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Relationships between Obesity markers and Semen Quality in adult Nigerians

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ABSTRACT

Likelihood of increased abnormal semen parameters among overweight and obese adult Nigerians because of the changing lifestyle exists. This study aimed to identify possible associations of obesity markers with sperm defects. 120 males (20-54years) were recruited after informed consent. Semen samples were collected from subjects by masturbation after 3-5 days of abstinence from sexual intercourse. Spermiogram was examined using WHO guidelines and 'Strict' criteria respectively. 10ml of blood was also obtained from each participant. Serum and seminal plasma were obtained by centrifugation of clotted blood and semen respectively. LH, FSH, Prolactin, Testosterone and Oestradiol were assayed using enzyme-immunoassay method whereas, cadmium, lead, Selenium and Zinc were assayed by atomic absorption spectrophotometry. Data were analyzed using SPSS20. WHtR and WHR were not significantly associated with abnormal spermatogram, changes in serum and seminal plasma Cd, Pb, Zn and Se levels. BMI showed irregular patterns in its association with spermatogram, endocrine status and toxic metals. There was a statistically significant difference in the distribution of waist circumference compared with sperm concentration (χ^2=13.55, p=0.009) and total sperm count (χ^2=11.26, p=0.02). Increased waist circumference was significantly associated with decrease in sperm concentration (β=-0.65, p=0.02) and normal morphology (β=-0.54, p=0.049), but an increase in mid-piece defects (β=0.92, p=0.001), tail defects (β=0.73, p=0.009), teratozoospermia index (β=0.95, p=0.001) and sperm deformity index (β=0.64, p=0.02) as well as decreased serum T/E_2 ratio (β=-0.55, p=0.04) but increased seminal plasma T/E_2 ratio (β=0.69, p=0.01), as well as increased serum Cd (β=0.81, p=0.002). Increased waist circumference was significantly associated with abnormal spermatogram, changes in endocrine status and Cd to highlight its role in male infertility.

Keywords: sperm quality, spermatogram, BMI, waist circumference, WHR, WHtR,

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ABBREVIATIONS

WHO	World Health Organization
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
WHtR	Waist-Height Ratio
WHR	Waist- Hip ratio
Cd	Cadmium
Pb	Lead
Zn	Zinc
Se	Selenium
BMI	Body Mass Index
T/E_2 ratio	Testosterone/Oestradiol
β	Standardized coefficient for multiple regression

INTRODUCTION

Obesity has become a global health problem reaching epidemic levels in both developed and developing countries [1]. Similarly, the incidence of infertility is growing at an alarming rate. In Western countries, subfertility is a serious health problem, affecting 10-15% of all couples trying to conceive [2]. Male factor subfertility accounts for 25-30% of all cases [3, 4]. Dyspermia is common in African males with mechanisms that are not well defined [5]. There is an increased likelihood of abnormal semen parameters among overweight and obese men [6]. The issues of fertility and obesity have remained largely equivocal. Although the incidence of overweight and obesity in men of reproductive age is rising which may affect fertility [7, 8] However, some studies have found no relationship between body mass index (BMI) in men and semen parameters [9, 10].

This study was therefore, designed to determine the possible relationship between body size and semen parameters of adult Nigerian men with a view to improving our understanding of the aetiology of poor semen quality as well as provide novel and rational approaches to preventing and treating infertility in men.

MATERIALS AND METHODS

Study design and selection of subjects

This prospective cross-sectional study was conducted using 120 male subjects after informed consent. The subjects were recruited from the Urology clinic of the University College Hospital, Ibadan and Urology department of the University of Port Harcourt Teaching Hospital, Port Harcourt and their environs. The study protocol was approved by the University College Hospital/ University of Ibadan ethical review committee prior to commencement of the study.

A baseline semen analysis was carried out for all subjects and this was repeated within two weeks following the World Health Organisation [11] guidelines. Semen was examined macroscopically for appearance, liquefaction, consistency and volume; and microscopically for concentration, motility and morphology.

Biological Sample Collection and Evaluation

Semen

Semen was collected in a clean, dry, sterilized, wide mouth, well stoppered glass vial by masturbation after 3-5days of abstinence. The sample was labelled with participant's identification number, date and time of collection and delivery, completeness of the collection. Physical characteristics of semen, sperm count, motility, viability, morphology and corresponding morphometry was measured at 400x and 1000x magnification after liquefaction of the sample following the WHO guidelines [11].

