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Effect of the metabolic syndrome on male reproductive function: a case-controlled pilot study

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Abstract

The metabolic syndrome (MetS) is a constellation of various risk factors. This study aimed to investigate the effect of MetS on testosterone and progesterone, and semen parameters, in a case-controlled pilot study. Male patients (n = 54) had body mass index, waist-to-hip ratio (WHR) and blood pressure recorded. Blood was analysed for HDL cholesterol, triglycerides and glucose. Saliva was assayed for free testosterone and free progesterone. Ejaculates were analysed for volume, sperm concentration, total sperm count, motility, vitality, mitochondrial membrane potential (MMP), DNA fragmentation and leucocyte concentration. Participants were divided into the control group (n = 28) and the MetS group (n = 26). Differences were found between the groups for body mass index, WHR, blood pressure, high-density lipoprotein (HDL), triglycerides and glucose. The MetS group showed significant reductions in sperm concentration (P = 0.0026), total sperm count (P = 0.0034), total motility (P = 0.0291), sperm vitality (P = 0.002), MMP (P = 0.0039), free testosterone (P = 0.0093) and free progesterone (P = 0.0130), while values for DNA fragmentation increased (P = 0.0287). Results indicate that patients with MetS have compromised sperm parameters in the absence of leucocytospermia. A reduction in free progesterone suggests that steroidogenesis cascades may be compromised. It is hypothesised that a systemic pro-inflammatory state with oxidative stress associated with MetS may provide a novel explanation.

Introduction

The metabolic syndrome is considered to be a collection of various metabolic risk factors that tend to cluster together, resulting in an increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (Beilby, **2004**). Although the exact aetiology and pathophysiology of MetS is still a matter of contention (Huang, **2009**), the syndrome comprises of various interrelated conditions that share complex and multifactorial underlying mediators, mechanisms and pathways. The common features that cluster together include central obesity, dyslipidaemia, hypertension, glucose intolerance (and T2DM) and a pro-inflammatory state, all well-documented risk factors of CVD (Taslim & Tai, **2009**).

Obesity, as a cardinal feature of MetS, has been associated with an increased incidence of male factor infertility. Although the effect of excess body fat on reproduction has been more extensively studied in females, there has been a recent increase in literature assessing the relationship between obesity and semen characteristics, male endocrine

changes, male sexual function and male factor infertility. Over the past decade, numerous studies have found an inverse correlation between increased obesity and semen quality that negatively affects male fertility, with an increased chance of subfertility among couples in which the male partner is obese. Various mechanisms for this relationship have been proposed and can be broadly divided into direct negative effects on spermatogenesis and sperm function, hormonal factors and physical factors (Eckel *et al.*, 2005; Hammoud *et al.*, 2008; Kasturi *et al.*, 2008). However, the effect of obesity on sperm parameters has not been conclusively resolved and is neither consistent, nor has there been a clear dose–response mechanism elicited (Kasturi *et al.*, 2008). Recent meta-analysis studies have also indicated conflicting results (MacDonald *et al.*, 2010; Sermondade *et al.*, 2012).

Various components of MetS have been attributed as a contributing factor to male infertility. Kasturi *et al.* (2008) comprehensively reviewed the literature involving the effect of the individual components on male fertility. The effect of individual parameters associated with MetS, such as dyslipidaemia, arterial hypertension and hyperglycaemia, have only had limited scientific investigation (Eckel *et al.*, 2005). Ramirez-Torres *et al.* (2000) found no correlation between sperm abnormalities and hypertension, glucose intolerance and diabetes mellitus. However, these authors did indicate a relationship between dyslipidaemia and sperm abnormalities. Shalaby *et al.* (2004) reported a potential role for dyslipidaemia in the development of infertility in male rats fed with a high cholesterol diet. Several studies have found an inverse relationship between blood pressure and total serum testosterone concentrations, which may result in impaired reproductive potential (Eckel *et al.*, 2005).

Type 2 diabetes mellitus, a known consequence of MetS, has increasingly been associated with male factor infertility in recent years, with complex and multifactoral factors involved. Palmer *et al.* (2012) reported a positive correlation between glycaemia and sperm DNA fragmentation, with a negative correlation with normal morphological spermatozoa, regardless of adiposity, in mice fed with a high fat diet. Poor semen quality, such as reduced sperm concentration and motility, abnormal morphology, mitochondrial DNA damage, nuclear DNA damage and increased seminal plasma abnormalities have been reported (La Vignera *et al.*, 2012). Advanced glycation end products are also increased in the reproductive tract of males with T2DM and are a likely role player in sperm cell damage (Mallidis *et al.*, 2009).

