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Bone mineral density is preserved in men with idiopathic infertility

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ABSTRACT

Background: Lower semen quality is associated with increased mortality and morbidity, which may include osteoporosis.

Objective: To assess whether infertile men have a lower bone mineral density (BMD) compared with fertile men at the time of fertility workup.

Methods: A total of 146 men from infertile couples with unexplained impaired semen quality, characterized by sperm concentration < 20 million/mL, progressive motility < 50% or < 12% morphologically normal spermatozoa. Men with infertility due to a genetic etiology or a condition that could cause testicular damage were excluded. A total of 271 men from couples with an ongoing naturally conceived pregnancy served as a control group. Lumbar, femoral, and total body BMD were measured by dual X-ray absorptiometry.

Results: Infertile men had similar BMD compared with fertile men (Beta coefficient (g/cm^2) and 95% confidence interval for the difference between the two groups: −0.02 (−0.05; 0.01) for lumbar BMD, −0.02 (−0.05; 0.01) for femoral neck BMD, −0.01 (−0.04; 0.02) for total femur BMD, and −0.01 (−0.03; 0.01) for total body BMD). Semen parameters were not associated with BMD measurements. Furthermore, BMD did not differ between infertile men with the lowest semen quality vs. infertile men with better semen quality nor between infertile men with low testosterone vs. fertile men with normal testosterone levels.

Conclusion: Bone mineral density is preserved in men with unexplained infertility at the time of fertility workup.

INTRODUCTION

Recent studies have shown that semen quality is an important marker for current and future health in young adult men (Eisenberg *et al.*, 2015). Men with lower sperm concentration are at higher risk of developing adverse health outcomes, especially cardiovascular disease and diabetes (Latif *et al.*, 2017, 2018). Moreover, these men also have a higher mortality risk. This is due to a variety of diseases and not only related to lifestyle factors (Jensen *et al.*, 2009; Eisenberg *et al.*, 2014).

Osteoporosis is associated with major morbidity and mortality in aging men. It is a frequent disease, with an estimated lifetime risk of an osteoporotic fracture of 20–25% for a man at age 50 (Laurent *et al.*, 2013). The origin of impaired bone health may, however, be much earlier in life, as the peak bone mass acquired in adolescence and early adulthood is a key determinant of future osteoporosis and fracture risk (Mora & Gilsanz, 2003). Thus, it may be possible to diagnose tendencies for impaired bone health already at a younger age.

Testosterone deficiency is a well-known risk factor for bone loss and osteoporosis in men (Rochira *et al.*, 2018). Infertile men have a higher risk of developing testosterone deficiency (Skakkebaek *et al.*, 2016), and a positive association between sperm counts and Leydig cell capacity for testosterone production has been observed (Andersson *et al.*, 2004; Jørgensen *et al.*, 2016; Olesen *et al.*, 2018).

Three studies have shown that infertile men have a lower bone mineral density (BMD) compared with healthy men from the general population (Karasek *et al.*, 2000; Yang *et al.*, 2012; Bobjer *et al.*, 2016). This association appeared more pronounced in men with low testosterone levels (Yang *et al.*, 2012; Bobjer *et al.*, 2016). However, male infertility is a heterogeneous condition, which includes chromosome disorders and endocrinopathies. As osteoporosis is a silent disease, it is important to identify men

who are at risk for developing bone loss. Reduced semen quality may thus be a useful and early marker to identify young adult men with impaired bone health.

In this study, we used data from a well-characterized cross-sectional cohort of men with idiopathic infertility to investigate whether they have a lower bone mineral density than fertile men and whether bone mineral density is associated with semen parameters.

METHODS

Study population

From 2013 to 2016, 149 men were included from the outpatient clinic of the Department of Growth and Reproduction, Rigshospitalet, Copenhagen. The men had all been referred for an andrological examination because of 'male factor infertility.' Inclusion criteria for participation in this study were age between 20 and 45 years old, the patient and his mother were born and raised in Denmark, and ICSI treatment planned following 'sperm wash' and having a semen sample with sperm concentration < 20 million/mL, progressive motility < 50% or <12% morphologically normal spermatozoa during routine clinical workup. Exclusion criteria were azoospermia, genetic disorders (Klinefelter syndrome, microdeletions of the Y chromosome), history of orchitis, epididymitis, testicular torsion, varicocelectomy, vasectomy, orchiectomy, chemotherapy or radiation therapy, and the presence of diseases requiring permanent treatment.

