

Body mass index in relation to semen quality and reproductive hormones in New Zealand men: a cross-sectional study in fertility clinics

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Submitted on March 14, 2013; resubmitted on August 8, 2013; accepted on September 9, 2013

STUDY QUESTION: Is there an association between body mass index (BMI) and routine semen analysis parameters in adult men?

SUMMARY ANSWER: No significant correlation was found between BMI and semen parameters measured with the exception of normal sperm morphology.

WHAT IS KNOWN ALREADY: Multiple cross-sectional studies have found inconsistent results, with two meta-analyses finding no correlation between BMI and semen parameters. A relationship between BMI and male reproductive hormones, particularly total testosterone, has been established in several studies and a systematic review.

STUDY DESIGN, SIZE, DURATION: Cross-sectional study of 511 men recruited at the time of semen analysis over 4 years (2008–2012).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Men presenting for semen analysis for any reason at participating fertility clinics in Auckland, New Zealand were recruited, with BMI measured or self-reported at this time. Exclusion criteria included azoospermia and pathological conditions of male genital tract. Conventional BMI categories were used (underweight < 18.5 kg/m², normal 18.5–24.99 kg/m², overweight 25.00–29.99 kg/m², obese ≥ 30 kg/m²). The routine semen analysis results for sperm concentration, total sperm count, sperm motility (total motility), sperm morphology, semen volume and total motile sperm (primary outcome) from one semen sample were recorded. Consent from 175 men was obtained to measure LH, FSH, estradiol, total testosterone, free testosterone and sex hormone-binding globulin (SHBG) in a blood sample (secondary outcome). Associations between BMI and these outcomes were assessed using Spearman correlation and analysis of variance, and a multiple linear regression analysis was performed. In addition, the relative risks for men having abnormal semen analysis results according to reference ranges of the World Health Organization, such as oligozoospermia, were calculated. This study has sufficient power to detect a doubling in abnormally low sperm concentration and total sperm count in overweight or obese men compared with men with normal BMI. Participation rate was not recorded.

MAIN RESULTS AND THE ROLE OF CHANCE: The body mass indices from measured and self-reported samples had an equivalent range of values which did not differ statistically. Median BMI was 27.1 kg/m² [10th–90th percentile: 22.8–32.9]. Overall, 72.8% of the study population were overweight or obese (BMI > 25 kg/m²), while 19 men (3.72%) had a BMI of 35–40 kg/m² and 7 men (1%) had a BMI of > 40 kg/m². No significant correlation was found between BMI and the semen parameters measured with the exception of normal sperm morphology ($r = 0.12$, $P = 0.024$), although this finding is derived from only 330 samples. Overweight and obese men showed no significantly increased relative risk of abnormal semen parameters. Of the reproductive hormones, significant negative relationships with BMI were found for total testosterone ($r = -0.35$, $P = <0.0001$), free testosterone ($r = -0.25$, $P = <0.0012$) and SHBG ($r = -0.44$, $P = <0.0001$). Multiple linear regression analysis also showed that BMI had a marginally significant effect on normal sperm morphology (effect estimate = 0.47, $P = 0.038$). In addition, < 2 days of abstinence was negatively associated with semen volume (effect estimate = -0.80, $P = 0.0074$) and summer season was negatively associated with sperm concentration (effect estimate = -14.9, $P = 0.020$).

LIMITATIONS, REASONS FOR CAUTION: The power of this study is limited by the relatively small overall sample size, although it does have one of the largest proportions of obese men (23.3%) in published cross-sectional studies. The study involved samples from men attending a fertility clinic, who are likely to have a lower semen quality and higher rate of pathology compared with the general population, therefore limiting the possible generalization of this study to all adult men.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings are consistent with those of other cross-sectional studies as well as two meta-analyses but do disagree in part with the most recent meta-analysis (which found significant odds ratios for oligozoospermia and azoospermia with increased BMI) and with studies measuring DNA fragmentation index. Therefore a definitive conclusion on the effect of BMI on semen quality remains uncertain while our data reinforce previous findings that BMI is negatively associated with male reproductive hormones.

STUDY FUNDING/COMPETING INTEREST(S): All funding for this study was from New Zealand academic and charitable sources including: Faculty of Medical and Health Sciences, University of Auckland (New Zealand), the Mercia Barnes Trust of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists and the Nurture Foundation for Reproductive Research. The authors have no conflicts of interest to declare.

Key words: BMI / obesity / semen / sperm count / reproductive hormones

Introduction

Subfertility is a serious health problem affecting the lives of at least 10% of the population in the developed world (Boivin *et al.*, 2007). While assisted reproduction technology (ART) has greatly improved the potential for subfertile couples to successfully conceive, it does not address the underlying cause of the couple's impaired fertility. ART is expensive and burdensome and not without associated morbidity (Chambers *et al.*, 2009). Therefore identifying potential modifiable risk factors for subfertility may ultimately lead to more satisfactory and cost-effective approaches to optimizing fertility such as lifestyle modification. Clarification of such risk factors would also supplement evidence for the mounting global public health efforts to encourage healthy lifestyles and disease prevention.