Seminal plasma was prepared from the whole liquefied semen after centrifugation at 500g for 15 minutes in IEC centrifuge (International Equipment Company, Boston, USA). Aliquot of the seminal plasma was separated into plastic sample containers and stored at -20^{o}C in lead free storage vial for heavy metal content analysis.

Blood

Blood sample (10 ml) was drawn from a large cubital vein in the sitting position from the subject between 8.00 am and 11.00 am as the semen sample was being submitted for analysis. The blood was collected directly into the vacuum tube and allowed to clot and retract completely before centrifugation at 500g in IEC centrifuge (International Equipment Company, Boston, USA) for 15 minutes. The serum sample was separated into plastic sample containers and stored at -20^{o}C until further analysis.

Lead, cadmium, zinc and selenium content of serum and seminal plasma was measured by atomic absorption spectrophotometer (AAS), Perkin-Elmer AAS model 703 (Perkin-Elmer Oak Brown, Illinois, USA) equipped with AS 60 atomic sampler and hollow cathode lamp. When the atoms in the vapour are excited, they return to the ground state by emitting light of the same wavelength. The amount of light absorbed by the metal is proportional to its concentration in the solution and is determined at a specific wavelength in the atomic absorption spectrophotometer (AAS). Lead was determined by the modified methods of Pleban and Mei [12] using atomic absorption spectrophotometry (AAS). Cadmium was determined by the modified methods of Ediger and Coleman [13] and Alfaro and Heaton [14], a modification of the method of Piper and Higgins [15] using atomic absorption spectrophotometer (AAS). Selenium in serum and seminal plasma was determined with atomic absorption spectrophometer (AAS) by the method of Pleban, Munyani and Beachum [16]. Zinc in serum and seminal plasma was determined by the method of Smith, Butrimovitz and Burdy [17] using atomic absorption spectrophotometry (AAS).

STATISTICAL ANALYSES

All the data obtained from the study participants were collated and analysed using the computer based software SPSS version 20 (SPSS Inc., USA). Descriptive statistics of the data obtained was done using cross-tabulation and χ^2-test whereas multiple regression analysis was used to calculate the interrelationships of obesity-associated biomarkers considered as possible explanatory variables (simultaneously introduced in the model) with respect to each of the measured semen parameter and reproductive hormones. The measured differences were considered to be statistically significant when $p<0.05$.

RESULTS

Tables 1-3 show comparative distributions of the sperm parameters and obesity-associated markers using the chi-squared test. In table 1, semen volume for all the participants in the various BMI groups was >1.7 ml and normal morphology was < 30%. There was a statistically significant difference in the sperm viability distribution when compared among BMI (χ^2=10.91, p = 0.03), but no significant difference in sperm concentration (χ^2=5.93, p = 0.21), total sperm count (χ^2=1.64, p = 0.80), sperm motility (χ^2=3.47, p = 0.18), and morphology using the strict criteria (χ^2=1.68, p = 0.43).

Table 1: The comparative distribution of the spermatogram and waist circumference using χ^2 after cross-tabulation

	BMI (18.5-24.9 kg/m^2)	BMI (25.0-29.9 kg/m^2)	BMI (≥30.0 kg/m^2)	χ^2, p
Semen volume				
<1.4 ml	0	0	0	
1.4-1.7 ml	0	0	0	
>1.7 ml	75(100.0%)	36(100.0%)	9(100.0%)	
Sperm viability				
<55%	39(55.7%)	9(30.0%)	4(50.0%)	10.91, **0.03**
55-63%	9(12.9%)	4(13.3%)	3(37.5%)	
>63%	22(31.4%)	17(56.7%)	1(12.5%)	
Sperm concentration				
<12.0 x 10^6/ml	12(17.1%)	5(16.7%)	4(50.0%)	5.93, 0.21
12-16 x 10^6/ml	13(18.6%)	6(20.0%)	0(0.0%)	
>16.0 x 10^6/ml	45(64.3%)	19(63.3%)	4(50.0%)	
Total sperm count				
<33 x 10^6	13(18.6%)	6(20.0%)	3(37.5%)	1.64, 0.80
33 - 46 x 10^6	9(12.9%)	4(13.3%)	1(12.5%)	
>46 x 10^6	48(68.6%)	20(66.7%)	4(50.0%)	
Sperm motility				
<38%	28(40.0%)	8(26.7%)	1(12.5%)	3.47, 0.18
38 - 42%	0	0	0	
>42%	42(60.0%)	22(73.3%)	7(87.5%)	
Normal morphology				
< 30%	70(100.0%)	30(100.0%)	8(100.0%)	
> 30%	0	0	0	
Strict criteria				
<3.0 %	3(4.3%)	0(0.0%)	0(0.0%)	1.68, 0.43
3.0-4.0 %	67(95.7%)	30(100.0%)	8(100.0%)	
>4.0 %	0	0	0	