Additionally, from 2012 to 2014, men of couples with a naturally achieved pregnancy were included as a control group (i.e. no use of fertility medication or assisted reproductive technology). The men were invited to participate when their pregnant female partners had their routine second-trimester examination, and 277 agreed to participate. Additional inclusion criteria were age 20–45 years, born and raised in Denmark and residence in the local referral area.

Men with missing dual X-ray absorptiometry (DXA) data ($n = 6$) or missing semen analysis ($n = 3$) were excluded from the analysis, leaving 146 infertile men and 271 fertile men in the analytical sample.

The local ethical committee approved the study protocol (Protocol number for fertile men: H-2-2012-090 and for infertile men: H-2-2012-091). All men gave written informed consent.

Physical examination and questionnaire

The men underwent a physical examination including assessment of possible presence of a varicocoele (grades 1–3) or hydrocele, enlargement of the epididymis and location, consistency and size of the testes assessed by palpation. Testis size was also assessed by ultrasound. Weight and height were measured, and body mass index (BMI) calculated. A standardized questionnaire similar to previous studies on male reproductive function was used (Jørgensen *et al.*, 2001, 2012). The questionnaire included information on previous and current diseases, history of fertility and infertility, and current lifestyle factors.

Semen analysis

For this study, all men produced one semen sample by masturbation in a room near the semen laboratory. The abstinence time was calculated from the self-reported last ejaculation time and the time the semen sample was delivered. The semen samples were kept at 37 °C until liquefaction. Semen analysis was carried out according to the WHO guidelines (World Health Organization, 2010). Semen volume was assessed by weighing.

Sperm motility was assessed in duplicate by placing 2 × 10 μL of well-mixed semen on a glass slide kept at 37 °C, covered with a 22 × 22 mm coverslip, and examined at ×400 magnification. Spermatozoa were classified as progressive motile, local motile, or immotile. The sperm concentration was also assessed in duplicates using a Bürker-Türk haemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). For assessment of the morphology of the spermatozoa, smears were air-dried at room temperature, fixed in ethanol and Papanicolaou stained. Sperm morphology was assessed according to strict criteria (Menkveld *et al.*, 1990). Total sperm count was calculated by multiplying sperm concentration and semen volume.

Blood samples and hormone analysis

A morning non-fasting venous blood sample was obtained from each participant. Alkaline phosphatase was measured by enzymatic absorption photometry (Cobas c702, Roche Diagnostics, Basel, Switzerland). Luteinizing hormone (LH), follicle stimulating hormone (FSH), and estradiol (E2) were measured by an immunofluorometric assay (Wallac, Turku, Finland). Total testosterone (total T) and sex hormone-binding globulin (SHBG) were measured by an enzyme-linked immunosorbent assay (Beckman Coulter, Wycombe, UK). Free testosterone (free T) was calculated from total testosterone and SHBG by using the Vermeulen equation (Vermeulen *et al.*, 1999). Inhibin B was measured by a specific two-sided enzyme-linked immunosorbent assay (Beckman Coulter). Inter- and intra-assay coefficients of variation (CVs) were, respectively, 3% and 2% for FSH, 2% and 3% for LH, 5% and 4% for SHBG, 5% and 4% for testosterone, <4% for E2, and 10% and 3% for inhibin B.

Dual X-ray absorptiometry

Bone mineral density (BMD) of the lumbar spine (L1–L4), dual femur (neck and total femur), and total body BMD was measured by using DXA (Lunar Prodigy, GE Healthcare, Chicago, IL, USA). For the femoral measurements, the average of the left and right scan was calculated. A built-in reference population was used to calculate Z- and T-scores. The Z-score indicates the number of standard deviations the BMD of a patient differs from age-matched controls, whereas a reference population of young healthy subjects is used to calculate the T-score. According to the WHO standards, osteopenia was defined by a T-score between −1.0 and −2.5 and osteoporosis as a T-score ≤ 2.5 (Ebeling, 2008). Calibration was done on a regular basis according to the manufacturer guidelines. The spine phantom was scanned daily to monitor device performance. The precision errors of these measurements were lower than 0.5%.

Statistical analysis

Baseline characteristics are presented as median and 5th–95th percentiles for continuous variables and frequencies for categorical variables. Differences between fertile and infertile men were assessed using *t*-tests for continuous variables and chi-squared tests for categorical variables. For semen parameters, differences between the two groups were tested by linear regression adjusted for abstinence time. To meet linear regression assumptions, semen parameters were log-transformed.

