging was measured using CMA_3 (Sigma-Aldrich), as described previously (Franco et al., 2012). The percentage of positive spermatozoa was determined by direct observation in four fields using a fluorescence microscope (Olympus BX 50), and the percentages of spermatozoa with abnormal chromatin packaging were determined. At least 200 spermatozoa were evaluated for each slide, with the appropriate filter.

2.7 | Determination of sperm apoptosis

Sperm apoptosis was measured using annexin V, a calcium-dependent phospholipid-binding protein with a high affinity for phosphatidylserine that is present in the inner leaflet of the sperm membrane, except in apoptotic spermatozoa, where phosphatidylserine is externalised. The sperm suspensions (1×10^6 cells/ml) were incubated in an appropriate binding buffer with 1 μl annexin V(green), 1 μl propidium iodide (PI) (red) (Dead Cell Apoptosis Kit with Annexin V Alexa Fluor® 488 & Propidium Iodide; Molecular Probes, Eugene, OR) and 1 μl a cell-permeable DNA stain Hoechst 33342 (blue) (Molecular Probes) at room temperature for 15 min in the dark. The PI dye is impermeable to live cells. After incubation, the suspension was centrifuged at 800 *g* for 10 min, and the pellet was mounted on poly-l-lysine-coated slides for examination under a fluorescence microscope (Olympus BX 50). From the analysis, subpopulations of spermatozoa could be identified: annexin V(−)/PI(−)—live intact sperm; annexin V(+)/PI(−)—early apoptotic cells; and annexin V(+/−)/PI(+)—necrotic cells. The percentages of early apoptotic cells (defined as the number of positive annexin V/negative PI spermatozoa divided by the total number of spermatozoa ×100) were determined. At least 200 spermatozoa were evaluated for each slide, with the appropriate filter.

2.8 | Determination of sperm MMP

Sperm MMP, an indicator of sperm mitochondrial functionality, was determined using MitoTracker Green FM (Molecular Probes). The live sperm suspensions were incubated in phosphate-buffered saline (PBS) containing 20 nmol/L MitoTracker for 20 min at 37°C. To stain the sperm DNA, the samples were subsequently incubated with a cell-permeable DNA stain Hoechst 33342 (Molecular Probes), for 10 min at 37°C. After incubation, the suspension was centrifuged at 800 *g* (10 min), and the pellet was mounted on a microscope slide. Green fluorescence in the midpiece indicated active mitochondria. Sperm samples were examined using a fluorescence microscope (Olympus BX 50), and the percentages of spermatozoa with altered MMP/mitochondrial damage (i.e., absence of green fluorescence) were determined. At least 200 spermatozoa were evaluated for each slide, with the appropriate filter.

2.9 | Quality control

To control for intra-observer and inter-observer variability, multiple fractions of semen samples were obtained from randomly selected patients. Each sample was observed at least three times by the same observer (blinded to subject identity). Intra-observer and Inter-observer variations of ≈0.5–1% and 0.5–7%, respectively, were obtained for each parameter analysed: semen parameters (according to the WHO guidelines), normality of the spermatozoon (as a whole), normality of nuclear structure, TUNEL-positive sperm, CMA_3-positive sperm, annexin V-positive spermatozoa and MitoTracker Green-positive spermatozoa. These variations are comparable to those of classical sperm quality parameters (Auger et al., 2000).

2.10 | Sample size

Sample size was calculated by performing a comparison between two proportions. A sample size of 250 subjects in each group has 80% power to detect an increase of 15% with a significance level of 0.05 (two-tailed).

2.11 | Statistical analysis

The data were analysed using the StatsDirect statistical software (Cheshire, UK). Potential confounders (age, abstinence time, smoking, alcohol, varicocele and vitamin use) were also assessed. Regression and correlation analyses with continuous variables (age, abstinence time, sperm volume, sperm pH, sperm concentration, percentage of spermatozoa with progressive motility, percentage of total sperm motility, percentages of normal spermatozoa, number of leucocytes and percentage of live spermatozoa (vitality)) were performed using the Spearman rank correlation test. For dichotomous variables (smoking, alcohol, varicocele and vitamin use), correlations were determined using logistic regression.

For group comparisons, the following BMI values were used as cut-off points to divide the subjects into three groups: ≤24.9 kg/m^2 (healthy weight), 25.0–29.9 kg/m^2 (overweight) and ≥30.0 kg/m^2 (obese) (WHO – World Health organization, 2016). As the number of men with BMI <18.5 kg/m^2 (underweight, $n = 1$) and with BMI≥40 kg/m^2 (very severely obese, $n = 35$) was very low, they were included in the healthy weight and obese groups, respectively. The Mann–Whitney U-test, Student t-test and chi-squared test were used, as appropriate.

The level of significance was set at $p < .05$.

3 | RESULTS

3.1 | General population characteristics

The regression analysis did not show a correlation between BMI and subject age, history of fathering at least one child (or generating a pregnancy that had ended in miscarriage), time of infertility, tobacco use, regular alcohol use, presence of varicocele and vitamin supplement use. Confirming the results observed with regression analysis, an equal distribution ($p > .05$) of the main characteristics was observed for all three BMI groups. Table 1 summarises the data.

In addition, a multiple regression analysis was performed to control for the these factors together and again no correlation with BMI was observed.

3.2 | Semen quality and general semen parameters (Table 2)

Regression analysis: The analysis did not show an influence of BMI on time of sexual abstinence, semen pH, sperm volume and number of leucocytes ($p > .05$). However, sperm concentration, sperm motility, sperm morphology and sperm vitality worsened as BMI increased ($p < .05$).