Hormonal changes that are associated with MetS and negatively affect various parameters of male fertility include reduced serum total testosterone (TT), free testosterone (FT) and sex hormone-binding globulin (SHBG), increased serum oestrogen, insulin (insulin resistance), leptin, FSH, LH and prolactin (Eckel *et al.*, 2005; Hammoud *et al.*, 2008). Conversely, the role of progesterone has not been extensively studied in males who are obese or diagnosed with MetS. Blanchette *et al.* (2006) reported a negative correlation between body weight, BMI and waist circumference (WC) with serum progesterone concentrations (as well as 17-hydroxyprogesterone, dehydroepiandrosterone sulphate, TT and dihydrotestosterone). Furthermore, the role of progesterone in male fertility has not been fully investigated, despite the fact that there is no great difference in serum

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progesterone concentrations between men and women, except during the luteal phase of the menstrual cycle and during pregnancy (Oettel & Mukhopadhyay, 2004).

On searching the scientific literature, it is apparent that the effect of MetS on male (in)fertility has not been sufficiently investigated. With various components of MetS tentatively linked to a reduced fertility potential in males, and limited studies in animal models have also indicated that MetS is associated with poor semen quality (Vartanian *et al.*, 2006; Mallidis *et al.*, 2011), it is hypothesised that male fertility may be compromised in the setting of this pathophysiological disorder. Although any negative relationship between MetS and male infertility is likely to be multifactorial and complex, a clear association first needs to be suggested by case-controlled investigations. Therefore, with reference to the impact of MetS on the hormonal system and male infertility, this case-controlled pilot study aimed to investigate peripheral concentrations of FT and free progesterone, and various semen function parameters, in men diagnosed with MetS.

Materials and methods

This study was approved by the Ethics Committee of the University of the Western Cape, Bellville, South Africa. Male participants aged 18–70 years of age were recruited randomly via word of mouth and advertisements distributed by hand, electronically and in local community newspapers, between October 2010 and January 2012. Therefore, the cohort represents those confined to the Western Cape region of South Africa, with multiple ethnic and cultural backgrounds. On pre-clinical screening via telephonic or electronic communication, participants with a vasectomy, diagnosed with any disorder of the prostate or reproductive system, or on any hormonal therapy (e.g. testosterone or insulin) were excluded from entering the study. Accepted participants attended one consultation and were individually counselled on the study background and design, and all participants signed an informed consent form to undergo a full medical consultation and clinical examination and allow for sample collection and relevant biochemical testing. All participants received detailed feedback on the results and were further advised appropriately on the recommended treatments or further investigations that may be required. Subjects were expected to be fasting for a minimum of 8 h before blood collection and abstain from sexual activity for 3–5 days before collection of a semen sample. All samples were collected in the morning between 7:00 hours and 10:00 hours. All of the relevant biological samples were collected at the consultation, specifically blood (via venous puncture), passive saliva and semen (via masturbation into a sterile container).

Relevant clinical data collected by a trained clinician included age, body mass index (BMI), waist and hip circumference (WHR) and blood pressure (BP). BMI was calculated by body weight (kilograms to nearest decimal point) divided by height (metres) squared. Waist circumference was measured in centimetres around the abdomen at the mid-point between the highest point of the iliac crest and the lowest point of the costal margin. The hip circumference (HC) was measured in centimetres around the level of the greater trochanter. WHR was determined by dividing WC by the HC. BP was measured twice on the dominant arm after a rest period of at least 15 min, and the mean between the readings was recorded. The blood samples were analysed for high-density lipoprotein (HDL) cholesterol, triglycerides and glucose concentrations. Saliva underwent analysis for

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free testosterone (FT) and free progesterone (FP) concentrations. Semen was analysed for total ejaculate volume, sperm concentration, total sperm count, sperm motility, vitality, mitochondrial membrane potential (MMP), DNA fragmentation (DF) and seminal leucocyte concentration.

Based on the clinical and laboratory data, participants were assessed for MetS according to the diagnostic criteria outlined in Table 1 (Alberti *et al.*, 2009). Participants with clinically apparent reproductive disorders (e.g. varicocoele; epididymitis), leucocytospermia (>1 x 10^6 ml^{-1}) or with a known or clinically detected acute or chronic inflammatory conditions not related to MetS were excluded from study. Further exclusion factors based on medical history and examination included a surgery or hospital admission for any reason within the last 6 months. Participants with unexplained (idiopathic) azoospermia had semen parameters removed from the study, but clinical, biochemical and hormonal parameters were included. Participants with a history of couple infertility were included in the study.