Table 1 Baseline characteristics

	Total cohort (n = 417) median (5th; 95th pct)	Infertile men (n = 146) median (5th; 95th pct)	Fertile men (n = 271) median (5th; 95th pct)	p-value
General				
Age (years)	32.8 (26.6; 41.0)	34.1 (26.9; 41.5)	32.5 (26.6; 39.7)	**0.002**
Weight (kg)	80.9 (66.2; 104.1)	82.1 (68.9; 111.5)	80.3 (65.7; 102.5)	**0.003**
Height (cm)	183.2 (173.6; 194.9)	184.0 (173.6; 195.2)	182.7 (174.0; 194.7)	0.245
BMI	23.8 (20.3; 29.9)	24.3 (21.0; 31.9)	23.7 (20.1; 29.5)	**0.006**
Waist circumference (cm)	85.0 (74.5; 103.0)	86.0 (75.0; 109.0)	84.5 (74.0; 100.0)	**0.004**
Alcohol (units/week)	9 (0; 27)	8 (0; 25)	9 (0; 29)	0.097
Smokers (%)	24.5	18.2	27.8	**0.031**
Physical activity (hours/week)	4.0 (0; 23.7)	3.0 (0; 24.1)	4.5 (0; 21.6)	0.827
Testis size on ultrasound (mL)	14.2 (8.7; 21.5)	13.0 (7.7; 20.9)	14.6 (9.2; 22.0)	**<0.001**
Any medication use in the last 3 months[a] (%)	15.5	16.8	14.8	0.599
Ever had cryptorchidism (%)	10.3	11.8	9.6	0.505
Treated for cryptorchidism (%)	5.6	8.2	4.2	0.122
Presence of grades 2–3 varicocoele[b] (%)	12.7	10.4	17.0	0.061
Hormones				
Total testosterone (nmol/L)	13.9 (7.4; 22.4)	14.2 (6.6; 22.4)	13.9 (8.1; 22.2)	0.860
SHBG (nmol/L)	34 (18; 61)	34 (18; 65)	34 (18; 59)	0.654
Calculated free testosterone (pmol/L)	275 (165; 399)	274 (139; 414)	277 (174; 394)	0.470
Estradiol (pmol/L)	74 (25; 150)	78 (38; 137)	70 (22; 154)	0.204
LH (U/L)	3.4 (1.6; 7.2)	4.2 (1.8; 9.1)	3.0 (1.5; 6.6)	**<0.001**
FSH (U/L)	3.5 (1.4; 10.1)	4.3 (1.5; 14.1)	3.2 (1.3; 7.1)	**<0.001**
Inhibin B (pg/ml)	168 (65; 299)	159 (43; 298)	175 (94; 299)	**0.006**
T/E2 ratio	0.19 (0.08; 0.50)	0.18 (0.08; 0.32)	0.20 (0.09; 0.65)	**0.003**
T/LH ratio	4.0 (1.8; 8.9)	3.3 (1.3; 8.1)	4.6 (2.2; 9.9)	**<0.001**
Total T < 10.5 nmol/L (%)	20.3	24.8	17.9	0.096
Total T < 8 nmol/L (%)	7.3	12.4	4.5	**0.003**
Semen parameters				
Semen volume (mL)	3.9 (1.9; 7.4)	3.9 (1.9; 7.2)	3.8 (2.0; 7.4)	0.995
Ejaculation abstinence (h)	68 (44; 194)	61 (37; 153)	72 (45; 225)	**0.012**
Time until motility analysis (min)	30 (10; 70)	30 (15; 80)	30 (10; 70)	0.837
Total count (million)	167 (7; 725)	41 (3; 442)	254 (33; 791)	**<0.001**
Concentration (million/mL)	44 (2; 172)	12 (1; 105)	63 (10; 195)	**<0.001**
Progressive motile (%)	56 (9; 81)	41 (4; 82)	60 (30; 80)	**<0.001**
Locally motile (%)	7 (1; 16)	7 (1; 16)	8 (1; 16)	0.783
Immotile (%)	36 (14; 81)	50 (15; 90)	32 (14; 58)	**<0.001**
Normal morphology (%)	6.5 (0.5; 16.5)	2.8 (0; 15.0)	8.5 (2.0; 17.0)	**<0.001**
Total count < 39 million (%)	21.1	49.3	5.9	**<0.001**
Concentration < 15 mil/mL (%)	23.3	52.7	7.4	**<0.001**
Progressive motility < 32% (%)	17.6	38.5	6.6	**<0.001**
Normal morphology < 4% (%)	30.6	63.1	13.0	**<0.001**