TABLE 1 Correlation between general population characteristics and BMI

Characteristic	Regression analysis: r/OR	95% Confidence Interval	p	BMI Group: Total n = 1824	≤24.9 kg/m² n = 370 (20.3%)	25–29.9 kg/m² n = 856 (46.9%)	≥30 kg/m² n = 598 (32.8%)	p
Age (years)[a]	r: −.04	−0.08 to 0.01	.11	37.9 ± 6.6	38.3 ± 7.0	37.8 ± 6.5	38.0 ± 6.4	.41
Fathered at least one child[b]	OR: .99	0.98 to 1.02	.92	31.4% (573/1824)	28.6% (106/370)	33.2% (284/856)	30.6% (183/598)	.25
Time of infertility (years)[a]	r: .00	−0.05 to 0.05	.96	4.1 ± 3.2	4.3 ± 3.6	4.1 ± 3.1	4.2 ± 3.3	.78
Tobacco use (%)[b]	OR: 1.02	0.99 to 1.06	.14	11.2% (204/1824)	10% (37/370)	10.7% (92/856)	12.5% (75/598)	.41
Regular alcohol use (%)[b]	OR: 1.02	0.99 to 1.04	.10	68.1% (1242/1824)	67.3% (249/370)	68.0% (582/856)	68.7% (411/598)	.89
Varicocele (%)[b]	OR: 0.98	0.96 to 1.02	.35	16.5% (301/1824)	18.9% (70/370)	15% (128/856)	17.2% (103/598)	.19
Vitamin supplement use (%)[b]	OR: 0.98	0.95 to 1.01	.20	16.9% (309/1824)	19.4% (72/370)	15.4% (132/856)	17.6% (105/598)	.20

r: Spearman's rank correlation coefficient; OD: odds ratio.
[a]Spearman's correlation.
[b]Logistic regression.

TABLE 2 Correlation between general semen parameters and BMI

Semen Parameter	Regression analysis: Spearman's Rank Correlation	95% Confidence Interval	p	BMI Group: Total	≤24.9 kg/m²	25–29.9 kg/m²	≥30 kg/m²	p
Abstinence (days)	r: .01	−0.03 to 0.06	.53	3.5 ± 1.4	3.5 ± 1.9	3.4 ± 1.0	3.5 ± 1.4	.96
pH	r: .04	−0.01 to 0.09	.10	8.0 ± 0.4	8.0 ± 0.3	8.0 ± 0.3	8.1 ± 0.4	.94
Volume (ml)	r: −.02	−0.07 to 0.02	.34	2.8 ± 1.6	2.8 ± 1.5	2.9 ± 1.6	2.8 ± 1.6	.53
Concentration (ml × 10^6)	r: −.18	−0.22 to −0.13	<.0001	72.4 ± 56.7	83.5 ± 62[a]	77.1 ± 56.3[b]	58.8 ± 51.1[a,b]	[a,b]<.0001
Progressive motility (rapid + slow progression) (%)	r: −.19	−0.23 to −0.14	<.0001	55.5 ± 15.7	58.6 ± 13.8[a]	56.8 ± 14.6[b]	51.5 ± 17.5[a,b]	[a,b]<.0001
Total motility (%)	r: −.18	−0.22 to −0.13	<.0001	62.7 ± 15.5	66.0 ± 13.2[a,b]	63.8 ± 14.8[a,c]	59.0 ± 17.1[b,c]	[a].01 [b,c]<.0001
Normal sperm structure (%)	r: −.11	−0.11 to −0.20	<.0001	0.6 ± 0.8	0.7 ± 0.9[a]	0.6 ± 0.8[b]	0.4 ± 0.7[a,b]	[a,b]<.0001
Leucocytes (×10^6/ml)	r: .03	−0.01 to 0.08	.13	0.4 ± 0.9	0.3 ± 0.6	0.4 ± 0.9	0.4 ± 1.1	.66
Vitality (%)	r: −.17	−0.22 to −0.12	<.0001	64.2 ± 14.3	67.6 ± 12.3[a,b]	65.0 ± 13.6[a,c]	60.8 ± 15.6[b,c]	[a].009 [b,c]<.0001

Values within rows with the same superscripted letter are significantly different.

BMI groups analysis: As in the regression analysis, an influence of BMI on time of sexual abstinence, semen pH, sperm volume and number of leucocytes was not observed ($p > .05$). However, sperm concentration, sperm motility, sperm morphology and the sperm vitality worsened with increase in BMI.

3.3 | Sperm DNA fragmentation, sperm chromatin packing, sperm apoptosis and sperm MMP (Table 3)

Regression analysis: An influence of BMI on sperm DNA fragmentation (percentage of TUNEL-positive sperm), sperm apoptosis (percentage of annexin V-positive sperm) and sperm chromatin packing (percentage of CMA_3-positive sperm) was not observed ($p > .05$). However, sperm MMP/mitochondrial damage worsened as BMI increased ($p < .05$).

BMI groups analysis: As in the regression analysis, BMI had no influence on sperm DNA fragmentation, sperm apoptosis and sperm chromatin packing ($p > .05$), but sperm MMP worsened with increase in BMI.

4 | DISCUSSION

Our results for a large population of men under investigation/treatment for infertility demonstrate a clear and significant reduction in semen quality as obesity increases. Despite the significant correlation between BMI and some semen parameters ($p < .05$), the correlation could be considered weak (Spearman's $r = -.17$ to $.26$). However, the correlation between BMI and some semen parameters was quite similar to the following results of various other authors: Bieniek et al. (2016): ejaculate volume, $r = -.04$, sperm concentration $r = -.08$, motility $r = -.07$ and morphology $r = -.04$, $p < .05$; Taha et al. (2016): sperm concentration $r = -.09$, $p = .01$; Alshahrani et al. (2016): sperm concentration $r = -10$, $p = .03$; Leisegang, Bouic, Menkveld, and Henkel (2014): sperm concentration $r^2 = -0.36$, total sperm count $r^2 = -0.33$, motility $r^2 = -0.07$, vitality $r^2 = -0.31$ and DNA fragmentation $r^2 = 0.39$, $p < .05$; and Hofny et al. (2010): abnormal sperm morphology $r = .04$, sperm concentration $r = -.43$ and sperm motility $r = .41$, $p < .05$. Regardless, all the correlations found in the present study were confirmed using group analysis, further supporting the significance of these findings.