The relationship between obesity and subfertility has already been well established in women. Overweight and obese women are more likely to experience ovulatory and menstrual disorders, consequently experiencing delayed fertility (Pasquali *et al.*, 2007). Furthermore, obese women have poorer outcomes when undergoing fertility treatment, for example experiencing lower pregnancy rates, increased likelihood of miscarriage and requiring higher doses of gonadotrophins (Maheshwari *et al.*, 2007). Finally, an overall increased risk of subfertility among overweight and obese women was demonstrated directly in a cross-sectional study of 47 835 Danish couples, with statistically significant odds ratios (OR) for a prolonged time to pregnancy of >12 months (Ramlau-Hansen *et al.*, 2007).

Male factor alone constitutes ~25–30% of all cases of subfertility (Hammoud *et al.*, 2006). Interestingly, there is some evidence that semen quality, primarily by the measure of sperm count, has declined in the latter half of the 20th century (Carlsen *et al.*, 1992; Swan and Elkin, 1999), although this hypothesis is still debated (Becker *et al.*, 1997; Saidi *et al.*, 1999; Bonde *et al.*, 2011) and several recent longitudinal studies of military conscripts have shown no temporal trend in sperm quantity in both Sweden (Axelsson *et al.*, 2011) and Denmark (Bonde *et al.*, 2011). Over the last 30 years, developed nations are simultaneously reporting a dramatically increased prevalence of overweight and obesity (Finucane *et al.*, 2011). The same study of Danish couples (above) reported an increased risk of subfertility which was independently associated with overweight and obesity in male partners (Ramlau-Hansen *et al.*, 2007).

It has therefore been suggested that overweight and obesity may represent a significant potentially reversible cause of male subfertility.

Recent research into the relationship between body mass index (BMI) and semen parameters has reported conflicting results, with an early systematic review with meta-analysis finding no relationship between these variables (MacDonald *et al.*, 2010). However, a more recent meta-analysis of 21 studies concluded that obesity did significantly increase the risk of oligozoospermia and azoospermia (Sermondade *et al.*, 2012). Although three large cross-sectional studies have found a significant negative relationship between BMI and sperm concentration or sperm count (Jensen *et al.*, 2004; Koloszar *et al.*, 2005; Qin *et al.*, 2007), many other large studies have not found such an association (Aggerholm *et al.*, 2008; Li *et al.*, 2009; Chavarro *et al.*, 2010; Duits *et al.*, 2010; Martini *et al.*, 2010; Eskandar *et al.*, 2012). Several of these studies, however, have been limited by small numbers of obese men in their sample, with three of the five largest studies, each with at least 1000 men, having samples with <10% of men with a BMI of >30 kg/m².

In terms of pathophysiology, a potential relationship between obesity and male subfertility is likely to have some mechanistic involvement of the male reproductive hormones. It has already been established that overweight and obesity are associated with significant reductions in the levels of total testosterone, free testosterone and sex hormone-binding globulin (SHBG) (MacDonald *et al.*, 2010). Many studies have also reported elevated levels of estrogens in men with increased BMI (MacDonald *et al.*, 2010).

This cross-sectional study sought to clarify the relationship between BMI and semen parameters as well as confirm the relationship between BMI and reproductive hormones in a population of men attending a fertility clinic.

Materials and Methods

Study population and participants

Over the period May 2008 to March 2012, men submitting semen samples for semen analysis or therapeutic procedures at three fertility clinics in Auckland, New Zealand were asked to participate in this study. Men were primarily recruited from Auckland's sole public fertility clinic with some recruited across two private fertility clinics. In addition, a small number of

men ($n = 8$, 1.6%) who had produced samples for community laboratory testing within the previous month, arranged in primary care by a general practitioner and performed by a separate publically funded laboratory, were contacted by the research assistants and asked to participate. A brief medical background was collected primarily by informal interview with the patient or alternatively from the participant's clinical notes or from a self-reported questionnaire.

Men with definitive pathological conditions likely to affect sperm quantity were excluded. This included hypogonadism requiring medical treatment, orchidectomy for any indication, testicular cancer, a history of undescended testis with or without orchidopexy and any medical condition obstructing sperm transit, such as congenital absence of the vas deferens. Men with extremely low sperm counts and azoospermia were excluded. This includes a history of documented azoospermia or a semen analysis result with sperm concentrations of <4 spermatozoa per $400\times$ high power field (which correlates with a sperm concentration $\sim 1 \times 10^6/\text{ml}$), as suggested by the *WHO Laboratory Manual for the Examination and Processing of Human Semen 2010* (WHO, 2010). Samples with delayed liquefaction or extremely viscous semen samples, as defined by the WHO Laboratory Manual 2010 as semen forming a thread >2 cm long when allowed to drop from a wide-bore pipette or lifted by a glass rod (WHO, 2010), were excluded. Samples with incomplete sample collection were excluded.

While no further exclusion criteria applied to the male participants recruited into this study, there were public funding criteria that restricted couple eligibility for fertility treatment based on characteristics of the female partner which may have influenced the population of males sampled from the public fertility clinic. These female partner restrictions included a BMI of >32 , age over 40 years and being a current smoker. These restrictions did not apply to couples receiving privately funded fertility treatment.

Our *post hoc* power calculation estimates that this study has sufficient power (0.80) to detect at least a doubling in abnormally low sperm concentration and total sperm count (relative risk ≥ 2.0) for overweight or obese men compared with men with a normal BMI, at the 5% level of significance.