Table 1. Criteria for the clinical diagnosis of the metabolic syndrome (MetS) as used in the study population (Alberti *et al.*, 2009). A minimum of three of the five criteria need to fulfilled to obtain a diagnosis of MetS (Alberti *et al.*, 2009). Cut-off values for waist circumference vary based on ethnic and genetic backgrounds

Measure	Categorical cut-off points (cm)
Waist circumference	Sub-Saharan African Male ≥ 94 Europid ≥ 94 Middle East & Mediterranean ≥ 94 Central & South Americans ≥ 90 Asian Male ≥ 90
Blood pressure (or antihypertensive medication)	Systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg
Fasting triglycerides (or relevant medication)	>1.70 mmol l^{-1}
HDL Cholesterol (or relevant medication)	<1.00 mmol l^{-1} in males
Fasting glucose	>5.5 mmol L^{-1}

All laboratory assays were conducted by an adequately trained technician who was blinded to the clinical data of each participant. Blinding was done by assignment of a random alpha-numeric code assigned to each participant. All results were collated by the clinician who diagnosed the participants and divided them into the relevant groups. Fifty-four males who met the screening criteria were accepted for sample collection based on the procedures outlined above, with two further respondents rejected due to a recent history of testosterone therapy. After sample analysis, a further one participant was subsequently excluded from data analysis based on leucocytospermia. Patients with MetS ($n = 26$) were then compared with the control group (CG) ($n = 28$) for the various parameters studied. The sample size was limited due to the pilot nature of the study

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design.

Selection bias was limited as no participants who met the criteria were excluded from data analysis, although idiopathic azoospermic participants had data removed from the seminal analysis for statistical analysis. Potential confounding variables were not included in detail by the data collection process, and analysis includes lifestyle factors (diet and physical activity), educational status and socio-economic background.

Serum cholesterol, triglycerides, insulin and glucose

Blood was collected in sodium fluoride and serum–separating tubule vacutainers® and transported immediately to PathCare Laboratories (Pathcare Park, Goodwood, South Africa) for analysis. HDL, triglycerides (lipogram) and glucose were assayed using the timed endpoint method.

Standard semen analysis

Seminal fluid was left at room temperature to liquefy and analysed within 60 min after ejaculation. Sperm count and motility were assessed using the Motility/Concentration module of the Sperm Class Analyzer®(SCA) system version 4.1.0.1 (Microptic S.L., Barcelona, Spain). The CASA system classified motility according to WHO standard (WHO, **2010**). For analysis, a Nikon Eclipse 50i microscope (IMP, Cape Town, South Africa) equipped with phase contrast optics and a heated stage was used. Sperm vitality was assessed using the eosin–nigrosin staining technique (WHO, **2010**). Leucocyte concentration was determined using the peroxidase staining technique as described by Politch *et al.* (**2007**).

Mitochondrial membrane potential (MMP)

Mitochondrial membrane potential was assessed as described previously (Henkel *et al.*, **2011**) using a Zeiss fluorescence microscope (Oberkochen, Germany) for analysis after staining spermatozoa with DePsipher staining kit (R&D Systems, Abingdon, UK). In brief, semen was diluted 1 : 5 ratio with human tubal fluid medium (Quinn *et al.*, **1985**) and centrifuged for 10 min at 500 x ***g***. The supernatant was discarded, and the pellet re-suspended in DePsipher staining solution and incubated for 20 min at 37 °C in the dark. The DePsipher sperm suspension was then centrifuged at 500xg, the supernatant discarded, and the pellet was re-suspended in 100 µl of pre-warmed 1X reaction buffer. The cells were observed immediately with fluorescence microscopy at 1000 times magnification. Spermatozoa exhibiting a green fluorescence within their mid-pieces were regarded as having disturbed MMP, while those spermatozoa showing red fluorescence were regarded as having intact MMP. The percentage of spermatozoa with disturbed MMP was calculated.