Because spermatogenesis requires a controlled testicular environment and intact endocrine signalling via the hypothalamic–pituitary–testicular axis, the impact of obesity on semen quality might be mainly attributed to endocrine mechanisms. In obese men, insulin causes a reduction in sex hormone-binding globulin (SHBG) levels, with a consequent increase in the androgens available for conversion to oestrogen by adipose tissue aromatase (Hajshafiha, Ghareaghaji, Salemi, Sadegh-Asadi, & Sadeghi-Bazargani, 2013; Håkonsen et al., 2011; MacDonald et al., 2010; Palmer, Bakos, Fullston, & Lane, 2012; Teerds, de Rooij, & Keijer, 2011). In addition, in all obese men, the serum levels of total and bioavailable testosterone and inhibin B are reduced, which is associated with a decrease in the luteinising hormone (LH) pulse amplitude (Crujeiras & Casanueva, 2015; Hajshafiha et al., 2013; Håkonsen et al., 2011; MacDonald et al., 2010; Palmer et al., 2012; Stewart et al., 2009; Tunc, Bakos, & Tremellen, 2011; Vermeulen, Kaufman, Deslypere, & Thomas, 1993). This hormonal profile suggests an increase in the oestrogen-induced pituitary negative feedback and a reduction in testosterone secretion by Leydig cells (Crujeiras & Casanueva, 2015; Hajshafiha et al., 2013; Hofny et al., 2010; Leisegang et al., 2014; Palmer et al., 2012; Tunc et al., 2011; Vermeulen et al., 1993). In addition, a preferential accumulation in the adipose tissue of toxic substances and fat-soluble endocrine disruptors might amplify these abnormalities (Katib, 2015; Sermondade et al., 2013). Furthermore, obese men are predisposed to an increase in scrotal temperature, due to the accumulation of fatty tissue around the scrotum, which may cause oxidative stress in the testicles with consequent adverse effects on semen parameters (Crujeiras & Casanueva, 2015; Fariello et al., 2012; Jung & Schill, 2000; Palmer et al., 2012).

However, the semen parameters analysed in the present study showed variable susceptibility to changes in BMI. Our results did not indicate a significant relationship between BMI and sperm pH or ejaculate volume, but the vitality decreased as the obesity increased. The correlation between pH and BMI is not usually analysed but tends

TABLE 3 Sperm DNA fragmentation, sperm chromatin packing, sperm apoptosis and sperm mitochondrial membrane potential (MMP) according to BMI

	Regression analysis			BMI group				
Parameter	Spearman's Rank Correlation	95% Confidence Interval	p	Total	≤24.9 kg/m^2	25–29.9 kg/m^2	≥30 kg/m^2	p
DNA fragmentation (%)	r: .01	−0.07 to 0.10	.76	14.5 ± 7.8	14.4 ± 7.5	14.5 ± 8.2	14.5 ± 7.4	.74
Apoptosis (%)	r: .01	−0.04 to 0.06	.81	19.2 ± 7.9	19.5 ± 8.1	19.2 ± 8.0	19.1 ± 7.6	.75
CMA_3 positivity (%)	r: .02	−0.2 to 0.07	.37	56.1 ± 15.2	56.3 ± 15.5	55.7 ± 15.1	56.4 ± 15.1	.73
Abnormal MMP (%)	r: .24	0.15 to 0.27	<.0001	26.0 ± 16.4	20.6 ± 13.1[a,b]	25.3 ± 15.7[a,c]	30.9 ± 18.3[b,c]	[a].0004 [b]<.0001 [c].0001

Values within rows with the same superscripted letter are significantly different.

to confirm our findings (Belloc et al., 2014). A negative correlation between vitality and BMI, which is more frequently assessed, was also reported by Leisegang et al. (2014), Andersen et al. (2015) and Taha et al. (2016). However, Fariello et al. (2012), Belloc et al. (2014), Eisenberg et al. (2014) and Luque et al. (2015) repudiate this association. Ejaculate volume has received considerable attention. The studies by Shayeb, Harrild, Mathers, and Bhattacharya (2011), Hammiche et al. (2012), Belloc et al. (2014), Eisenberg et al. (2014) and Bieniek et al. (2016) revealed a significant decline in ejaculate volume with increasing BMI. However, similar to our results, a large number of studies failed to demonstrate a significant relation between BMI and semen volume (Alshahrani et al., 2016; Chavarro, Toth, Wright, Meeker, & Hauser, 2010; Duits, Van Wely, Van Der Veen, & Gianotten, 2010; Fariello et al., 2012; Gutorova, Kleshchyov, Tipisova, & Osadchuk, 2014; La Vignera, Condorelli, Vicari, & Calogero, 2012; Leisegang et al., 2014; Martini et al., 2010; Pauli et al., 2008; Qin et al., 2007; Rybar, Kopecka, Prinosilova, Markova, & Rubes, 2011). In addition, two meta-analyses showed no significant relationship between BMI and semen volume (Campbell et al., 2015; MacDonald et al., 2010).

A significant number of studies (Aggerholm, Thulstrup, Toft, Ramlau-Hansen, & Bonde, 2008; Bandel et al., 2015; Duits et al., 2010; Dupont et al., 2013; Hajshafiha et al., 2013; La Vignera et al., 2012; MacDonald et al., 2013; Martini et al., 2010; Pauli et al., 2008; Qin et al., 2007; Rybar et al., 2011; Shayeb et al., 2011) did not detect any relationship between sperm concentration and BMI. Reinforcing these data, two meta-analyses showed no significant relationship between BMI and sperm concentration (Campbell et al., 2015; MacDonald et al., 2010). However, the results reported in the literature exhibit wide variation. In contrast to the just-reported findings, in our study, the sperm concentration exhibited a significant negative correlation with BMI, which is not an isolated finding. The results of other studies, including some recent ones, agree with ours (Alshahrani et al., 2016; Bakos, Henshaw, Mitchell, & Lane, 2011; Belloc et al., 2014; Bieniek et al., 2016; Braga et al., 2012; Eisenberg et al., 2014; Fariello et al., 2012; Hammiche et al., 2012; Hammoud et al., 2008; Hofny et al., 2010; Jensen et al., 2004; Luque et al., 2015; Sermondade, Faure, et al., 2012; Stewart et al., 2009; Taha et al., 2016; Tunc et al., 2011). In addition, in their meta-analysis, Sermondade et al. (2013) found that overweight and obesity were associated with an increased prevalence of azoospermia or oligozoospermia.