Ethics approval

Ethics approval was granted by the Northern Y Regional Ethics Committee of the New Zealand Ministry of Health (NTY/07/07/077). Participants were offered written information about the study and informed of the study hypothesis. Informed consent was obtained, with all participants signing a standardized consent form.

Measurements

When a research assistant was present at the time of recruitment, height and weight were measured by the research staff. At other times, men were asked to measure their height and weight themselves at the clinic with equipment provided. The same wall-mounted height measuring tape and electronic scales were used for height and weight measurements at each fertility clinic. Measurements were made with shoes off, pockets emptied and jackets removed. A small proportion ($n = 75$, 15%) of men self-reported their height and weight, which was the case for men recruited at the fertility clinics who were not measured and men recruited from community laboratory testing. In addition, because of the public-funding access criteria excluding female partners with a BMI of >32 , it was possible that this would indirectly limit the BMI range of our male sample, and so the female partner's height and weight were measured or documented from clinical records. Conventional BMI categories were used (underweight <18.5 kg/m², normal 18.5–24.99 kg/m², overweight 25.00–29.99 kg/m², obese ≥ 30 kg/m²).

Semen analysis data were extracted from the routine semen analysis (RSA) reports of all participants. Parameters recorded were semen volume, sperm concentration, total sperm count, sperm motility (total motility), total motile

sperm and sperm morphology (percentage normal forms). Data were recorded from a single semen sample only. Men were instructed by referring clinicians to submit semen samples after a period of abstinence of at least 2 full days but no more than 7 days, although participants were not excluded if this was not adhered to. Semen samples were all collected on-site in a private room near the laboratory. Samples were collected in a wide-mouthed clean, clear plastic container. Any incomplete specimen collections were repeated at another time or otherwise excluded from the study.

The following methods describe the routine procedures for semen analysis at the primary fertility laboratory used in this study: Upon receipt, semen samples were either left on a bench at room temperature or rotated slowly on a two-dimensional circular rotator at room temperature, for ~ 30 min but no more than 60 min. Semen volume was measured by aspiration into a graduated plastic Eppendorf 5.0 ml pipette (Eppendorf AG, Hamburg, Germany). For sperm motility assessment, the semen sample was mixed thoroughly via repeated aspiration, a 10 μl aliquot was immediately placed onto a clean glass slide with coverslip then applied and then motility pattern was characterized for 200 spermatozoa at room temperature. No replicate count was performed for sperm motility. For sperm concentration measurement, the semen sample was mixed as for sperm motility assessment and then 50 μl aliquots of semen were diluted in unstained fixative with a dilution factor of 1:2 to 1:20, with dilution determined by the estimated sperm concentration on the initial undiluted wet film used for sperm motility assessment as guided by the WHO Laboratory Manual 2010 (WHO, 2010). A 10 μl aliquot of diluted semen was then pipetted onto an improved Neubauer Haemocytometer and 200 sperm were counted to calculate sperm concentration. Replicate counts were not performed. Positive-displacement Eppendorf Research pipettes were used for all pipetting (Eppendorf AG, Hamburg, Germany). Total sperm count was simply calculated as the product of sperm concentration and semen volume. For sperm morphology, Papanicolaou staining was performed off-site at the Auckland City Hospital clinical laboratory with slides then returned for interpretation. One slide was initially prepared for each semen sample at each laboratory by air-drying and then fixation in 95% ethanol. Upon return, the stained slides were examined under oil immersion at $\times 1000$ magnification with assessment of at least 200 spermatozoa. Slides were examined once with no replicate assessment. Only the percentage of normal/abnormal forms of spermatozoa was recorded.

Semen analyses were performed primarily at the clinical laboratory attached to Auckland's sole public fertility clinic. However, semen analyses used in this study were performed at a total of four other laboratories: two laboratories at Auckland's two private fertility clinics and two community laboratories for the few community samples that were used. The methods of semen analysis used at these other laboratories were essentially identical to the methods described above for the primary laboratory, although the other laboratories all performed in-house Papanicolaou staining rather than off-site staining. Replicate measurements of sperm concentration, sperm motility and sperm morphology were not performed at these other laboratories. Interpretation of semen morphology was according to the WHO Laboratory Manual current at the time, although some laboratories reported simple interval ranges rather than absolute values for sperm morphology results, which could not be readily incorporated into our data collection.

The primary laboratory in this study participates in the regional external quality control scheme for Australasia, the External Quality Assurance Schemes for Reproductive Medicine (EQARSM) based in Northlands, Western Australia, which involves external quality assessments four times annually. Internal quality control is not formally undertaken for semen analysis results. The other laboratories do not participate in the EQARSM.

In addition, the participants were asked to voluntarily complete a blood test for the reproductive hormones LH, FSH, estradiol, total testosterone, free testosterone and SHBG. Blood sampling was available on site between the hours of 08:00 and 15:00, with patients instructed to have

their blood tests between 08:00 and 09:00 in the morning. All hormone testing was performed by one clinical laboratory in Auckland, New Zealand. Estradiol was measured by radioimmunoassay (RIA) using the Spectra Estradiol RIA coated-tube RIA (Orion Diagnostica Oy, Espoo, Finland). SHBG was also measured by RIA on the Roche e601 module (Roche Diagnostics N.Z. Ltd, Auckland, New Zealand). Total testosterone, LH and FSH were all measured by electrochemiluminescence assays on the Roche e602 module (Roche Diagnostics N.Z. Ltd, Auckland, New Zealand). Free testosterone was calculated according to the Vermeulen formula using total testosterone, SHBG and albumin (Vermeulen *et al.*, 1999).