Footnote:
p *significance value*
* *Significance at p<0.05*

Table 4 showed that an increase in BMI was significantly associated with increase in sperm viability (β = 0.39, p = 0.01), sperm concentration (β = 0.31, p = 0.04), total sperm count (β = 0.42, p = 0.004), % sperm motility (β = 0.38, p = 0.01), normal morphology (β = 0.35, p = 0.02), but a significant decrease in head defects (β = -0.35, p = 0.02), tail defects (β = -0.47, p = 0.002), cytoplasmic droplets (β = -0.36, p = 0.02), teratozoospermia index (β = -0.39, p = 0.008) and sperm deformity index (β = -0.42, p = 0.004). In table 5, increase in BMI was significantly associated with a decrease in serum Prolactin (β = -0.32, p = 0.03) only, and significantly associated with a decrease in serum cadmium level (β = -0.41, p = 0.005) as shown in table 6.

Table 2: The comparative distribution of the spermatogram and waist circumference using χ^2 after cross-tabulation

	WAIST (<93.9 cm)	WAIST (93.9 – 101.5 cm)	WAIST (≥101.6 cm)	χ^2, p
Semen volume				
<1.4 ml	0(0.0%)	0(0.0%)	0(0.0%)	
1.4-1.7 ml	0(0.0%)	0(0.0%)	0(0.0%)	
>1.7 ml	99(100.0%)	16(100.0%)	5(100.0%)	
Sperm vitality				
<55%	42(45.7%)	8(66.7%)	2(50.0%)	6.89, 0.14
55-63%	13(14.1%)	1(8.3%)	2(50.0%)	
>63%	37(40.2%)	3(25.0%)	0(0.0%)	
Sperm concentration				
<12.0 x 10^6/ml	13(14.1%)	5(41.7%)	3(75.0%)	13.55, **0.009**
12-16 x 10^6/ml	17(18.5%)	2(16.7%)	0(0.0%)	
>16.0 x 10^6/ml	62(67.4%)	5(41.7%)	1(25.0%)	
Total sperm count				
<33 x 10^6	14(15.2%)	6(50.0%)	2(50.0%)	11.26, **0.02**
33 - 46 x 10^6	12(13.0%)	1(8.3%)	1(25.0%)	
>46 x 10^6	66(71.7%)	5(41.7%)	1(25.0%)	
Sperm motility				
<38%	31(33.7%)	6(50.0%)	0(0.0%)	3.42, 0.18
38 - 42%	0(0.0%)	0(0.0%)	0(0.0%)	
>42%	61(66.3%)	6(50.0%)	4(100.0%)	
Normal morphology				
< 30%	92(100.0%)	12(100.0%)	4(100.0%)	
> 30%	0(0.0%)	0(0.0%)	0(0.0%)	
Strict criteria				
<3.0 %	3(3.3%)	0(0.0%)	0(0.0%)	0.54, 0.77
3.0-4.0 %	89(96.7%)	12(100.0%)	4(100.0%)	
>4.0 %	0(0.0%)	0(0.0%)	0(0.0%)	