In our study, both the total and progressive sperm motility exhibited a significant negative correlation with BMI. However, a comparison of results with other studies is again problematic due to conflicting results. Hammoud et al. (2008), Hofny et al. (2010), Martini et al. (2010), Hammiche et al. (2012), Braga et al. (2012), La Vignera et al. (2012), Fariello et al. (2012), Dupont et al. (2013), Belloc et al. (2014), Luque et al. (2015), Tang et al. (2015), Umul et al. (2015) and Taha et al. (2016) all published studies that reported a significant increase in the prevalence of spermatozoa with a reduction in total and/or progressive sperm motility in parallel to an increase in BMI. However, other studies did not detect any association between BMI and sperm motility (Alshahrani et al., 2016; Duits et al., 2010; Eisenberg et al., 2014; Gutorova et al., 2014; Hajshafiha et al., 2013; Pauli et al., 2008; Rybar et al., 2011; Shayeb et al., 2011). In addition, Bandel et al. (2015) found that obese men had a higher percentage of progressive motile spermatozoa than did normal-weight men. The available meta-analyses reflect such contradictory results. Although MacDonald et al. (2010) did not detect a significant correlation between sperm motility and BMI in their meta-analysis, Campbell et al. (2015) showed a small but significant decrease in motility for obese men in the overall population (general population and infertile couples), with a nonsignificant trend of a decrease in the infertile population.

Our MSOME results for evaluating morphology showed a significant decrease in the percentage of morphologically normal spermatozoa as BMI increased. Unfortunately, few studies used MSOME as a criterion for morphological analysis, which makes the interpretation of the observed correlation challenging. Some studies applied different morphological criteria (e.g., morphology by the WHO criteria or Kruger's strict criteria), and the results agree with ours and also indicate a correlation between an increase in obesity and poorer sperm morphology (Bieniek et al., 2016; Hofny et al., 2010; La Vignera et al., 2012; Luque et al., 2015; MacDonald et al., 2013; Shayeb et al., 2011; Taha et al., 2016). However, different authors failed to find any relationship (Alshahrani et al., 2016; Bakos et al., 2011; Belloc et al., 2014; Chavarro et al., 2010; Duits et al., 2010; Dupont et al., 2013; Eisenberg et al., 2014; Fariello et al., 2012; Hajshafiha et al., 2013; Leisegang et al., 2014; Martini et al., 2010; Pauli et al., 2008; Qin et al., 2007; Umul et al., 2015). The available meta-analyses make these divergences even more evident. MacDonald et al. (2010) observed that the largest study included found no significant association between BMI and sperm morphology, although the results from smaller studies varied. On the contrary, Campbell et al. (2015) found that in their meta-analysis of studies using the WHO criteria, when the analysis was restricted to the clinical assisted reproduction technology (ART) population, the poorer morphology for obese men compared with normal-weight men was statistically significant. However, when the analysis also included studies that were conducted with the overall population, a nonsignificant decrease was found. When studies using Kruger's criteria were subjected to meta-analysis, the results showed no significant differences.

Several issues should be considered when evaluating the discrepancies among studies on the BMI–semen quality relationship. The sample size might possibly explain the variation among studies because an analysis of larger populations might yield more consistent data. Nevertheless, studies with samples composed of more than 1,000 individuals reported variable results. Similarly to us, some authors found significant abnormalities in the analysed set of semen parameters as BMI increased (motility, concentration and morphology) (Bieniek et al., 2016; Tsao et al., 2015); however, several others reported a selective decline in some semen parameters (Belloc et al., 2014; Jensen et al., 2004; Paasch, Grunewald, Kratzsch, & Glander, 2010; Shayeb et al., 2011) or did not find any significant relationship between semen parameters and BMI (Aggerholm et al., 2008; Bandel et al., 2015; Duits et al., 2010). In essence, these results reproduce the distribution of the results obtained from the analysis of smaller populations. The reproductive status of a population (fertile/overall

population or subfertile) might also influence the results. However, once again, studies either demonstrate or refute the correlation between an increase in BMI and poorer semen quality, even when using selective approaches (some parameters only) in studies targeting the subfertile population (Alshahrani et al., 2016; Belloc et al., 2014; Bieniek et al., 2016; Eisenberg et al., 2014; Luque et al., 2015; Tang et al., 2015) and in studies of the overall population (Aggerholm et al., 2008; Andersen et al., 2015; Bandel et al., 2015; Jensen et al., 2004; Kort et al., 2006; Taha et al., 2016; Tsao et al., 2015). This issue is complicated by the actual degree of reliability of the classification of the population reproductive status because subfertile individuals are usually identified as members of couples under assessment/treatment for infertility.

Differences in the methods used to assess the sperm quality among laboratories and in statistical analysis methods likely contribute to the differences between these studies. The study of sperm morphology provides an illustrative example of the high inter-rater variability and the differences in the standards used for categorisation. One should still consider confounding factors, which might further affect the sperm quality. Similar to our study, most studies also control for factors such as age, abstinence, use of recreational drugs and infection in the analysis of the relationship between semen parameters and BMI. However, additional observations of other unusual factors could provide more important information about controversial results. Differences in the genetic backgrounds of various ethnic populations should also be considered. In addition, the possibility of harder to control/assess factors, such as diet type and action of pollutants, should also be taken into account.

Male obesity has been associated with a reduction in pregnancy and live birth rates (Bakos et al., 2011; Campbell et al., 2015), and one plausible explanation for these results is that obese men may have more spermatozoa with damaged DNA. Defects in protamination and apoptosis were suggested as an explanation for sperm DNA fragmentation (Agarwal, Virk, Ong, & du Plessis, 2014; Sakkas & Alvarez, 2010). Our study, which used a CMA_3 (which binds to the same DNA sites as protamines) assay, failed to detect any relationship between protamination level/chromatin packing and BMI. Using this same technique (the CMA_3 assay), Rybar et al. (2011) also did not detect an effect of BMI on protamination. Similarly, using annexin V, we did not find any relationship between apoptosis and BMI. In contrast, La Vignera et al. (2012) found a statistically significant higher percentage of early apoptotic spermatozoa in overweight and obese men than in normal-weight men; this parameter was assessed also using annexin V. However, only a small number of patients (50 control, 50 overweight and 50 obese) were included. The paucity of published studies hinders the comparison of results; nonetheless, obesity seems to have little or no influence on both apoptosis and sperm protamination. Additional studies on this topic are welcome.