Statistical analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC, USA). The body mass indices from men measured at the clinic and from men who self-reported their BMI were assessed for difference by Student's *t*-test. Differences in the median semen parameters and median reproductive hormones across the conventional BMI categories were assessed using Kruskal–Wallis one-way analysis of variance. Correlation between BMI and semen parameters and between BMI and reproductive hormones as continuous variables was assessed by Spearman's rank correlation. Where these two analyses were equivalent, only Spearman's rank correlation is reported in the results. These correlations were also assessed with regression coefficients estimated from the regression analysis described below. The relative risk for men having semen parameters in the abnormal reference range (according to World Health Organization (WHO) reference limits; specified in Table II) was calculated for both overweight and obese men separately compared with men of normal weight. This was performed with both crude data and data adjusted for confounders as part of the multiple linear regression model described below.

Additionally, a multiple linear regression analysis was performed using the SAS General Linear Model Procedure to assess the predictive effect on semen parameters of independent variables including BMI and other background variables: age, reason for semen analysis (IVF/ICSI), intrauterine insemination (IUI) or RSA, period of abstinence (<2 days versus ≥ 2 days), paternity history (0 previous pregnancies versus ≥ 1 previous pregnancies), season (summer versus winter), alcohol consumption (0 units per week, <6 units per week or ≥ 6 units per week), smoking status (smoker versus non-smoker) and history of sexually transmitted infection (STI) (history of chlamydia or gonorrhoea versus no STI history). These background variables were all readily available in the clinical records and most have an established effect on semen analysis results (as for age, period of abstinence, season, STI history). Alcohol consumption and smoking status were selected as these are the two most commonly used recreational drugs in New Zealand and paternity history was selected as this, to some degree, distinguishes men with proven fertility from men with known or potential subfertility.

Correlation between reproductive hormones and semen parameters was assessed by Spearman's rank correlation. Correlation between male BMI and female partner BMI was also assessed in light of the restriction on female BMI for publicly funded fertility treatment.

Results

Population

Men ($n = 511$) were recruited into the study with a mean age of 36.8 years [10th–90th percentile: 30–44]. The majority of these men ($n = 426$, 83.4%) were recruited from Auckland's public fertility clinic. The remainder were recruited from private fertility clinics ($n = 77$, 15.0%) or from the community laboratory samples ($n = 8$, 1.6%). The median period of abstinence preceding semen sample collection was 3 days [10th–90th

percentile = 2–6], with only 34 men (6.7%) reporting an abstinence period of <2 days.

A reliable participation rate is not available because, although this was intended to be recorded, study staff were not always present on site to document men who declined.

The clinical indications for the semen analyses were: RSA for investigation of infertility ($n = 203$, 39.7%), IVF ($n = 151$, 29.6%), ICSI ($n = 84$, 16.4%), IUI or trial wash for IUI ($n = 58$, 11.4%) and RSA for non-fertility reasons, including pre-vasectomy testing and sperm donation ($n = 15$, 2.9%). The reasons for infertility documented for each couple were: unexplained infertility ($n = 167$, 32.7%), female factor(s) infertility ($n = 149$, 29.2%), undocumented/unknown ($n = 65$, 12.7%), male factor(s) infertility ($n = 58$, 11.4%), mixed male and female factor(s) ($n = 42$, 8.2%) and recurrent pregnancy loss ($n = 15$, 2.9%). Finally, 15 patients (2.9%) were patients without subfertility, undergoing pre-vasectomy testing, sperm donation and PGD.

BMI was measured by staff in 348 men (68%) or by the men themselves at clinic in 71 cases (14%). The remainder of BMI measurements were self-reported ($n = 75$, 15%) or unspecified (17, 3%). The body mass indices from measured and self-reported samples were not significantly different, i.e. the two subpopulations had an equivalent range of BMI data and therefore were considered one overall study population for the purposes of statistical analysis.

The median BMI was 27.1 kg/m² [10th–90th percentile: 22.8–32.9 kg/m²] and the BMI distribution across BMI categories is presented in Table I. Overall, 72.8% of the study population were overweight or obese (BMI >25 kg/m²). Nineteen men (3.72%) had a BMI of 35–40 kg/m², and 7 men (1%) had a BMI of >40 kg/m². None had a BMI of <18.5 kg/m².

Average semen parameters and average reproductive hormone levels are outlined in Table I. Not all semen parameters could be included from the semen analysis report for all men because some measurements were not performed or reporting differed across laboratories. This was especially the case for sperm morphology, which was available in only 330 cases, due to sperm morphology not being assessed in many cases according to laboratory policy or differences in reporting as described above. BMI was recorded from 329 of the female partners to the men in this study with a median female partner BMI of 23.7 kg/m² [10th–90th percentile: 20.0–30.4 kg/m²]. There was a statistically significant relationship between male BMI and the female partner's BMI, with a Spearman correlation coefficient of 0.30 ($P < 0.0001$) (Fig. 1).