Footnote:
p *significance value*
* *Significance at p<0.05*

Table 3: The comparative distribution of the spermatogram and waist/hip ratio using χ^2 after cross-tabulation

	waist/Hip ratio (<0.90)	waist/Hip ratio (>0.90)	χ^2, p
Semen volume			
<1.4 ml	0(0.0%)	0(0.0%)	
1.4-1.7 ml	0(0.0%)	0(0.0%)	
>1.7 ml	57(100.0%)	63(100.0%)	
Sperm vitality			
<55%	27(50.9%)	25(45.5%)	2.39, 0.30
55-63%	5(9.4%)	11(20.0%)	
>63%	21(39.6%)	19(34.5%)	
Sperm concentration			
<12.0 x 10^6/ml	8(15.1%)	13 (23.6%)	2.53, 0.28
12-16 x 10^6/ml	12(22.6%)	7(12.7%)	
>16.0 x 10^6/ml	33(62.3%)	35(63.6%)	
Total sperm count			
<33 x 10^6	8(15.1%)	14(25.5%)	1.94, 0.38
33 - 46 x 10^6	8(15.1%)	6(10.9%)	
>46 x 10^6	37(69.8%)	35(63.6%)	
Sperm motility			
<38%	16(30.2%)	21(38.2%)	0.77, 0.38
38 - 42%	0(0.0%)	0(0.0%)	
>42%	37(69.8%)	34(61.8%)	
Normal morphology			
< 30%	53(100.0%)	55(100.0%)	
> 30%	0(0.0%)	0(0.0%)	
Strict criteria			
<3.0 %	1(1.9%)	2(3.6%)	0.31, 0.58
3.0-4.0 %	52(98.1%)	53(96.4%)	
>4.0 %	0(0.0%)	0(0.0%)	

Footnote:
p *significance value*
* *Significance at p<0.05*

There was a statistically significant difference in the distribution of waist circumference compared with sperm concentration (χ^2=13.55, p = 0.009) and total sperm count (χ^2=11.26, p = 0.02) as shown in table 2. Increase in waist circumference was significantly associated with a decrease in sperm concentration (β = -0.65, p = 0.02) and

normal morphology (β = -0.54, p = 0.049), but an increase in mid-piece defects (β = 0.92, p = 0.001), tail defects (β = 0.73, p = 0.009), teratozoospermia index (β = 0.95, p = 0.001) and sperm deformity index (β = 0.64, p = 0.02) as shown in table 4. Table 5 showed that increased waist circumference was significantly associated with decreased serum T/E_2 ratio (β = -0.55, p = 0.04) but increased seminal plasma T/E_2 ratio (β = 0.69, p = 0.01). Table 6 showed that increased waist circumference was significantly associated with increased serum Cd (β = 0.81, p = 0.002).

Table 4: The relationships between obesity-associated biomarkers and spermatogram in adult Nigerian men using a Linear Multiple Regression model

	BMI (β, p)	**Waist/Height (β, p)**	**Waist (β, p)**	**Waist/Hip (β, p)**
Semen Volume	(0.26, 0.08)	(-0.38, 0.11)	(0.23, 0.38)	(-0.20, 0.13)
Sperm viability	**(0.39, 0.01)***	(0.04, 0.86)	(-0.33, 0.25)	(0.004, 0.98)
Sperm count	**(0.31, 0.04)***	(0.03, 0.89)	**(-0.65, 0.02)***	(0.24, 0.09)
Total sperm count	**(0.42, 0.004)***	(-0.24, 0.33)	(-0.44, 0.12)	(0.18, 0.20)
Sperm motility	**(0.38, 0.01)***	(0.29, 0.26)	(-0.42, 0.14)	(-0.14, 0.31)
Normal morphology	**(0.35, 0.02)***	(0.34, 0.18)	**(-0.54, 0.049)***	(-0.23, 0.10)
Head defects	**(-0.35, 0.02)***	(-0.33, 0.19)	(0.54, 0.05)	(0.23, 0.10)
Mid-piece defects	(-0.26, 0.07)	(-0.47, 0.06)	**(0.92, 0.001)***	(-0.06, 0.67)
Tail defects	**(-0.47, 0.002)***	(-0.14, 0.56)	**(0.73, 0.009)***	(-0.12, 0.37)
Cytoplasmic droplets	**(-0.36, 0.02)***	(0.07, 0.78)	(0.25, 0.38)	(-0.05, 0.75)
Teratozoospermic index	**(-0.39, 0.008)***	(-0.38, 0.12)	**(0.95, 0.001)***	(-0.12, 0.39)
Sperm deformity index	(-0.42, **0.004**)*	(-0.34, 0.17)	(0.64, **0.02**)*	(0.15, 0.26)
Footnote: *p* *significance value* * *Significance at p<0.05*				