The TUNEL assay using fluorescence microscopy in our study was performed using a large population, and an increased risk of sperm DNA fragmentation was not observed in obese or overweight men. Some other published studies have reported conflicting results, possibly because of the heterogeneity of the techniques (some measured denatured DNA rather than proper DNA fragmentation) and the small samples used in some studies. Different from our results, Kort et al. (2006), who used the sperm chromatin structure assay (SCSA), and Taha et al. (2016), who used flow cytometry based on the fluorescence emission from individual spermatozoon stained with PI, reported an increased sperm DNA fragmentation rate in overweight and obese patients. Leisegang et al. (2014), who used the TUNEL assay with fluorescence microscopy, observed that obesity was associated with increased sperm DNA fragmentation. Chavarro et al. (2010) and Fariello et al. (2012), who used the comet assay method, La Vignera et al. (2012), who used the TUNEL assay with flow cytometry, and Dupont et al. (2013), who used the TUNEL assay with fluorescence microscopy, observed higher sperm DNA damage in obese men but not in overweight men. However, these studies were conducted using a relatively small population (ranging from 150 to 520), and some of these studies do not adjust for confounders such as age and tobacco. A recent meta-analysis (Campbell et al., 2015) reported a statistically significant increase in the percentage of spermatozoa with DNA fragmentation in obese men compared with normal-weight men, but only four studies with a small population could be included.

Nevertheless, many studies agree with ours. For infertile populations, Tunc et al. (2011), who used the TUNEL assay with fluorescence microscopy, and Smit, Romijn, Wildhagen, Weber, and Dohle (2010), Hammiche et al. (2012), Rybar et al. (2011) and Thomsen et al. (2014), who used the SCSA method, did not find any significant association between BMI and sperm DNA integrity, but only small populations (ranging from 81 to 612) were used for these studies. With general populations and the SCSA method, Håkonsen et al. (2011, 2012), Eisenberg et al. (2014) and Andersen et al. (2015) also reported increased sperm DNA fragmentation in overweight and obese patients, but these populations were also small (ranging from 43 to 501). However, Bandel et al. (2015) used the SCSA method in a study that was based on semen samples from 1503 men from a general population and found that high BMI is not associated with increased sperm DNA fragmentation. With these controversial results, further evaluation of the relation between obesity and DNA fragmentation is needed, but DNA fragmentation may not be the answer to the reduction in clinical finding following infertility treatment in obese/overweight men.

Although reactive oxygen species (ROS) participate in essential activities, such as the acrosome reaction, an imbalance between the semen antioxidative capacity and ROS production results in oxidative stress, which is a major cause of sperm function damage (Agarwal et al., 2014; Aitken, Jones, & Robertson, 2012; Fariello et al., 2012; Treulen, Uribe, Boguen, & Villegas, 2015). Increased ROS production has been associated with alterations in the mitochondrial membrane permeability, possibly leading to loss in the MMP and eventually to DNA fragmentation and the death of both somatic cells and spermatozoa (Agarwal et al., 2014; Aitken et al., 2012; Kroemer, Galluzzi, & Brenner, 2007; Malić Vončina et al., 2016; Treulen et al., 2015). We found a significant positive correlation between altered MMP and BMI. Fewer studies have assessed the impact of obesity on MMP, but all of them agree with our findings. La Vignera et al. (2012), who

assessed MMP by JC-1 staining, Fariello et al. (2012), who used the deposition of diaminobenzidine (DAB), and Leisegang et al. (2014), who used a DePsipher staining kit, demonstrated that the percentage of spermatozoa that had altered MMP was significantly higher in obese men than in normal-weight men. In addition, obese men presented with a higher percentage of spermatozoa with low MMP than overweight men (La Vignera et al., 2012). In contrast to our results, all these studies reported a concomitant increase in sperm DNA fragmentation in parallel as obesity increased. Differences in sample size (42–305 vs. 1,824 men) or in the techniques employed might account for this discrepancy. However, such interconnection (MMP loss/alteration) might not be the only factor affecting sperm DNA fragmentation (Lobascio et al., 2015; Malić Vončina et al., 2016).

Abnormalities in mitochondrial function might affect the sperm motility due to possible oxidative phosphorylation inhibition; for the latter, this change will result in decreased ATP production and thus a reduction in the availability of energy. Supporting this possible correlation, in our study, MMP was negatively correlated with vitality ($r = -.026$, $p < .0001$) and motility (total: $r = -.31$, $p < .0001$; progressive: $r = -.29$, $p < .0001$). Leisegang et al. (2014) report the same finding.

The major strength of this study is the large sample size. In addition, the study population comprised men from different age groups and included a large number of overweight and obese men, and the analysis controlled for intra- and inter-technician variability. The limitations were that BMI was the only studied measure of obesity and that the data were cross-sectional. The number of patients presenting overweight or obesity is very important (79.7%). Furthermore, because this study was conducted using couples who sought fertility treatment, it could be biased towards infertility. Caution should be used if generalising these results to the general population.

In conclusion, increased BMI in infertile men may negatively affect sperm quality. BMI does not seem to be associated with sperm DNA fragmentation, sperm apoptosis or sperm protamination but is associated with increased mitochondrial damage. Given the adverse consequences of obesity and the possible negative role of male BMI, the benefits of weight reduction should be discussed when counselling couples interested in fertility treatment. Analogous to some clinical conditions such as cardiovascular diseases or diabetes, it is believed that weight loss can benefit male fertility, helping to restore normality of hormonal profiles (ASRM, 2015; El Bardisi et al., 2016). However, existing data on the benefits of weight loss are unclear, and it should be noted the lack of well-designed studies that demonstrate improvement in seminal quality with BMI reduction (ASRM, 2015; El Bardisi et al., 2016). On the other hand, abrupt weight loss with restriction of intake/absorption of important nutrients can lead to worsening seminal quality (El Bardisi et al., 2016; Sermondade, Massin, et al., 2012).