Semen parameters

No statistically significant differences or correlation in sperm concentration and total sperm count in relation to BMI were detected (Table II, Supplementary data, Figure S1). Normal sperm morphology did increase with increasing BMI, with a Spearman correlation coefficient of $r = 0.12$ ($P = 0.024$) and regression coefficient of $r = 0.47$ ($P = 0.038$), although sperm morphology measurements were available from only 330 samples (not shown). None of the relative risks for overweight or obese men having abnormal semen parameters were significant, including risk of oligozoospermia (Table II).

The multiple linear regression analysis showed that BMI had a marginally significant effect on normal sperm morphology in this model (effect estimate = 0.47, $P = 0.038$), consistent with the weak positive correlation described above. Two other independent variables had a

Table 1 Average outcome data and correlation results for a population of men in New Zealand attending fertility clinics.

Outcome		Samples (n)	Median result [10th–90th percentile]				Spearman correlation		Regression analysis	
			Overall, n = 511 (100%)	BMI 18.5–24.99, n = 139 (27.2%)	BMI 25–29.99, n = 253 (49.5%)	BMI ≥30, n = 119 (23.3%)	Correlation coefficient	P-value	Regression coefficient	P-value
Semen analysis parameters	Semen volume (ml)	510	2.9 [1.46–5.2]	2.8 [1.3–5.2]	3.0 [1.5–5.2]	2.9 [1.4–5.2]	0.02	0.72	0.003	0.87
	Sperm concentration (× 10 ⁶ /ml)	511	48 [7.9–147]	52.5 [7.4–139]	50.0 [9.3–152]	42.0 [7.4–116.5]	–0.05	0.26	–1.15	0.15
	Total sperm count (× 10 ⁶)	511	128.8 [21.5–413]	128.1 [21.1–413]	135.0 [24.1–455]	122.1 [15.4–407]	–0.04	0.41	–1.32	0.58
	Sperm motility, total (%)	509	65 [40.0–81.0]	64.0 [35.0–80.0]	64.5 [40.0–81.0]	67.0 [44.0–82.0]	0.07	0.12	0.18	0.40
	Total motile sperm (× 10 ⁶)	509	82.9 [9.2–273.8]	83.7 [10.4–264.9]	84.1 [10.1–279]	78.1 [8.0–302.4]	–0.03	0.53	–0.79	0.63
	Morphology (% normal)	330	19.0 [7.0–40]	16.0 [7.0–34.0]	20.0 [39.0–6.0]	22.0 [8.0–45.0]	0.12*	0.024*	0.47*	0.038*
Reproductive hormones	FSH (IU/l)	175	4.5 [2.5–10.1]	4.5 [2.3–11.3]	4.5 [2.5–8.7]	4.6 [2.5–10.6]	0.01	0.92	0.07	0.30
	LH (IU/l)	175	3.9 [2.0–6.7]	4.0 [2.5–7.5]	3.9 [1.9–6.0]	3.5 [1.8–6.8]	–0.04	0.61	0.01	0.76
	Total testosterone (nmol/l)	176	13.9 [8.8–21.9]	15.9 [10.2–24.2]	14.0 [8.9–21.4]	11.7 [7.3–17.3]	–0.35*	<0.0001*	–0.38*	0.0006*
	Free testosterone (pmol/l)	167	366 [244–526]	412 [308–552.0]	381 [243–516]	332 [238–504]	–0.25*	0.001*	–0.51*	0.045*
	Estradiol (pmol/l)	166	74.0 [52.0–105]	72.5 [51.0–94.0]	73.5 [51.5–104]	75.5 [56.0–108]	0.08	0.30	0.30	0.49
	Estradiol test. ratio	163	5.3 [3.4–9.1]	4.3 [2.8–7.5]	5.2 [3.6–9.1]	6.4 [4.2–11.5]	0.38*	<0.0001*	0.15*	0.02*
SHBG (mmol/l)	170	23.0 [12.0–42.0]	29.0 [16.0–47.0]	22.0 [13.0–40.0]	18.0 [10.0–31.0]	–0.44*	<0.0001*	–0.97*	<0.0001*	

SHBG, sex hormone-binding globulin.

*Statistically-significant ($P < 0.05$).

statistically significant effect on semen parameters: <2 days of abstinence was negatively associated with semen volume slightly (effect estimate = -0.80, *P* = 0.0074) and summer season was negatively associated with sperm concentration (effect estimate = -14.9, *P* = 0.020). Both were similarly related to total sperm count with comparable *P*-values, although this parameter is not an independent variable, as it is simply the calculated product of sperm concentration and semen volume.

Reproductive hormones

In total, 175 (34%) men consented to and completed a blood test for reproductive hormones. The majority of blood samples were taken in the morning (72.3% before 12:00 p.m.), although the average time of blood sample was 11:12 a.m. with only 22.3% of blood samples taken before 10:00 a.m. Statistically significant relationships between BMI and the reproductive hormones total testosterone, free testosterone and SHBG were detected (Table I). No significant relationships were found for FSH, LH, SHBG and estradiol.