Table 5: The relationships between obesity-associated markers and reproductive hormones in adult Nigerian men using a Linear Multiple Regression model

	BMI (β, p)	**Waist/Height (β, p)**	**Waist (β, p)**	**Waist/Hip (β, p)**
Serum Prolactin	**(-0.32, 0.03)***	(-0.15, 0.53)	(0.26, 0.32)	(0.08, 0.57)
Serum LH	(0.21, 0.16)	**(-0.49, 0.04)***	(0.10, 0.72)	(0.21, 0.12)
Serum FSH	(0.10, 0.50)	(-0.15, 0.52)	(0.23, 0.40)	(0.07, 0.58)
Serum Testosterone	(0.22, 0.14)	(0.21, 0.39)	(-0.53, 0.05)	(0.04, 0.76)
Serum Oestradiol	(-0.13, 0.38)	(-0.20, 0.40)	(0.52, 0.05)	(-0.05, 0.05)
Serum T/E_2	(0.23, 0.12)	(0.09, 0.69)	**(-0.55, 0.04)***	(0.07, 0.61)
Seminal plasma E_2	(0.16, 0.28)	(-0.002, 0.99)	(-0.22, 0.41)	(0.20, 0.14)
Serum T/LH	(0.02, 0.90)	(0.31, 0.21)	(-0.40, 0.15)	(0.02, 0.87)
Seminal plasma T	(-0.06, 0.68)	(-0.15, 0.55)	(0.09, 0.73)	(-0.04, 0.75)
Seminal plasma T/ E_2	(-0.18, 0.25)	(-0.29, 0.23)	**(0.69, 0.01)***	(-0.23, 0.08)
Footnote: *p* *significance value* * *Significance at p<0.05*				

In Table 4. The relationships between obesity-associated markers and the sperm parameters was evaluated. Thus, there was no significant association between the different sperm parameters and waist/height ratio (WHtR) and waist/hip ratio (WHR). In Table 5, an increase in WHtR was significantly associated with a decrease in serum LH (β = -0.49, p = 0.04), but increased WHR was not significantly associated with any changes in the hormone levels. In Table 6, increased WHR and WHtR were not significantly associated with any changes in the serum and seminal plasma toxic metals and essential micronutrient levels.

Table 6: The relationships between obesity-associated markers and trace elements in serum and seminal plasma of adult Nigerian men using a Linear Multiple Regression model

	BMI (β, p)	Waist/Height (β, p)	Waist (β, p)	Waist/Hip (β, p)
Serum Cadmium	**(-0.41, 0.005)***	(-0.44, 0.06)	**(0.81, 0.002)***	(-0.02, 0.85)
Seminal plasma Cadmium	(-0.03, 0.83)	(-0.12, 0.61)	(0.20, 0.46)	(-0.05, 0.74)
Serum lead	(-0.22, 0.14)	(0.10, 0.67)	(0.08, 0.76)	(0.12, 0.39)
Seminal plasma lead	(-0.19, 0.21)	(0.11, 0.66)	(0.05, 0.85)	(0.01, 0.92)
Serum Zinc	(0.03, 0.85)	(0.13, 0.58)	(0.06, 0.82)	(9-0.24, 0.08)
Seminal plasma Zinc	(0.10, 0.50)	(0.15, 0.55)	(-0.14, 0.61)	(-0.15, 0.28)
Serum Selenium	(0.14, 0.37)	(0.08, 0.73)	(-0.19, 0.50)	(0.04, 0.78)
Seminal plasma Selenium	(0.11, 0.46)	(0.06, 0.80)	(-0.29, 0.28)	(0.07, 0.60)
Serum Zn/Cd	(0.29, 0.06)	(0.03, 0.89)	(-0.26, 0.34)	(-0.02, 0.90)
Seminal plasma Zn/Cd	(0.16, 0.29)	(-0.02, 0.94)	(-0.06, 0.82)	(-0.06, 0.68)
Serum Zn/Pb	(0.21, 0.15)	(0.05, 0.85)	(-0.12, 0.66)	(-0.21, 0.11)
Seminal plasma Zn/Pb	(0.22, 0.14)	(0.08, 0.75)	(-0.20, 0.46)	(-0.13, 0.33)
Serum Se/Cd	(0.26, 0.08)	(-0.01, 0.97)	(-0.23, 0.39)	(0.60, 0.65)
Seminal plasma Se/Cd	(0.13, 0.38)	(0.05, 0.85)	(-0.17, 0.53)	(0.01, 0.92)
Serum Se/Pb	(0.07, 0.64)	(0.24, 0.33)	(-0.35, 0.20)	(0.10, 0.45)
Seminal plasma Se/Pb	(0.19, 0.21)	(0.04, 0.86)	(-0.27, 0.33)	(-0.02, 0.90)