ACKNOWLEDGEMENT

The authors wish to thank American Journal Experts for revising the English text.

ORCID

J. G. Franco http://orcid.org/0000-0001-5082-0240

REFERENCES

Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health*, *32*, 1–17.

Aggerholm, A. S., Thulstrup, A. M., Toft, G., Ramlau-Hansen, C. H., & Bonde, J. P. (2008). Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertility and Sterility*, *90*, 619–626.

Aitken, R. J., Jones, K. T., & Robertson, S. A. (2012). Reactive oxygen species and sperm function–in sickness and in health. *Journal of Andrology*, *33*, 1096–1106.

Alshahrani, S., Ahmed, A. F., Gabr, A. H., Abalhassan, M., & Ahmad, G. (2016). The impact of body mass index on semen parameters in infertile men. *Andrologia*, *48*, 1125–1129.

Andersen, J. M., Herning, H., Aschim, E. L., Hjelmesæth, J., Mala, T., Hanevik, H., … Witczak, O. (2015). Body mass index is associated with impaired semen characteristics and reduced levels of anti-müllerian hormone across a wide weight range. *PLoS ONE*, *10*, e0130210.

Auger, J., Eustache, F., Ducot, B., Blandin, T., Daudin, M., Diaz, I., … Jouannet, P. (2000). Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. *Human Reproduction*, *15*, 2360–2368.

Bakos, H. W., Henshaw, R. C., Mitchell, M., & Lane, M. (2011). Paternal body mass index is associated with decreased blastocyst development and reduced live birth rates following assisted reproductive technology. *Fertility and Sterility*, *95*, 1700–1704.

Bandel, I., Bungum, M., Richtoff, J., Malm, J., Axelsson, J., Pedersen, H. S., … Giwercman, A. (2015). No association between body mass index and sperm DNA integrity. *Human Reproduction*, *30*, 1704–1713.

Belloc, S., Cohen-Bacrie, M., Amar, E., Izard, V., Benkhalifa, M., Dalléac, A., & de Mouzon, J. (2014). High body mass index has a deleterious effect on semen parameters except morphology: Results from a large cohort study. *Fertility and Sterility*, *102*, 1268–1273.

Bieniek, J. M., Kashanian, J. A., Deibert, C. M., Grober, E. D., Lo, K. C., Brannigan, R. E., … Jarvi, K. A. (2016). Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of subfertile men. *Fertility and Sterility*, *106*, 1070–1075.

Braga, D. P., Halpern, G., Figueira, R. C., Setti, A. S., Iaconelli, A. Jr, & Borges, E. Jr (2012). Food intake and social habits in male patients and its relationship to intracytoplasmic sperm injection outcomes. *Fertility and Sterility*, *97*, 53–59.

Campbell, J. M., Lane, M., Owens, J. A., & Bakos, H. W. (2015). Paternal obesity negatively affects male fertility and assisted reproduction outcomes: A systematic review and meta-analysis. *Reproductive Biomedicine Online*, *31*, 593–604.

Chavarro, J. E., Toth, T. L., Wright, D. L., Meeker, J. D., & Hauser, R. (2010). Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men at- tending an infertility clinic. *Fertility and Sterility*, *93*, 2222–2231.

Crujeiras, A. B., & Casanueva, F. F. (2015). Obesity and the reproductive system disorders: Epigenetics as a potential bridge. *Human Reproduction Update*, *21*, 249–261.

Duits, F. H., Van Wely, M., Van Der Veen, F., & Gianotten, J. (2010). Healthy overweight male partners of subfertile couples should not worry about their semen quality. *Fertility and Sterility*, *94*, 1356–1359.

Dupont, C., Faure, C., Sermondade, N., Boubaya, M., Eustache, F., Clement, P., … Levy, R. (2013). Obesity leads to higher risk of sperm DNA damage in infertile patients. *Asian Journal of Andrology*, *15*, 622–625.

EASO - European Association for the Study of Obesity (2014). *Obesity, perception and policy: multi-country review and survey of policymakers 2014*. London: C3 Collaborating for Health.

Eisenberg, M. L., Kim, S., Chen, Z., Sundaram, R., Schisterman, E. F., & Buck Louis, G. M. (2014). The relationship between male BMI and waist circumference on semen quality: Data from the LIFE study. *Human Reproduction*, *29*, 193–200.

El Bardisi, H., Majzoub, A., Arafa, M., AlMalki, A., Al Said, S., Khalafalla, K., … Sabanegh, E. Jr (2016). Effect of bariatric surgery on sêmen parameters and sex hormone concentrations: A prospective study. *Reproductive BioMedicine Online*, *33*, 606–611.

Fariello, R. M., Pariz, J. R., Spaine, D. M., Cedenho, A. P., Bertolla, R. P., & Fraietta, R. (2012). Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. *BJU international*, *110*, 863–867.

Flegal, K. M., Graubard, B. I., Williamson, D. F., & Gail, M. H. (2007). Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA*, *298*, 2028–2037.

Flegal, K. M., Kit, B. K., Orpana, H., & Graubard, B. I. (2013). Association of all-cause mortality with overweight and obesity using standard body mass index categories: A systematic review and meta-analysis. *JAMA*, *309*, 71–82.

Franco, J. G. Jr, Mauri, A. L., Petersen, C. G., Massaro, F. C., Silva, L. F., Felipe, V., … Vagnini, L. D. (2012). Large nuclear vacuoles are indicative of abnormal chromatin packaging in human spermatozoa. *International Journal of Andrology*, *35*, 46–51.

GHO - Global Health Observatory data repository (2016). *Overweight and obesity*. accessed December 1, 2016. Retrieved from http://www.who.int/gho/ncd/risk_factors/overweight/en/

Gutorova, N. V., Kleshchyov, M. A., Tipisova, E. V., & Osadchuk, L. V. (2014). Effects of overweight and obesity on the spermogram values and levels of reproductive hormones in the male population of the European north of Russia. *Bulletin of Experimental Biology and Medicine*, *157*, 95–98.