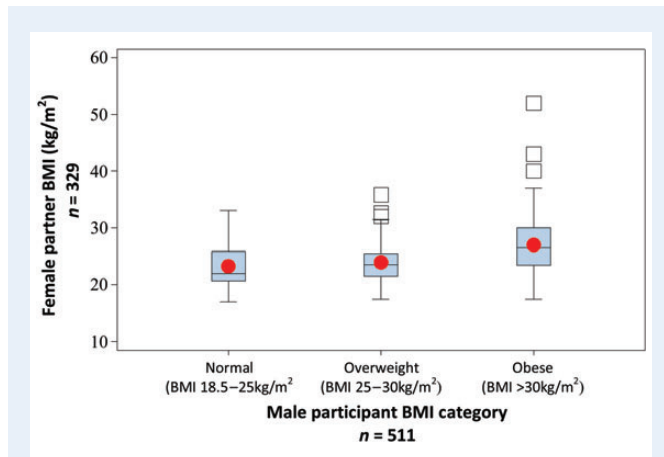


Figure 1 Box plot of male versus female partner's BMI. Note: Borders of large shaded box represent 25th and 75th percentiles, internal line within large shaded box represents median, central dot represents mean, external whisker lines represent 1.5 × interquartile range (IQR) and small unshaded squares represent outliers outside of 1.5 × IQR.

LH and FSH had statistically significant negative relationships with sperm concentration, total sperm count, total motile sperm and, in the case of LH, sperm morphology (Table III). In addition, total testosterone was significantly correlated with sperm morphology (Table III) although not with other semen parameters. Estradiol, free testosterone and SHBG had no statistically significant relationships with any of the semen parameters.

Discussion

This study has shown no relationship between BMI and most semen parameters, including sperm concentration and total sperm count. These results reinforce the conclusions of the majority of other cross-sectional studies and one of the two previously published systematic reviews with meta-analysis, which found that any relationship between BMI and semen parameters must be so marginal as to be undetectable in large population studies (MacDonald *et al.*, 2010). Our results are in mixed agreement with a more recently published meta-analysis (Sermondade *et al.*, 2012), which, while also unable to find a significant correlation between BMI and semen concentration, did find a statistically significant OR for the clinical diagnoses of oligozoospermia or azoospermia in overweight and obese men. The one statistically significant correlation between BMI and semen analysis parameters in this study was with sperm morphology, where a positive correlation was found between BMI and normal sperm morphology. This contrasts with previous studies where in almost all cases no statistically significant correlation with BMI has been detected, although one other study has also reported a positive albeit very weak correlation between BMI and normal sperm morphology (Qin *et al.*, 2007) whereas two other studies have reported statistically significant ORs for low normal sperm morphology in overweight and obese men (Hammoud *et al.*, 2008; Shayeb *et al.*, 2011). The reliability of this finding, however, is limited as it is derived from a small data subset of 330 samples.

There are a total of nine studies published with larger population samples than this study (Jensen *et al.*, 2004; Qin *et al.*, 2007; Aggerholm *et al.*, 2008; Duits *et al.*, 2010; Li *et al.*, 2009; Martini *et al.*, 2010; Paasch *et al.*, 2010; Sekhavat and Moein, 2010; Shayeb *et al.*, 2011). Our finding of an absence of any correlation between BMI and most semen parameters, particularly sperm concentration and total sperm count, is in agreement with six of these nine studies (Qin *et al.*, 2007; Aggerholm *et al.*, 2008; Duits *et al.*, 2010; Li *et al.*, 2009; Martini *et al.*, 2010;

Table II Relative risks for semen parameter measurements below WHO reference limits in overweight and obese men.

Semen parameter	Reference limit (WHO)	Crude relative risk [95% confidence interval]		Adjusted relative risk [95% confidence interval]	
		Overweight	Obese	Overweight	Obese
Semen volume	<1.5 (ml)	0.92 [0.50–1.68]	0.93 [0.46–1.92]	0.89 [0.45–1.76]	0.99 [0.46–2.17]
Sperm concentration	<15 (× 10 ⁶ /ml) ^a	1.02 [0.64–1.65]	1.06 [0.61–1.85]	1.10 [0.61–1.99]	1.17 [0.59–2.32]
Total sperm count	<39 (× 10 ⁶)	0.96 [0.61–1.52]	1.31 [0.80–2.15]	1.02 [0.58–1.81]	1.59 [0.87–2.89]
Sperm motility	<40% (% total motility)	0.86 [0.46–1.63]	0.75 [0.33–1.66]	0.89 [0.40–1.99]	0.96 [0.38–2.47]
Morphology	<4% (% normal)	2.20 [0.24–19.4]	1.45 [0.09–22.7]	4.97 [0.38–64.4]	0.97 [0.03–35.7]

WHO, World Health Organization.
^aDiagnostic threshold for oligozoospermia.

Table III Relationship between male reproductive hormones and semen parameters.