DNA fragmentation

Washed spermatozoa were centrifuged at 500 x ***g*** and re-suspended in PBS (Oxoid, Basingstoke, Hampshire, UK), and a smear on a Superfrost® slide (Mentzel, Braunschweig, Germany) was made and allowed to air dry. Subsequently, DNA fragmentation was assessed by the terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) assay according to Henkel *et al.* (**2004**). Manual counting was carried out using a Zeiss fluorescence microscope, and the results expressed as a percentage of

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cells showing fragmented DNA (TUNEL-positive cells).

Saliva steroid hormone analysis

Saliva samples were collected by passive drooling into a sterile Eppendorf container and stored at −20 °C until testing. Testosterone and progesterone assays were performed using commercial ELISA kits supplied by IBL International GMBH (Hamburg, Germany).

Statistical analysis

Statistical analysis was performed using the MedCalc software (Version 12.0; Mariakerke, Belgium). After testing for normal distribution using the Kolmogorov–Smirnov test, appropriate statistical tests, either parametric (Pearson correlation, independent samples t-test) or nonparametric (Spearman Rank correlation, Mann–Whitney U-test), were performed. A P -value of <0.05 was considered as significant.

Results

Based on the clinical criteria for the diagnosis of the MetS (Table 1), 54 male subjects who were confirmed eligible for the study were placed either in the CG (n = 28; mean age: 37.4 ± 9.3 years) or in the MetS group (n = 26; mean age: 39.9 ± 10.6 years). With regard to age, both groups did not differ (P = 0.3407) from one another. Details regarding potential confounders such as age groups, BMI groups, demographics, number of MetS diagnostic criteria, lipid-lowering medication, participants diagnosed with T2DM, smokers, sperm concentrations and fertility history are summarised in Table 2. Statistical analysis indicated a significant difference between the groups for BMI > 35 (morbidly obese category), those on medications relevant to MetS and those on cholesterol medications. No significant difference was found between the groups for other potential confounding factors identified, including smoking.

Table 2. Details of various parameters of both the control group and metabolic syndrome (MetS) group represented as percentages rounded to the nearest decimal point. Recent history of subfertility was defined as an inability to achieve a conception with regular sexual intercourse for 12 months. Significance was calculated using Fischer's exact test, and significant differences were considered when $P < 0.05$. Proven fertility was defined as a live birth within the last 2 years of the consultation date. BMI = body mass index; MetS = metabolic syndrome; T2DM = type 2 diabetes mellitus

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Variable	Control group (n = 28) (%)	Metabolic syndrome group (n = 26) (%)	P-value
Age			
18–29	10.7	15.4	0.7072
30–39	60.7	43.3	0.4899
40–49	17.9	19.2	0.1671
50–59	7.1	7.7	1.0000
60–70	3.6	14.4	0.6153
BMI			
18–24.9	32.1	3.8	0.0657
25–29.9	57.1	19.2	0.0699
30–34.9	10.7	23.1	0.4741
>35	0	53.8	0.0002
Demographics			
Caucasian	64.3	65.4	1.0000
Coloured	21.4	30.8	0.7651
Black	14.3	3.8	0.3625
Asian	0	0	–
MetS diagnostic criteria (table 1)			
0 criterion	32.1	–	–
1 criterion	46.4	–	–
2 criterions	21.5	–	–
3 criterions	–	69.2	–
4 criterions	–	19.2	–
5 criterions	–	11.5	–
MetS medications	7.1	46.2	0.0152
Hypertension	3.6	23.1	0.1063
Cholesterol	3.6	38.5	0.0168
Triglycerides	0	0	–
Glucose	0	7.7	0.4909
Other medications			
COX inhibitors	0	11.5	0.2368
T2DM	0	11.5	0.2368
Smokers	10.7	19.2	0.7072
Recent history of subfertility	7.1	30.8	0.8848
Proven fertility	28.6	11.5	0.3268
Sperm concentrations			
Azoospermia	7.1	7.7	1.0000
Oligozoospermia	14.2	42.3	0.1421
Normospermia	78.7	50.0	0.3833
Asthenozoospermia	17.8	50.0	0.1030

As expected, patients in the MetS group were found to have a significantly increased BMI, WC, WHR, as well as systolic and diastolic BP as compared to the CG. Significantly decreased values were found for HDL cholesterol, while serum triglycerides and serum glucose concentrations were significantly higher in the MetS group (Table 3).

