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## ORIGINAL ARTICLE

# Obesity leads to higher risk of sperm DNA damage in infertile patients

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There has been a growing interest over the past few years in the impact of male nutrition on fertility. Infertility has been linked to male overweight or obesity, and conventional semen parameter values seem to be altered in case of high body mass index (BMI). A few studies assessing the impact of BMI on sperm DNA integrity have been published, but they did not lead to a strong consensus. Our objective was to explore further the relationship between sperm DNA integrity and BMI, through a 3-year multicentre study. Three hundred and thirty male partners in subfertile couples were included. Using the terminal uridine nick-end labelling (TUNEL) assay, we observed an increased rate of sperm DNA damage in obese men (odds ratio (95% confidence interval): 2.5 (1.2–5.1)).

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**Keywords:** male infertility; obesity; overweight; sperm; sperm DNA fragmentation

## INTRODUCTION

Infertility is a public health problem concerning 15% of couples of child-bearing age. Male factors are involved in almost 50% of cases. Many studies have highlighted impairments of sperm quality for patients with a high or very high body mass index (BMI), notably a decrease in seminal sperm concentration.<sup>1–4</sup> Two recent meta-analyses including, respectively 14 and 21 studies, demonstrated an increased risk of azoospermia or oligozoospermia in overweight or obese males.<sup>5,6</sup>

Sperm DNA integrity is another factor that may be affected in obese subjects, possibly resulting from increased damage due to oxidative stress. A reduced number of studies have assessed the impact of BMI on sperm DNA integrity, with controversial results due to low numbers of cases studied and disparity in the techniques used.<sup>1–4,7–9</sup> The objective of the present study was to explore this relationship further through a 3-year multicentre study.

## MATERIALS AND METHODS

Data collection involved four centres during the period 2009–2012. Three hundred and thirty male partners of subfertile couples, presenting for semen analysis, were included, after providing written and informed consent. Height and weight were recorded on the day of semen collection and the BMI was calculated ( $\text{kg m}^{-2}$ ). Patients provided information on their tobacco consumption. Patients who smoked more than two cigarettes per day were designated as smokers.

Semen samples were collected by masturbation into a sterile plastic cup in the laboratory. Samples were allowed to liquefy at room temperature for 30 min and conventional semen quality (semen volume, sperm concentration and mobility) was evaluated according to WHO guidelines.<sup>10</sup> Sperm morphology was assessed by using David's criteria.<sup>11</sup>

Sperm DNA fragmentation was detected with the terminal uridine nick-end labelling (TUNEL) technique with an *In Situ* Cell Death Detection Kit (Fluorescein, Roche Applied Science, Meylan, France). Briefly, after trypsinization (Trypsin EDTA, Eurobio, Les Ulis, France), spermatozoa were immediately fixed in Carnoy solution (2:1 methanol/acetic acid) and stored at  $-20^\circ\text{C}$  for at least 60 min. After being washed with phosphate-buffered solution, sperm pellets were permeabilized for 20 min with 0.1% (*v/v*) Triton X-100 (Sigma-Aldrich, Saint Quentin Fallavier, France) in sodium citrate solution (sodium citrate dihydrate, VWR, Fontenay sous Bois, France;  $3.4\text{ mmol l}^{-1}$ , pH 6.8) and washed again with phosphate-buffered solution. The cells were then incubated with FITC-labelled dUTP and a terminal deoxyribonucleotidyl transferase (TUNEL solution). The positive control sample was treated with  $100\ \mu\text{l}$  of DNase ( $1\ \text{U}\ \mu\text{l}^{-1}$ ) (Fermentas, Saint Léon Rot, France) for 1 h at  $37^\circ\text{C}$  before incubation with the TUNEL solution and the deoxyribonucleotidyl transferase enzyme was omitted for the negative control. Cells were then washed twice in phosphate-buffered solution and spread over glass slides. The slides were dried at room temperature in the dark

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and DAPI solution (VECTASHIELD Mounting Medium with DAPI, 1.5 µg ml<sup>-1</sup>; Vector Laboratories, Paris, France) was added to the spermatozoa. Slides were examined by fluorescence microscopy. At least 200 spermatozoa were assessed blindly by two biologists and the total DNA fragmentation rate was calculated as the number of FITC-positive cells divided by the total number of sperm nuclei (labelled with DAPI).