Values are reported as median and 5th–95th percentile for continuous variables and percentage for categorical variables. Differences between the two groups were assessed by using a T-test for continuous variables and a chi-squared test for categorical variables. For semen parameters, differences between the two groups were assessed by linear regression adjusted for abstinence time with the log-transformed semen parameters as independent variable. BMI, body mass index; E2, estradiol; T, testosterone; SHBG, sex hormone-binding globulin. p-values in bold are significant. [a]Medication use last 3 months: taken any medication for at least one week in the three months prior to participation. [b]Grade 2 or 3 varicocoele detected during physical examination.

Associations between age and BMD measurements and semen parameters and BMD measurements in infertile men were illustrated graphically, with the addition of reference lines indicating the mean BMD ± 2 standard deviations of the fertile men from the control group.

Differences in BMD measurements between infertile and fertile men were assessed by linear regression for continuous variables and logistic regression for categorical variables (unadjusted and adjusted for age, BMI, smoking, alcohol intake, and physical activity). Associations between log-transformed semen parameters and BMD measurements were also assessed by linear regression (unadjusted and adjusted for abstinence time, time to semen analysis, age, BMI, smoking, alcohol intake, and physical activity).

Additionally, infertile men were categorized according to semen quality as previously described (Damsgaard *et al.*, 2016), as a reduction in one semen parameter can have a negative effect on fertility, even though the other parameters are in the normal range. Low semen quality was defined as a sperm concentration < 15 million/mL or motility < 32% or normal morphology < 4%. All other men were classified as having better sperm quality. Differences between the groups were analyzed by linear regression (unadjusted and adjusted for age, BMI, smoking, alcohol, and physical activity). For semen parameters, the model was also adjusted for abstinence time.

A p-value < 0.05 was considered statistically significant. Data were analyzed using STATA version 13.1 (StataCorp, College Station, TX, USA).

RESULTS

The analytical sample consisted of 146 infertile men and 271 fertile men. Infertile men were older and had a higher BMI and waist circumference compared with fertile men. The number of smokers was lower in the infertile group. Total and free T and E2 levels were similar in both groups, but LH was

Table 2 Comparison of bone mineral density and alkaline phosphatase between fertile and infertile men

	Infertile men (n = 146) median (5th–95th pct)	Fertile men (n = 271) median (5th–95th pct)	Beta coefficient/odds ratio (95% CI) for difference between the two groups with the fertile men as the reference group	
			Unadjusted	Adjusted
Spine				
L1–L4 BMD (g/cm²)	1.23 (1.03; 1.45)	1.24 (1.00; 1.50)	−0.01 (−0.04; 0.02)	−0.02 (−0.05; 0.01)
L1–L4 Z-score	−0.05 (−1.71; 1.80)	0.08 (−1.68; 2.22)	−0.19 (−0.43; 0.05)	−0.15(−0.40; 0.10)
L1–L4 T-score	0.11 (−1.59; 1.91)	0.20 (−1.81; 2.35)	−0.10 (−0.35; 0.15)	−0.14 (−0.39; 0.11)
L1–L4 T-score < −1 (%)	16.2	15.7	1.04 (0.59; 1.80)	1.04 (0.58; 1.86)
L1–L4 T-score ≤ −2.5 (%)	0.7	1.5	0.47 (0.05; 4.21)	0.42 (0.04; 4.07)
Femur				
Femoral neck BMD (g/cm²)	1.09 (0.88; 1.30)	1.10 (0.88; 1.33)	−0.02 (−0.05; 0.01)	−0.02 (−0.05; 0.01)
Femoral neck Z-score	0.06 (−1.35; 1.59)	0.31 (−1.34; 1.89)	−0.17 (−0.38; 0.04)	−0.18 (−0.39; 0.03)
Femoral neck T-score	0.14 (−1.45; 1.80)	0.22 (−1.50; 1.99)	−0.15 (−0.36; 0.07)	−0.17 (−0.38; 0.05)
Femoral neck T-score < −1 (%)	13.1	10.7	1.26 (0.68; 2.33)	1.33 (0.70; 2.53)
Femoral neck T-score ≤ −2.5 (%)	0	0		
Total femur BMD (g/cm²)	1.11 (0.92;1.37)	1.12 (0.91; 1.39)	−0.00 (−0.03; 0.03)	−0.01 (−0.04; 0.02)
Total femur Z-score	0.14 (−1.43; 2.07)	0.23 (−1.30; 2.06)	−0.06 (−0.28; 0.15)	−0.08 (−0.30; 0.14)
Total femur T-score	0.20 (−1.33; 2.14)	0.21 (−1.42; 2.30)	−0.03 (−0.25; 0.20)	−0.07 (−0.29; 0.16)
Total femur T-score < −1 (%)	13.1	11.9	1.12 (0.61; 2.05)	1.16 (0.62; 2.20)
Total femur T-score ≤ −2.5 (%)	0	0		
Total body				
Total body BMD (g/cm²)	1.34 (1.18; 1.56)	1.34 (1.14; 1.55)	0.01 (−0.02; 0.03)	−0.01 (−0.03; 0.01)
Total body Z-score	1.18 (−0.35; 2.90)	1.15 (−0.39; 2.99)	−0.03 (−0.24; 0.19)	−0.09 (−0.31; 0.13)
Total body T-score	1.42 (−0.24; 3.52)	1.36 (−0.62; 3.45)	0.10 (−0.15; 0.35)	−0.07 (−0.30; 0.15)
Alkaline phosphatase (U/L)	57 (38–80)	57 (38–84)	−1.41 (−4.38; 1.56)	−1.61 (−4.69; 1.46)