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Table 3. Clinical and biochemical parameters taken in the study. All statistical analysis performed with parametric student's t-tests. BP = blood pressure; HDL = high-density lipoprotein

	Control group				Metabolic syndrome group				
Parameter	*n*	Mean ± SD	Median	Range	*n*	Mean ± SD	Median	Range	*P*-value
Age	28	37.4 ± 9.3	35.5	24–67	26	39.9 ± 10.6	37.5	26–63	0.3407
BMI (kg/m^2)	28	25.8 ± 3.2	25.9	18.6–32.3	26	34.6 ± 5.4	35.6	22.3–44.0	<0.0001
Waist-to-Hip Ratio	28	0.92 ± 0.07	0.94	0.80–1.02	26	1.03 ± 0.05	1.03	0.96–1.17	<0.0001
Waist circumference (cm)	28	92.7 ± 8.8	96.0	73–104	26	117.7 ± 12.9	115.0	89–142	<0.0001
Systolic BP (mmHg)	28	120.2 ± 9.7	120.0	110–140	26	133.1 ± 11.1	135.0	110–160	<0.0001
Diastolic BP (mmHg)	28	79.6 ± 6.7	80.0	70–95	26	90.0 ± 8.0	90.0	75–110	<0.0001
Serum HDL Cholesterol (mmol/L)	28	1.21 ± 0.36	1.21	0.57–2.24	26	0.94 ± 0.26	0.89	0.58–2.04	0.0004
Serum Triglycerides (mmol/L)	28	1.05 ± 0.52	0.93	0.48–2.70	26	1.76 ± 1.07	1.57	0.47–5.33	0.0040
Serum Glucose (mmol/L)	28	4.9 ± 0.6	4.9	3.8–7.0	26	5.6 ± 1.3	5.3	4.4–10.7	0.0219

All 54 participants provided semen sample for analysis. Idiopathic azoospermia was identified in four participants (CG: $n = 2$; MetS: $n = 2$). These samples were eliminated from the statistical analysis of the sperm parameters, although these participants still had clinical and hormonal data included. A total of 50 participants were therefore included for sperm analysis, 26 in the CG (mean age: 36.7 ± 9.1 years) and 24 in the MetS (mean age: 39.9 ± 11.0 years). Age groups did not significantly differ ($P \geq 0.1$). The results of the semen analysis are summarised in Table 4.

Table 4. Semen parameters taken in the study. Although the age groups for these parameters are matching, significant differences between the control and metabolic syndrome groups were found. All statistical analysis performed with parametric student's t-tests, except those indicated with * (Mann–Whitney nonparametric tests). MMP = mitochondrial membrane potential

	Control group				Metabolic syndrome group				
Parameter	*n*	Mean ± SD	Median	Range	*n*	Mean ± SD	Median	Range	*P*-value
Age	26	36.7 ± 9.1	35	24–67	24	39.9 ± 11.0	37	26–63	0.2689
Ejaculation Volume (ml)	26	2.7 ± 1.0	2.7	1.0–5.0	24	2.3 ± 1.6	2.0	0.4–7.0	0.3242
Sperm Concentration (1x10^6/ml)	26	43.2 ± 25.4	38.9	8.8–110.8	24	24.6 ± 14.6	22.1	7.5–57.1	0.0026
Total Sperm Count (1 x 10^6)	26	122.0 ± 108.2	91.7	13.2–465.4	24	59.3 ± 57.1	46.9	3.7–243.7	0.0034*
Progressive Motility (%)	26	31.0 ± 17.6	32.6	0.0–59.5	24	21.7 ± 18.3	21.0	0.0–70.1	0.0725
Total Motility (%)	26	54.8 ± 20.2	55.6	18.5–90.1	24	41.8 ± 20.6	46.5	1.1–74.9	0.0291
Vitality (%)	26	67.0 ± 16.0	66.5	29.0–92.0	24	47.2 ± 25.0	53.5	6.0–88.0	0.0020
Disturbed MMP (%)	19	40.3 ± 24.5	35.0	8.0–91.0	23	62.4 ± 22.3	65.0	21.0–93.0	0.0039
TUNEL-pos. (%)	19	17.8 ± 12.1	15.0	3–45	21	29.8 ± 20.4	28.0	5–83	0.0287

The MetS group showed a highly significant lower sperm concentration ($P = 0.0026$) and total sperm count ($P = 0.0034$), with no difference in ejaculate volume ($P = 0.3242$). Total and progressive motility were higher in the CG compared with the MetS group. While the difference was significant ($P = 0.0291$) for total motility, only a tendency ($P = 0.0725$) was observed for progressive motility. Sperm vitality was significantly lower in the MetS group ($P = 0.002$). The leucocyte concentration for all patients in both groups was >100 000 per ml showing no difference between the groups.