Reproductive hormone	Semen parameter	Spearman correlation coefficient	P-value
FSH (n = 175)	Sperm concentration ($\times 10^6$ /ml)	-0.33	<0.0001*
	Total sperm count ($\times 10^6$)	-0.37	<0.0001*
	Sperm motility (%)	-0.15	0.051
	Total motile sperm ($\times 10^6$)	-0.38	<0.0001*
	Morphology (% normal)	-0.18	0.061
LH (n = 175)	Sperm concentration ($\times 10^6$ /ml)	-0.26	0.001*
	Total sperm count ($\times 10^6$)	-0.24	0.002*
	Sperm motility (%)	-0.04	0.60
	Total motile sperm ($\times 10^6$)	-0.22	-0.004*
	Morphology (% normal)	-0.36	0.0001*
Total testosterone (n = 176)	Sperm concentration ($\times 10^6$ /ml)	0.01	0.85
	Total sperm count ($\times 10^6$)	0.02	0.78
	Sperm motility (%)	0.02	0.81
	Total motile sperm ($\times 10^6$)	0.40	0.60
	Morphology (% normal)	-0.20	0.03*

*Statistically-significant ($P < 0.05$).

Shayeb et al., 2011). This includes the two studies with the largest population sample, consisting of 2035 men (Shayeb et al., 2011) and 1989 men (Aggerholm et al., 2008), both of which had high proportions of overweight and obese men (57.4 and 47.1%, respectively). Two of these large studies reported a statistically significant negative correlation between BMI and semen parameters generally (Jensen et al., 2004; Sekhavat and Moein, 2010). Paasch et al. (2010) reported no statistically significant correlations, except for between BMI and total sperm count in men aged 20–30 years.

This study is moderately sized compared with other population studies investigating the relationship between BMI and semen parameters. From the overall pool of at least 40 studies investigating the same scientific question (Sermondade et al., 2012), there are a total of nine larger studies in the published literature (described above), with sample sizes ranging from 794 (Martini et al., 2010) to 2035 (Shayeb et al., 2011) with five of these having population samples of between 1000 and 2035 men (Jensen et al., 2004; Aggerholm et al., 2008; Duits et al., 2010; Li et al., 2009; Shayeb et al., 2011). However, half of these studies had limited numbers of obese men, with 10% or less of the sample having a BMI of >30 kg/m² (Jensen et al., 2004; Qin et al., 2007; Aggerholm et al., 2008; Duits et al., 2010; Li et al., 2009). One of these studies investigated young Danish military recruits with a prevalence of obesity of 3.8% (Jensen et al., 2004) and another investigated healthy Chinese volunteers with a prevalence of obesity 1.2% (Li et al., 2009). The present study has one of the highest rates of overweight and obesity of all published cross-sectional studies of BMI and semen parameters, with 372 men (72.8%) of the men having a BMI of >25 kg/m², including 119 (23.3%) obese men (BMI >30). With the high rates of overweight and obesity in our study sample and a relatively high mean age of 36.8 years, this sample is one of the most reflective of men in developed countries seeking medical treatment for subfertility.

This study is one of many studies that have investigated men recruited specifically from fertility clinics, representing a potentially subfertile population. These studies form a heterogeneous collection with a large number of studies based on smaller sample sizes. The findings of this study are of variable consistency with the range of results reported by

these other studies of subfertile populations. Of studies with 500 or more men, 3 agree with our findings that there is no correlation between BMI and any semen parameters (Duits et al., 2010; Relwani et al., 2011; Eskander et al., 2012). Conversely, 3 studies with sample sizes of at least 500 men have reported negative correlations between BMI and at least one semen parameter: total sperm count (Paasch et al., 2010), 'normal-motile sperm' (Kort et al., 2006) and sperm motility (Martini et al., 2010). A further two studies reported significant OR for abnormal semen parameters: low semen volume and low normal sperm morphology (Shayeb et al., 2011) and low sperm concentration (Hammoud et al., 2008).

The major limitation of this study is that it samples men from fertility clinics, with the majority of participants recruited being investigated or treated for couple subfertility. Therefore this sample is certain to have a higher rate of subfertile men, with presumably poorer semen analysis results, compared with the general population. While this study excluded men with definitive pathological conditions of the male genital tract, there are potentially other undefined pathological factors in this sample that would affect their semen quality apart from obesity. In any case, if BMI and semen parameters were truly correlated, then this relationship should still be evident across the BMI range in this sample. The BMI distribution of this study population is also very similar to the BMI distribution of the general population of adult men in New Zealand, with New Zealand men having rates of normal weight, overweight and obesity of 30.2, 41.3 and 27.7%, respectively (University of Otago and New Zealand Ministry of Health 2011). Furthermore, while sampling men from fertility clinics may limit the generalizability of this study's conclusion to the whole male population, this does at least make the conclusions of this study directly relevant to the clinical population seen in fertility medicine. As the male BMI and female partner's BMI were significantly correlated, the upper BMI distribution of this sample of men may also have been limited by the BMI threshold restriction applied to female partners in order to access public funding for fertility treatment in New Zealand, possibly limiting the number of highly obese men recruited into this study, although this would not be expected to affect the overall relationship across the BMI range, as explained above.