### Statistical analyses

Data are summarized as the median, the first quartile and third quartile for continuous data. Categorical variables are presented as absolute and relative frequencies. Patients were assigned to different groups according to their BMI: normal weight (BMI: 18.5–24.9 kg m<sup>-2</sup>), overweight (25.0–29.9 kg m<sup>-2</sup>) or obese (≥30.0 kg m<sup>-2</sup>).<sup>12</sup> Association between sperm parameter values and BMI was analysed by using linear regression. Secondly, data were categorized according to the DNA fragmentation rate as normal (<14%), borderline (14%–29%) and pathological (≥30%). Ordinal logistic regression was performed to assess the impact of BMI across DNA fragmentation categories. Since age and tobacco can affect sperm DNA integrity,<sup>1,13</sup> unadjusted and adjusted analyses for age and smoking status were performed. Data were analysed by the free available R statistical software version 2.14.1.

### RESULTS

The mean age and BMI were 37.6±6.2 years and 25.8±4.0 kg m<sup>-2</sup>, respectively. Abstinence was not different between groups (3.9±1.8, 4.3±4.9 and 3.8±2.8 days, respectively, for normal, overweight and obese patients). No association between BMI and total sperm count or percentage of morphologically normal spermatozoa could be established. In contrast, sperm motility was low in obese patients compared with that in normal and overweight patients (mean differences (95% CI): -7.2 (-12.7, -1.7) and mean differences (95% CI): -3.8 (-7.6, 0.1), respectively), even after adjustment for age and smoking status (mean differences (95% CI): -8.6 (-14.6, -2.6) and -2.7 (-6.9, 1.49), respectively). Compared with men with normal BMI, the DNA fragmentation rate was significantly higher in obese men, (mean differences (95% CI): 3.9 (0.2, 7.6)), but not in overweight men (mean differences (95% CI): 1.1 (-1.4, 3.6)). Comparable results were obtained after adjustment for age and tobacco use (obese vs. normal: mean differences (95% CI): 4.2 (0.4, 7.9)); overweight vs. normal: mean differences (95% CI): 1.2 (-1.4, 3.8)) (Table 1).

In a second analysis, data were stratified according to the DNA fragmentation rate as normal (<14.0%), borderline (14.0%–29.9%) and pathological (≥30.0%). Compared with men with normal BMI,

OR (95% CI) for moving from one DNA fragmentation category to the next was 1.3 (0.8, 2.1) for overweight and 2.4 (1.2, 4.7) for obese men. After adjustment for age and smoking status, OR (95% CI) for moving from one DNA fragmentation category to the next was 1.4 (0.8, 2.4) for overweight and 2.5 (1.2, 5.1) for obese men (Table 2).

### DISCUSSION

Over the past few years, there has been a growing interest on the link between male nutrition and infertility. It is important to evaluate the potential effect of overweight or obesity on DNA integrity, since lower pregnancy rates or higher miscarriage rates have been reported in cases of increased sperm DNA fragmentation.<sup>13</sup> Six studies have been published with conflicting results because of the heterogeneity of the techniques and the small samples used in some studies (Table 3). Kort *et al.*<sup>3</sup> reported an increased sperm DNA fragmentation rate, determined by the sperm chromatin structure assay (SCSA), in overweight and obese patients. Chavarro *et al.*<sup>1</sup> and Farriello *et al.*<sup>8</sup> using the Single Cell Gel Electrophoresis assay method (comet assay), and LaVignera *et al.*,<sup>9</sup> using the TUNEL assay with flow cytometry, observed higher sperm DNA damage in obese, but not in overweight men. Tunc *et al.*,<sup>4</sup> Hammiche *et al.*<sup>2</sup> and Rybar *et al.*<sup>7</sup> using respectively the TUNEL<sup>4</sup> and SCSA<sup>2,7</sup> methods, did not find any significant association between BMI and sperm DNA integrity, but only small populations (ranging from 81 to 175) were used in these studies.