For the determination of MMP and DNA fragmentation, a total of 19 samples in the CG (MMP: mean age: 37.5 ± 10.4 years; DNA fragmentation: mean age: 36.2 ± 6.6 years) and

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23 and 21 samples in the MetS group, respectively (MMP: mean age: 40.4 ± 10.9 years; DNA fragmentation: mean age: 40.3 ± 11.6 years), were analysed. Therefore, a total of 42 samples underwent MMP determination, and 40 samples underwent DNA fragmentation assays. Missing data for MMP (9 samples in the CG; 3 samples in the MetS group) and DF (9 samples in the CG; 5 samples in the MetS group) were due to unsuitable sperm concentrations and/or ejaculation volume for adequate smears, preparation and counting. For both parameters, the ages groups were matching ($P > 0.1$). Results show significantly higher percentages of spermatozoa with disturbed MMP ($P = 0.0039$) and DNA damage ($P = 0.0287$) in the MetS group (Table 4).

A total of 41 participants underwent free testosterone (FT) and free progesterone (FP) assays, with 22 participants in the CG (mean age: 37.8 ± 9.3 years) and 19 participants in the MetS group (mean age: 39.7 ± 9.7 years). Missing data for the assays (6 samples in the CG; 7 samples in the MetS group) were due to inadequate saliva sampling. Both age groups were matching ($P > 0.5$). In the MetS group, significantly lower concentrations for both free testosterone ($P = 0.0093$) and free progesterone ($P = 0.0130$) were detected (Table 5).

Table 5. Saliva hormone results. Patients in the metabolic syndrome group show significantly lower hormonal levels, for both testosterone and progesterone, compared with the control group, with matching age groups. All statistical analysis performed with Mann–Whitney nonparametric tests

	Control group				Metabolic syndrome group				
Parameter	*n*	Mean ± SD	Median	Range	*n*	Mean ± SD	Median	Range	*P*-value
Saliva-free testosterone (pg ml^{-1})	22	240.1 ± 119.8	211.5	74.0–519.7	19	148.2 ± 75.5	116.7	59.1–315.8	0.0093
Saliva-free progesterone (pg ml^{-1})	22	83.2 ± 33.8	84.3	29.4–144.4	19	58.2 ± 23.7	50.6	24.6–122.7	0.0130
T/P ratio	22	2.96 ± 1.12	2.77	1.36–6.02	19	2.65 ± 1.11	2.61	0.86–5.46	0.3466

Apart from the expected standard correlations among BMI, WHR and lipids, as well as sperm motility, viability and disturbed MMP (not shown), the most evident and interesting correlations were found between the saliva concentrations of FT and FP in the CG ($r^2 = 0.5540$; $P = 0.0075$) and the MetS group ($r^2 = 0.3632$; $P = 0.1264$).

Discussion

The metabolic syndrome is a poorly understood pathophysiological phenomenon, associated with complex metabolic, hormonal and immune dysfunction resulting in various deleterious effects on patients. MetS is considered a systemic inflammatory condition that is associated with numerous chronic complications, including, but not limited to, CVD and T2DM (Beilby, **2004**). The basic results of this study, consisting of a cohort of males of reproductive age, have reinforced the known clinical and biochemical features of MetS, showing highly significant differences between the CG and MetS group in BMI, (WC) and WHR, blood pressure, HDL cholesterol, triglycerides and glucose concentrations.

The effect of obesity on sperm parameters has not been conclusively resolved. Various studies have indicated that obesity is associated with a reduction in sperm count and

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concentration, motility, vitality, morphology and DNA integrity (Jensen *et al.*, **2004**; Fejes *et al.*, **2005**, **2006**; Kort *et al.*, **2006**; Stewart *et al.*, **2009**; Hofny *et al.*, **2010**; Håkonsen *et al.*, **2011**). In contrast, other researchers have not found similar relationships (Aggerholm *et al.*, **2008**; Pauli *et al.*, **2008**; Chavarro *et al.*, **2010**; Rybar *et al.*, **2011**). A limited meta-analytical review by MacDonald *et al.* (**2010**) investigated the association of BMI with hormonal and semen parameters and found no negative association between increased body weight and male seminal analysis. Sermondade *et al.* (**2012**) have recently published a meta-analysis indicating that being overweight or obese is associated with an increased risk of azoospermia and oligozoospermia. Furthermore, there is strong evidence for reduced testosterone with increased BMI. Thus, the negative effect of obesity on optimal sperm parameters determined by the World Health Organisation (WHO, **2010**) is neither consistent, nor is there a clear dose–response mechanism (Hammoud *et al.*, **2008**). As obesity is a cardinal component of MetS, it is hypothesised that inconsistent results in obesity studies may be related to the underlying metabolic pathophysiology associated with MetS.