Hajshafiha, M., Ghareaghaji, R., Salemi, S., Sadegh-Asadi, N., & Sadeghi-Bazargani, H. (2013). Association of body mass index with some fertility markers among male partners of infertile couples. *International Journal of General Medicine*, *6*, 447–451.

Håkonsen, L. B., Spano, M., Bonde, J. P., Olsen, J., Thulstrup, A. M., Ernst, E., & Ramlau-Hansen, C. H. (2012). Exposures that may affect sperm DNA integrity: Two decades of follow-up in a pregnancy cohort. *Reproductive Toxicology*, *33*, 316–321.

Håkonsen, L. B., Thulstrup, A. M., Aggerholm, A. S., Olsen, J., Bonde, J. P., Andersen, C. Y., … Ramlau-Hansen, C. H. (2011). Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reproductive Health*, *8*, 24.

Hammiche, F., Laven, J. S., Twigt, J. M., Boellaard, W. P., Steegers, E. A., & Steegers-Theunissen, R. P. (2012). Body mass index and central adiposity are associated with sperm quality in men of subfertile couples. *Human Reproduction*, *27*, 2365–2372.

Hammoud, A. O., Wilde, N., Gibson, M., Parks, A., Carrell, D. T., & Meikle, A. W. (2008). Male obesity and alteration in sperm parameters. *Fertility and Sterility*, *90*, 2222–2225.

Hofny, E. R., Ali, M. E., Abdel-Hafez, H. Z., Eel-D, K., Mohamed, E. E., Abd El-Azeem, H. G., & Mostafa, T. (2010). Semen parameters and hormonal profile in obese fertile and infertile males. *Fertility and Sterility*, *94*, 581–584.

Jensen, T. K., Andersson, A. M., Jorgensen, N., Andersen, A. G., Carlsen, E., Petersen, J. H., & Skakkebaek, N. E. (2004). Body mass index in relation to semen quality and reproductive hormones among 1558 Danish men. *Fertility and Sterility*, *82*, 863–870.

Jung, A., & Schill, W. B. (2000). Male infertility. Current life style could be responsible for infertility. *MMW Fortschritte der Medizin*, *142*, 31–33.

Katib, A. (2015). Mechanisms linking obesity to male infertility. *Central European Journal of Urology*, *68*, 79–85.

Kort, H. I., Massey, J. B., Elsner, C. W., Mitchell-Leef, D., Shapiro, D. B., Witt, M. A., & Roudebush, W. E. (2006). Impact of body mass index values on sperm quantity and quality. *Journal of Andrology*, *27*, 450–452.

Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, *87*, 99–163.

Kyrou, I., Randeva, H. S., & Weickert, M. O. (2014). Clinical problems caused by obesity. In L. J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J. M. Hershman, C. Koch, M. Korbonits, R. McLachlan, M. New, J. Purnell, R. Rebar, F. Singer, & A. Vinik (Eds.), *Endotext [Internet]*. South Dartmouth, MA: MDText.com Inc.

La Vignera, S., Condorelli, R. A., Vicari, E., & Calogero, A. E. (2012). Negative effect of increased body weight on sperm conventional and nonconventional flow cytometric sperm parameters. *Journal of Andrology*, *33*, 53–58.

Leisegang, K., Bouic, P. J., Menkveld, R., & Henkel, R. R. (2014). Obesity is associated with increased seminal insulin and leptin alongside reduced fertility parameters in a controlled male cohort. *Reproductive Biology and Endocrinology*, *12*, 34.

Lobascio, A. M., De Felici, M., Anibaldi, M., Greco, P., Minasi, M. G., & Greco, E. (2015). Involvement of seminal leukocytes, reactive oxygen species, and sperm mitochondrial membrane potential in the DNA damage of the human spermatozoa. *Andrology*, *3*, 265–270.

Luque, E. M., Tissera, A., Gaggino, M. P., Molina, R. I., Mangeaud, A., Vincenti, L. M., … Martini, A. C. (2015). Body mass index and human sperm quality: Neither one extreme nor the other. *Reproduction, Fertility, and Development*, in Press, https://doi.org/10.1071/RD15351.

MacDonald, A. A., Herbison, G. P., Showell, M., & Farquhar, C. M. (2010). The impact of body mass index on semen parameters and reproductive hormones in human males: A systematic review with meta-analysis. *Human Reproduction Update*, *16*, 293–311.

MacDonald, A. A., Stewart, A. W., & Farquhar, C. M. (2013). Body mass index in relation to semen quality and reproductive hormones in New Zealand men: A cross-sectional study in fertility clinics. *Human Reproduction*, *28*, 3178–3187.

Malić Vončina, S., Golob, B., Ihan, A., Kopitar, A. N., Kolbezen, M., & Zorn, B. (2016). Sperm DNA fragmentation and mitochondrial membrane potential combined are better for predicting natural conception than standard sperm parameters. *Fertility and Sterility*, *105*, 637–644.

Martini, A. C., Tissera, A., Estofan, D., Molina, R. I., Mangeaud, A., De Cuneo, M. F., & Ruiz, R. D. (2010). Overweight and seminal quality: A study of 794 patients. *Fertility and Sterility*, *94*, 1739–1743.

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2014). Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA*, *311*, 806–814.

Oliveira, J. B. A., Petersen, C. G., Mauri, A. L., Vagnini, L. D., Baruffi, R. L. R., & Franco, J. G. Jr (2014). The effects of age on sperm quality: An evaluation of 1,500 semen samples. *JBRA Assisted Reproduction*, *18*, 34–41.

Paasch, U., Grunewald, S., Kratzsch, J., & Glander, H. J. (2010). Obesity and age affect male fertility potential. *Fertility and Sterility*, *94*, 2898–2901.

Palmer, N. O., Bakos, H. W., Fullston, T., & Lane, M. (2012). Impact of obesity on male fertility, sperm function and molecular composition. *Spermatogenesis*, *2*, 253–263.