This study relied on semen analysis data from clinical laboratories performing routine semen analyses for clinical service, rather than measurements from research laboratories or tests conducted specifically for dedicated scientific research. There are several limitations to the validity of our data, which principally include deficiencies in laboratory methods, deficiencies in quality control at the participating laboratories and the amalgamation of data from multiple laboratories. The laboratory processes for semen analysis in this study had mixed compliance with the WHO Laboratory Manual standards, i.e. some test methods were performed in complete compliance with the WHO Laboratory Manual standards while some were not. Notable deviations from WHO standards include the measurement of semen volume by aspiration, which is discouraged in the WHO Laboratory Manual 2010 due to its potential to underestimate semen volume although it does remain one of the most commonly used methods worldwide (Cooper *et al.*, 2010), and a failure to perform replicate measurements for sperm motility, sperm concentration and sperm morphology. Regrettably, quality control was limited, with little formal internal quality control performed. Most of the laboratories did not participate in the EQARSM, although the primary laboratory in this study did have an active involvement in this programme. Finally, the use of multiple laboratories is problematic as semen analysis measurements exhibit significant inter-laboratory variation, principally for sperm motility and morphology (Jørgensen *et al.*, 1997). Reassuringly, the primary laboratory performed 83.4% of the semen analyses and in any case laboratory methods were very similar between laboratories; therefore, this is unlikely to have an impact on the final results.

Despite the lack of any statistically significant correlation between BMI and semen parameters, this study did find a significant effect of BMI on the male reproductive hormones total testosterone and free testosterone as well as SHBG. This is consistent with the majority of previously published cross-sectional studies which have found statistically significant negative correlations (MacDonald *et al.*, 2010). In contrast, this study did not find any statistically significant relationship between BMI and estradiol, where previously studies have frequently, although not consistently, detected positive correlations (MacDonald *et al.*, 2010). No statistically significant relationships were found between BMI and the hormones LH or FSH in this study or in most previous studies (MacDonald *et al.*, 2010).

It is of notable interest that BMI appears to have a consistently significant impact on the reproductive hormone profile of men (MacDonald *et al.*, 2010), including lowered total testosterone (MacDonald *et al.*, 2010), yet there is limited evidence for any effect on semen parameters. Spermatogenesis is a process driven primarily by testosterone, in the form of free testosterone, and FSH (Zirkin, 1998). Overall semen quality involves further processes as well such as sperm maturation and concentration in the epididymis, and accessory gland secretion (Cornwall, 2009). It seems that these processes of sperm and semen production are adequately maintained in obese men despite their deranged reproductive hormone potential. There are many possible explanations for why this may be the case, including the levels of testosterone not having a specifically quantifiable role in sperm and semen production or other adaptive factors occurring to maintain homeostasis. The maintenance of a normal FSH level in obese men, as evidenced by the consistent lack of statistically significant correlations between BMI and FSH (MacDonald *et al.*, 2010), may also explain this discrepancy.

Although semen analysis represents the gold standard of male fertility assessment, it remains possible that overweight and obesity are

associated with male subfertility through mechanisms not quantified in the RSA. Ultimately semen analysis is only one indirect predictor of the more clinically important outcome of overall fertility rate. Consistent with this, data from large population studies on couple subfertility have identified BMI as an independent risk factor for subfertility (Sallmén *et al.*, 2006; Nguyen *et al.*, 2007; Ramlau-Hansen *et al.*, 2007). Two studies investigating DNA fragmentation index (DFI) as a measure of the genetic quality of sperm have yielded statistically significant positive correlations between BMI and DFI, suggesting that overweight and obesity may still reduce the quality of semen even if sperm count and other parameters remain unchanged (Kort *et al.*, 2006; Chavarro *et al.*, 2010). In addition, BMI was found to have a statistically significant correlation with increased seminal oxidative stress assessed using the Nitro Blue Tetrazolium assay as well as increased seminal macrophage activation via seminal plasma neopterin in 81 men (Tunc *et al.*, 2011). Obesity has also been repeatedly associated with erectile dysfunction (Pasquali *et al.*, 2007). Finally, obesity has well established and substantial negative outcomes for most other areas of health. Therefore, overweight and obese men presenting with subfertility should ultimately still be strongly advised to reduce their weight to a healthier level.

This cross-sectional study of BMI and semen parameters complements the range of cross-sectional studies already published. The strength and consistency of the results of this study add to the growing evidence that any relationship between BMI and the semen parameters assessed in RSA, if present, is marginal. In any case, the ultimate advice offered to men in a fertility setting is unlikely to be substantially adjusted based on these conclusions. Already one prospective trial of weight loss in 43 obese men has yielded positive results with statistically significant increases in semen parameters (Håkonsen *et al.*, 2011). Therefore, future research would benefit most from longitudinal studies and clinical trials of weight loss in men and investigations of other measures of semen quality, such as DFI.

Supplementary data

Supplementary data are available at <http://humrep.oxfordjournals.org/>.

Acknowledgements

We acknowledge Dr Virginia Griffiths, Ariane Heynen, Josie Rishworth and Craig Riddell who assisted with patient recruitment. We are especially thankful to the staff at Fertility Plus, particularly the embryology staff, who accommodated the study and assisted in the recruitment of the majority of participants. We also thank the other fertility clinics in Auckland, Fertility Associates and Repromed, and the community laboratory Labtests for their contribution to participant recruitment.

Authors' roles

A.A.M. was responsible for the majority of data collection and manuscript preparation. A.W.S. undertook all statistical analysis. C.M.F. was responsible for initial study design and overall study oversight. All authors collaborated for data interpretation, manuscript editing and critical discussion.

Funding

Funding for this study was from the Faculty of Medical and Health Sciences, University of Auckland, New Zealand and the Mercia Barnes Trust of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists and the Nurture Foundation for Reproductive Research.

Conflict of interest

The authors have no conflicts of interests to declare.

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