The effect of MetS on male fertility has not been previously evaluated in a similar case-controlled study and remains relatively unknown. This small pilot study aimed to determine whether MetS has a negative relationship with male fertility potential. The results of this study indicate significantly compromised sperm parameters in males clinically diagnosed with MetS. However, it should be noted that although the mean sperm concentration in the MetS group was significantly lower than in the CG, the mean concentration of 24.6 million ml^{-1} (Table 4) within this group is above the threshold considered normal according to parameters defined by WHO (**2010**). A similar association is also seen with the total sperm count, total motility and vitality in the MetS group. Together with the other data of the semen analysis, these results reveal an overall reduction in sperm number and function, but not necessarily an absolute indication for male infertility in patients with MetS.

Since MetS is considered a systemic inflammatory condition with increased serum levels of C-reactive protein, inflammatory cytokines (Tamakoshi *et al.*, **2003**) and reactive oxygen species (ROS) (Furukawa *et al.*, **2004**), the observation that patients with MetS have increased damage to mitochondrial function and spermatozoa DNA appears conceivable, as both ROS and inflammatory cytokines have been repeatedly shown to cause damage to the sperm plasma membrane and DNA (Henkel, **2005**; Martinez *et al.*, **2007**). This is particularly interesting as the study design excluded patients with leucocytospermia, and the mean numbers of leucocytes present in the ejaculates of males from both control and MetS group were $>0.1 \times 10^6$ ml^{-1}. Although numerous studies have indicated a reduction in sperm count, motility and reduced male fertility potential (Dousset *et al.*, **1997**; Lampiao & du Plessis, **2008**; Tronchon *et al.*, **2008**), it is unclear whether the potential negative effects of pro-inflammatory cytokines caused this effect as the results of this study indicates.

Although speculative, and assuming that increased serum concentrations of cytokines and ROS may potentially cross into the testicular tissue via the blood–testes barrier and/or gain access to seminal fluid through secretions of the seminal vesicles and prostate during ejaculation, inflammatory cytokines may potentially reduce sperm concentration, motility

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and fertilising capabilities. This would provide a novel explanation for reduced fertility potential in obese, MetS and T2DM males, and would provide an interesting avenue for future research into possible underlying mechanisms associated with these results. Yet, apart from these potential direct effects of cytokine action, indirect effects on spermatogenesis via effects on Sertoli cells or peritubular cells should not be neglected.
In adult males, MetS correlates with reduced serum testosterone concentrations and raised gonadotropins, thus reflecting primary hypogonadism (Kupelian *et al.*, **2006**; Guay, **2009**; Saad & Gooren, **2009**). It is emerging that reduced serum testosterone in nonobese men, including those with asymptomatic androgen deficiency, increases the risk of developing MetS (Boyanov *et al.*, **2003**; Kupelian *et al.*, **2006**), with further lines of evidence suggesting that the clinical administration of testosterone can improve many of the characteristics associated with the syndrome (Saad & Gooren, **2009**).

Salivary testosterone assays are a noninvasive method that offers both technical and cost advantages and is a reliable substitute for serum calculated free or bioavailable testosterone (Yasuda *et al.*, **2008**). Calculated free testosterone levels may not accurately reflect the concentration of metabolically active (bioavailable) testosterone, as reduced testosterone and hypogonadism are associated with an increase in SHBG (Morales *et al.*, **2000**; Feldman *et al.*, **2002**; Morales, **2002**; Yasuda *et al.*, **2008**). Therefore, in this study, saliva sampling was utilised for accuracy of bioavailable hormone concentrations and cost-effectiveness. Furthermore, few, if any, studies have looked at saliva hormone concentrations in males with MetS, with well-documented decline in serum concentrations and calculations. A significant difference was found between the groups for free testosterone (FT) in saliva, corresponding to literature reports demonstrating hypogonadism reflected in the serum of male patients with MetS.