Median and 5th–95th percentile values for continuous variables and percentage for categorical variables. To assess differences between groups, linear regression was used for continuous variables and logistic regression for categorical variables. The analysis was performed unadjusted and adjusted for age, BMI, smoking, alcohol, and physical activity. BMD, bone mineral density, CI, confidence interval.

higher in the infertile group. As expected, infertile men had higher levels of FSH, lower inhibin B, and lower semen parameters (Table 1). Overall, BMD was not associated with age (Figure S1).

Bone mineral density measurements did not differ between the two groups (Table 2). The number of men with osteopenia was similar for infertile and fertile men (16.2% vs. 15.7% at the lumbar level, 13.1% vs. 10.7% for the femoral neck, and 13.1% vs. 11.9% for total femur). There were very few participants with a lumbar BMD in the osteoporotic range (n = 4 in the fertile group and n = 1 in the infertile group) and no participants with femoral osteoporosis (Table 2). The level of alkaline phosphatase was not different between the two groups (Table 2).

In the fertile men, there were no associations between BMD measurements and any semen parameter (Table S1). Infertile men had a slightly lower lumbar, femoral, and total body BMD with increasing sperm concentration and with increasing total sperm count, but this was not significant after adjusting for confounders (Figures 1 and 2 and Table S1). A similar pattern was observed for lumbar and total body BMD and progressive motility (Figure S2 and Table S1). There was no association between sperm morphology and BMD measurements (Figure S3 and Table S1).

When categorizing the men according to semen quality (hereby combining the three semen parameters in one), infertile men with the lowest semen quality had higher BMD than infertile men with better semen quality, but this association disappeared after adjustments (Table S2).