Pauli, E. M., Legro, R. S., Demers, L. M., Kunselman, A. R., Dodson, W. C., & Lee, P. A. (2008). Diminished paternity and gonadal function with increasing obesity in men. *Fertility and Sterility*, *90*, 346–351.

Practice Committee of the American Society for Reproductive Medicine (2015). Obesity and reproduction: A committee opinion. *Fertility and Sterility*, *104*, 1116–1126.

Qin, D. D., Yuan, W., Zhou, W. J., Cui, Y. Q., Wu, J. Q., & Ga, E. S. (2007). Do reproductive hormones explain the association between body mass index and semen quality? *Asian Journal of Andrology*, *9*, 827–834.

Rybar, R., Kopecka, V., Prinosilova, P., Markova, P., & Rubes, J. (2011). Male obesity and age in relationship to semen parameters and sperm chromatin integrity. *Andrologia.*, *43*, 286–291.

Sakkas, D., & Alvarez, J. G. (2010). Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertility and Sterility*, *93*, 1027–1036.

Sermondade, N., Faure, C., Fezeu, L., Lévy, R., Czernichow, S., & Obesity-Fertility Collaborative Group (2012). Obesity and increased risk for oligozoospermia and azoospermia. *Archives of Internal Medicine*, *172*, 440–442.

Sermondade, N., Faure, C., Fezeu, L., Shayeb, A. G., Bonde, J. P., Jensen, T. K., … Czernichow, S. (2013). BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Human Reproduction Update*, *19*, 221–231.

Sermondade, N., Massin, N., Boitrelle, F., Pfeffer, J., Eustache, F., Sifer, C., … Levy, R. (2012). Sperm parameters and male fertility after bariatric surgery: Three case series. *Reproductive Biomedicine Online*, *24*, 206–210.

Shayeb, A. G., Harrild, K., Mathers, E., & Bhattacharya, S. (2011). An exploration of the association between male body mass index and semen quality. *Reproductive Biomedicine Online*, *23*, 717–723.

Silva, L. F., Oliveira, J. B., Petersen, C. G., Mauri, A. L., Massaro, F. C., Cavagna, M., … Franco, J. G. Jr (2012). The effects of male age on sperm analysis by motile sperm organelle morphology examination (MSOME). *Reproductive Biology and Endocrinology*, *10*, 19.

Smit, M., Romijn, J. C., Wildhagen, M. F., Weber, R. F., & Dohle, G. R. (2010). Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. *Fertility and Sterility*, *94*, 1748–1752.

Stewart, T. M., Liu, D. Y., Garrett, C., Jorgensen, N., Brown, E. H., & Baker, H. W. (2009). Associations between andrological measures, hormones and semen quality in fertile Australian men: Inverse relationship between obesity and sperm output. *Human Reproduction*, *24*, 1561–1568.

Taha, E. A., Sayed, S. K., Gaber, H. D., Abdel Hafez, H. K., Ghandour, N., Zahran, A., & Mostafa, T. (2016). Does being overweight affect seminal variables in fertile men? *Reproductive Biomedicine Online*, *33*, 703–708.

Tang, W. H., Zhuang, X. J., Ma, L. L., Qiao, J., Hong, K., Zhao, L. M., … Jiang, H. (2015). Correlation between body mass index and semen quality in male infertility patients. *Turkish Journal of Medical Sciences*, *45*, 1300–1305.

Teerds, K. J., de Rooij, D. G., & Keijer, J. (2011). Functional relationship between obesity and male reproduction: From humans to animal models. *Human Reproduction Update*, *17*, 667–683.

Thomsen, L., Humaidan, P., Bungum, L., & Bungum, M. (2014). The impact of male overweight on semen quality and outcome of assisted reproduction. *Asian Journal of Andrology*, *16*, 749–754.

Treulen, F., Uribe, P., Boguen, R., & Villegas, J. V. (2015). Mitochondrial permeability transition increases reactive oxygen species production and induces DNA fragmentation in human spermatozoa. *Human Reproduction*, *30*, 767–776.

Tsao, C. W., Liu, C. Y., Chou, Y. C., Cha, T. L., Chen, S. C., & Hsu, C. Y. (2015). Exploration of the association between obesity and semen quality in a 7630 male population. *PLoS ONE*, *10*, e0119458.

Tunc, O., Bakos, H. W., & Tremellen, K. (2011). Impact of body mass index on seminal oxidative stress. *Andrologia*, *43*, 121–128.

Umul, M., Kose, S. A., Bilen, E., Altuncu, A. G., Oksay, T., & Guney, M. (2015). Effect of increasing paternal body mass index on pregnancy and live birth rates in couples undergoing intracytoplasmic sperm injection. *Andrologia*, *47*, 360–364.

Vagnini, L., Baruffi, R. L., Mauri, A. L., Petersen, C. G., Massaro, F. C., Pontes, A., … Franco, J. G. Jr (2007). The effects of male age on sperm DNA damage in an infertile population. *Reproductive Biomedicine Online*, *15*, 514–519.

Vermeulen, A., Kaufman, J. M., Deslypere, J. P., & Thomas, G. (1993). Attenuated luteinizing hormone (LH) pulse amplitude but normal LH pulse frequency, and its relation to plasma androgens in hypogonadism of obese men. *Journal of Clinical Endocrinology and Metabolism*, *76*, 1140–1146.

WHO – World Health organization (2016). *Fact sheet: Obesity and overweight*. Updated June 2016 Retrieved from http://www.who.int/mediacentre/factsheets/fs311/en/

WHO– World Health organization (2010). *WHO laboratory manual for the examination and processing of human semen*, 5th edn. Geneva: World Health Organization.

Yoon, P. W., Bastian, B., Anderson, R. N., Collins, J. L., Jaffe, H. W., & Centers for Disease Control and Prevention (CDC) (2014). Potentially preventable deaths from the five leading causes of death–United States, 2008-2010. *MMWR Morbidity and Mortality Weekly Report*, *63*, 369–374.

How to cite this article: Oliveira JBA, Petersen CG, Mauri AL, et al. Association between body mass index and sperm quality and sperm DNA integrity. A large population study. *Andrologia*. 2018;50:e12889. https://doi.org/10.1111/and.12889