Although the role of progesterone in female reproductive physiology and pathology has been extensively elicited, literature on progesterone in male reproductive function is scanty. Traditionally, progesterone in males has been viewed as an unimportant precursor hormone in male physiology and has only recently begun to be recognised as an important modulator of male endocrine function (Oettel & Mukhopadhyay, **2004**). Progesterone is synthesised predominantly in the Leydig cells and adrenal glands in men, sourced from cholesterol via pregnenolone. Testosterone is synthesised predominantly from progesterone via its immediate metabolite, 17α-hydroxyprogesterone (17α-OH-P). 17α-OH-P is further hydrolysed into androstenedione, which is itself hydrolysed into testosterone following the Δ^5-steroid pathway. Pregnenolone, as a precursor to progesterone, can also be converted into 17α-OH-pregnenolone, which is hydrolysed into dehydroepiandrosterone, a precursor of androstenedione, and hence testosterone, via the Δ^4-steroid pathway (Sherbet *et al.*, **2003**; Midzak *et al.*, **2009**).

Progesterone has not been previously studied in men diagnosed with MetS. For similar reasons related to FT, we assayed progesterone in saliva samples. The results of this study indicate that saliva concentrations of free progesterone (FP) are reduced in MetS-positive males. These results correlate with those published by Blanchette *et al.* (**2006**), who reported a negative correlation between BMI and serum progesterone. Considering the reduced FT concentrations in saliva, it would be logical, albeit premature, to suggest that testosterone production might be reduced in MetS due to a restriction in the progesterone

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precursor supply based on the Δ^5-steroid pathway. Yet, this idea would agree with Isidori *et al.* (**1999**), who found that serum 17-OH-P is reduced in obese patients compared with controls and moderately obese (BMI 30–40) patients compared with massively obese (BMI > 40) patients.

Blanchette *et al.* (**2006**) also reported a negative correlation between serum 17-OH-P and BMI. However, testosterone may be sourced via the Δ^4-steroid pathway, bypassing the need for progesterone and 17-OH-P. This would imply that cholesterol conversion into pregnenolone may be affected by MetS, although the molecular mechanisms associated with these results are unlikely to be this simplistic. Nevertheless, this assumption is supported by the fact that in men of the CG, saliva testosterone concentrations are significantly and positively correlated with the progesterone concentrations, but not in the MetS group.

One can further speculate that clues to the possible mechanisms that would explain, at least partially, the reduction in testosterone and progesterone concentrations may be found by considering the low-grade systemic inflammation associated with MetS, as transcription and function of the steroidogenic enzymes may be modulated by the action of various cytokines. A review by Payne & Youngblood (**1995**) indicated that pro-inflammatory interleukin (IL)-1 and tumour necrosis factor-alpha (TNF-α) cause a reduction in cholesterol side-chain cleavage cytochrome ($P450_{scc}$; CYP11A) and $P450_{17\alpha}$ (CYP17) mRNA expression in rat Leydig cells, associated with reduced LH- or cAMP-stimulated testosterone production. IL-1β-induced hCG-stimulated testosterone inhibition in rat Leydig cells is reversed with the co-culture of an IL-1 antagonist (Sherbet *et al.*, **2003**; Hales *et al.*, **2005**). Similar results were found in mouse Leydig cell lines incubated with IL-1 and TNF-α (Payne & Youngblood, **1995**). If confirmed, these mechanisms would indicate that a pro-inflammatory state associated with MetS may reduce pregnenolone production in the mitochondria and downregulate the steroidogenic cascade, resulting in both a reduction in systemic progesterone and testosterone as reported in this study. However, at this point in time, the results of this study are limited by the sample size in each group due to the pilot study nature of the research, and the results cannot be generalised across all patients with MetS. However, as inflammatory cytokines act as paracrine and pleiotropic regulators in spermatogenesis via complex interactions with Sertoli cells, in addition to steroidogenesis (Białas *et al.*, **2009**; Guazzone *et al.*, **2009**; Yao *et al.*, **2009**), it is conceivable that any alteration in cytokine concentrations within the reproductive tract related to a chronic inflammatory state may further provide a novel explanation for the reduction in sperm count and function observed in this study.

In conclusion, the results of this pilot study suggest that males diagnosed with MetS have reduced fertility potential in the absence of leucocytospermia and clinical detection of varicocoele. Reduced FT would in part explain some of these findings. FP concentrations have also been found to be lower in patients with MetS, and the implications of this require further attention. These results warrant further investigation into confirming these conclusions and understanding possible mechanisms that may explain potential subfertility in male patients with MetS.

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