In the present work, with the TUNEL assay performed on a large population, an increased risk of sperm DNA damage was observed in obese, but not in overweight men, confirming previous observations.<sup>1,8,9</sup> This increased risk was confirmed after adjustment for age and tobacco consumption, whereas these adjustments were usually lacking in previous studies, despite the fact that age and tobacco consumption are known to increase sperm DNA fragmentation.<sup>1,13</sup>

Although there were no significant differences in sperm concentration or percentage of morphologically normal spermatozoa between BMI classes, sperm motility was also significantly diminished in obese men as previously described.<sup>9</sup>

The negative impact of male obesity on sperm DNA integrity could lead to serious consequences for fertility.<sup>14</sup> Indeed, sperm DNA alteration has been linked to impaired fertilization,<sup>13</sup> altered embryo and blastocyst development,<sup>15</sup> lower implantation rates and a higher incidence of miscarriage.<sup>16</sup> Moreover, a recent link between high paternal BMI and decreased live birth outcomes after assisted reproduction treatment has been highlighted,<sup>17–20</sup> possibly due to reduced blastocyst development, reduced implantation rates and higher pregnancy loss.<sup>19</sup>

**Table 1** Conventional semen parameter values and sperm DNA fragmentation rate across BMI categories

	Normal (18.5–24.9 kg m <sup>-2</sup> ) (n=151)	Overweight (25.0–29.9 kg m <sup>-2</sup> ) (n=137)	Obese (≥30.0 kg m <sup>-2</sup> ) (n=43)	Linear regression	Normal	Overweight	Obese
	Median (Q1–Q3)				Mean difference (95% CI)		
Total sperm count (TSC) (10 <sup>6</sup> )	74 (30–173.2)	73.5 (27.04–111.7)	32.48 (7.198–134.4)	Univariate	0 (reference)	-36.7 (-93.6, 20.2)	-10.4 (-88.4, 67.6)
				Adjusted	0 (reference)	-43.1 (-103.8, 17.6)	-7.6 (-88.8, 73.6)
Motility (%)	45 (30–50)	40 (30–50)	35 (30–50)	Univariate	0 (reference)	-3.8 (-7.6, 0.1)	-7.2 (-12.7, -1.7)
				Adjusted	0 (reference)	-2.7 (-6.9, 1.49)	-8.6 (-14.6, -2.6)
Normal morphology (%)	21 (11.5–29.25)	16 (8–27)	18.5 (13.25–31.75)	Univariate	0 (reference)	-3.3 (-7.0, 5.34)	-0.8 (-1.4, 4.8)
				Adjusted	0 (reference)	-1.3 (-6.3, 3.7)	0.5 (-6.3, 3.7)
DNA fragmentation rate (%)	13 (10–15)	13 (7.4–19)	13 (13–21.9)	Univariate	0 (reference)	1.1 (-1.4, 3.6)	3.9 (0.2, 7.6)
				Adjusted	0 (reference)	1.2 (-1.4, 3.8)	4.2 (0.4, 7.9)

Abbreviation: BMI, body mass index.