Footnote:
p *significance value*
* *Significance at p<0.05*

DISCUSSION

Studies have suggested that the incidence of overweight and obesity in men of reproductive ages is rising and this may affect fertility [7]. Eisenberg et al [18] in their landmark LIFE study, evaluated the relationship between male BMI, waist circumference and semen quality and found that overweight and obesity are associated with a higher prevalence of low ejaculate volume, sperm concentration and total sperm count. In another study, Hammische et al., [7] reported that sperm concentration and total motile sperm count in men of sub-fertile couples are detrimentally affected by a high BMI and central adiposity. Christofolini et al., [19] investigated the influence of BMI and abdominal circumference on seminal parameters and found no statistically significant relationship. Thomsen et al., [20] investigated whether increased male BMI affects sperm quality and the outcome of assisted reproduction in couples with an overweight or obese man and a non-obese partner. They reported no statistically significant effect of male BMI on sperm concentration, seminal volume and sperm motility. Chitra & Prasad [21] studied the relationship between body mass status and semen quality and reported that semen volume, sperm count, sperm motility and morphologically normal sperms showed a negative correlation with all anthropometric measures.

In this study, we evaluated the relationships between various obesity markers and the sperm parameters, endocrine status, heavy metals in order to elucidate their contributions to the rising incidence of poor semen quality. A significant association between the different sperm parameters and waist/height ratio (WHtR), and waist/hip ratio (WHR) was not found and BMI did not show a very clear pattern in its association with the spermatogram, endocrine status and toxic metals. WHR and WHtR were not significantly associated with any changes in the serum and seminal plasma toxic metals and essential micronutrient levels, therefore suggesting that these parameters are not very sensitive indicators of the relationships between anthropometry and sperm quality. Belloc et al., [22] evaluated the influence of body mass index (BMI) on semen characteristics in a cohort study of a large patient sample size and found that increased BMI was associated with decreased semen quality, affecting volume, concentration, and motility. The percentage of normal forms was not decreased. MacDonald et al [23] investigated the association between body mass index (BMI) and routine semen analysis parameters in adult men and found no significant correlation between BMI and semen parameters with the exception of normal sperm morphology.

In a study conducted by Petty et al., [24] to determine if an increase in BMI is associated with an increase in semen parameter abnormalities found that there was no statistically significant association between BMI and any of the individual semen parameters tested. However, when the data looked at globally rather than on the effects on individual parameters (total number of normal motile sperm cells—NMS), functional sperm cells decreased with increasing BMI. Collectively these data suggest that obesity has a multifactorial effect on male fertility; possibly due to relationships with the hormone cascade, body composition and potentially testis temperature regulation. Sermondade et al., [8] in their systematic review and collaborative meta-analysis reported that overweight and obesity were associated with an increased prevalence of azoospermia or oligozoospermia. Similarly, Hakonsen et al., [25] found obesity to be associated with poor semen quality and altered reproductive hormonal profile in a

cohort of morbidly obese men. In their cross-sectional study of the association of BMI with testosterone in infertile males among local populations in Pakistan, Fehmida et al., [26] found a significant correlation between reduced testosterone levels and raised BMI levels to suggest obesity as a risk factor for male infertility. In this study, increased waist circumference was significantly associated with poor spermatogram i.e. a decrease in sperm concentration and normal morphology, but an increase in mid-piece defects, tail defects, teratozoospermia index and sperm deformity index. Increased waist circumference was also significantly associated with decreased serum T/E_2 ratio but increased seminal plasma T/E_2 ratio, as well as increased serum Cd. This suggests a relationship between adiposity, sperm quality, endocrine status and environmental toxins.

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Conflict of Interest
We wish to state categorically that there are no conflicts of interests with our employers or any other party.

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