**Table 2** Prevalence of subjects in each BMI and sperm DNA fragmentation category

	n (%)			Ordinal logistic regression	OR (95% CI)		
DNA fragmentation rate <14.0%	110 (72.8%)	93 (67.9%)	22 (51.2%)	Univariate	1 (reference)	1.3 (0.8, 2.1)	2.4 (1.2, 4.7)
DNA fragmentation rate 14.0%–29.9%	30 (19.9%)	30 (21.9%)	16 (37.2%)	Adjusted	1 (reference)	1.4 (0.8, 2.4)	2.5 (1.2, 5.1)
DNA fragmentation rate ≥30%	11 (7.3%)	14 (10.2%)	5 (11.6%)				

Abbreviation: BMI, body mass index.

ORs for moving from one DNA fragmentation category to the next. Results are presented as unadjusted, and adjusted for age and tobacco use.

**Table 3** Summary of the main studies comparing BMI and sperm DNA fragmentation

	Population	N	Technique	Observations
Kort <i>et al.</i> (2006) <sup>3</sup>	Male partner in subfertile couples	520	SCSA (flow cytometry)	Higher sperm DNA fragmentation rate in overweight and obese men compared with men with normal BMI
Chavarro <i>et al.</i> (2010) <sup>1</sup>	Male partner in subfertile couples	413	COMET assay	Higher sperm DNA fragmentation rate in obese men compared with men with normal BMI
Fariello <i>et al.</i> (2012) <sup>8</sup>	Male partner in subfertile couples	305	COMET assay	
LaVignera <i>et al.</i> (2012) <sup>9</sup>	Healthy non smoking men	150	TUNEL assay (flow cytometry)	BMI
Tunc <i>et al.</i> (2010) <sup>4</sup>	Male partner in subfertile couples	81	TUNEL assay (slides)	No association between BMI and DNA fragmentation
Rybar <i>et al.</i> (2011) <sup>7</sup>	Male partner in subfertile couples	153	SCSA (flow cytometry)	
Hammiche <i>et al.</i> (2011) <sup>2</sup>	Male partner in subfertile couples	175	SCSA (flow cytometry)	

Abbreviations: BMI, body mass index; COMET assay, also know as single-cell gel electrophoresis assay; SCSA, sperm chromatin structure assay; TUNEL, terminal uridine nick-end labelling.

Further studies are needed to investigate the underlying mechanisms involved.<sup>21</sup> Oxidative stress may be the key mechanism linking overweight or obesity with male infertility, inducing increased sperm DNA damage. Indeed, BMI and abdominal obesity have been correlated to systemic oxidative stress assessed in plasma and urine.<sup>22</sup> In addition, the testicular microenvironment is also exposed to oxidative stress and a positive correlation between BMI and seminal oxidative stress has been observed.<sup>4</sup>

Finally, consequences of paternal overweight or obesity may not be limited to fertility. Indeed, offspring development may be altered by paternal health and nutrition, as recently demonstrated in animal models.<sup>23</sup> A link between oxidative stress, sperm DNA fragmentation and sperm DNA methylation has been demonstrated in men,<sup>24</sup> and these findings may play a role in subsequent offspring development and epigenetic markers.

### CONCLUSION

Male obesity is associated with an increased risk of sperm DNA damage and lower sperm motility and thus reduced sperm quality. BMI, which is an easy and widely-used indicator of nutritional status, should be considered in male fertility assessment and prognosis, both in spontaneous conception and after assisted reproductive techniques. Overweight or obese male patients of couples planning to conceive should be informed and educated on this subject. Whether weight normalization could improve sperm DNA integrity should also be further investigated.

### AUTHOR CONTRIBUTIONS

CD, CF and NS participated in study conception and design, acquisition, interpretation and analysis of data, and drafting of the manuscript. MB and VL participated in study design, performed statistical analyses and participated in critical revision of the manuscript for intellectual content. FE, PC, PB, IB, ICD, BB and PCP participated in acquisition of data and critical revision of the manuscript for intellectual content. RL participated in study conception and design,

interpretation of data, critical revision of the manuscript for intellectual content and supervised the study.

### COMPETING FINANCIAL INTERESTS

All authors declare that there are no competing financial interests.

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