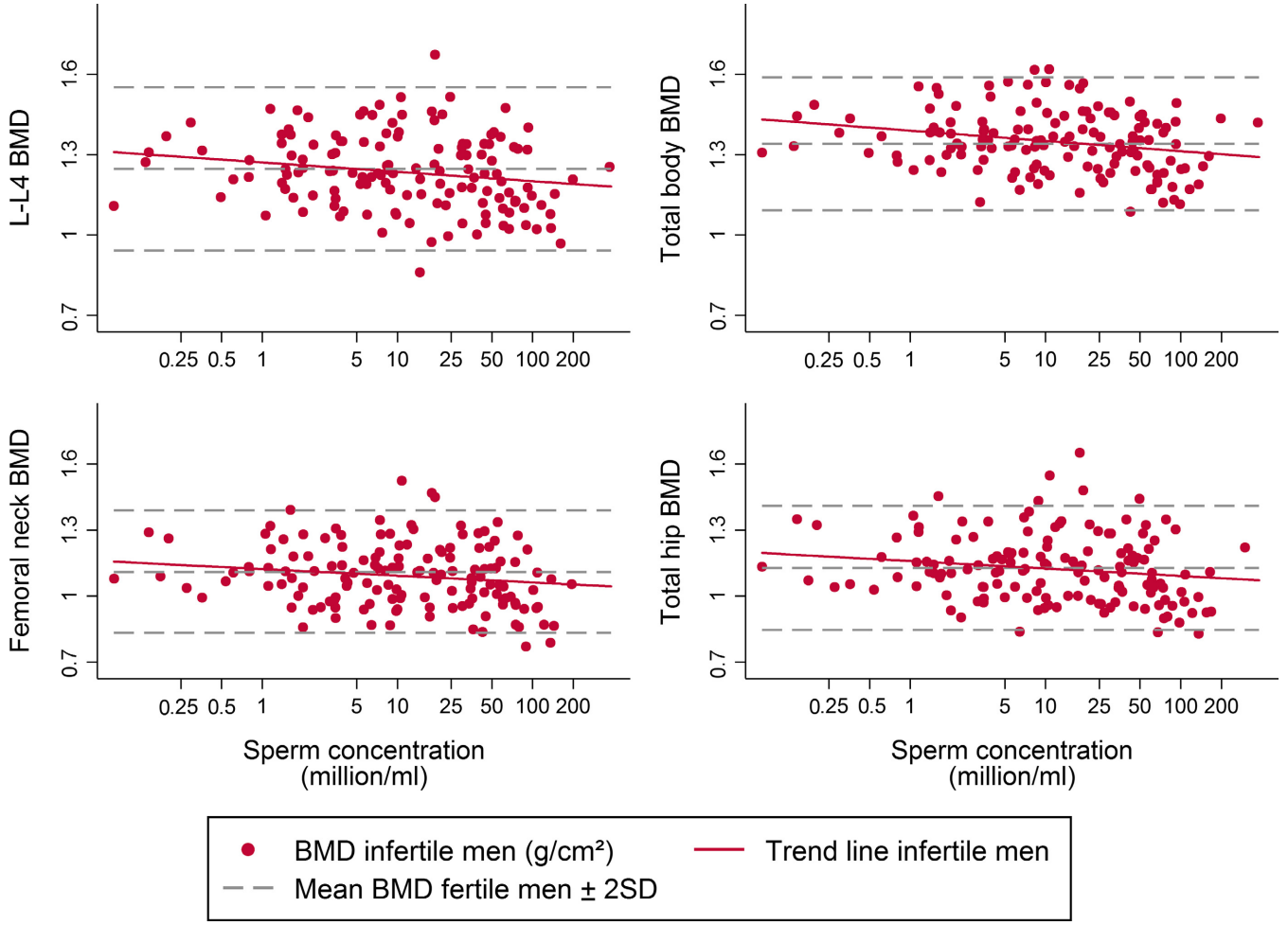
In a subanalysis comparing fertile men with normal testosterone levels (>10.5 nmol/L) to infertile men with low testosterone levels (<10.5 nmol/L), there were no differences in BMD measurements (Table S3). Bivariate plots of total T/LH and free T/LH illustrate the Leydig cell function of the infertile and fertile men in relation to the normal ranges from healthy Danish men (age range 18–50) (Aksglaede *et al.*, 2007) (Figure S4). Of the infertile men, 40% were outside the reference range for total T/LH, whereas this was 20% for the fertile men. For free T/LH, 41% of the infertile and 19% of the fertile men were outside the reference range. Neither for total T/LH, nor for free T/LH, did BMD measurements differ when comparing men in the reference range to men outside the reference range (data not shown).

DISCUSSION

In this cross-sectional study, we did not detect any difference in bone mineral density measurements between idiopathic infertile and fertile men at the time of fertility evaluation. Furthermore, semen parameters were not associated with BMD measurements. This could possibly be explained by the fact that our focus was on men with idiopathic infertility, of whom most had normal testosterone levels.

Our results are in contrast to previously published studies investigating bone health in infertile men (Karasek *et al.*, 2000; Yang *et al.*, 2012; Bobjer *et al.*, 2016). A small Polish study showed that men with oligozoospermia had lower lumbar BMD and T-score compared with young healthy men. This was, however, not adjusted for age (Karasek *et al.*, 2000). Also in a large Chinese cohort, infertile men (defined as time to first pregnancy exceeding 12 months) had a lower lumbar spine and total hip BMD compared with age-matched men from the general population (Yang *et al.*, 2012). A recent Swedish study excluding men

Figure 1 Scatter plot of sperm concentration and bone mineral density measurements in infertile men. The dashed lines represent mean and ± 2 SD of fertile men as a reference range. Beta coefficients and confidence intervals of these associations are shown in Table S1.



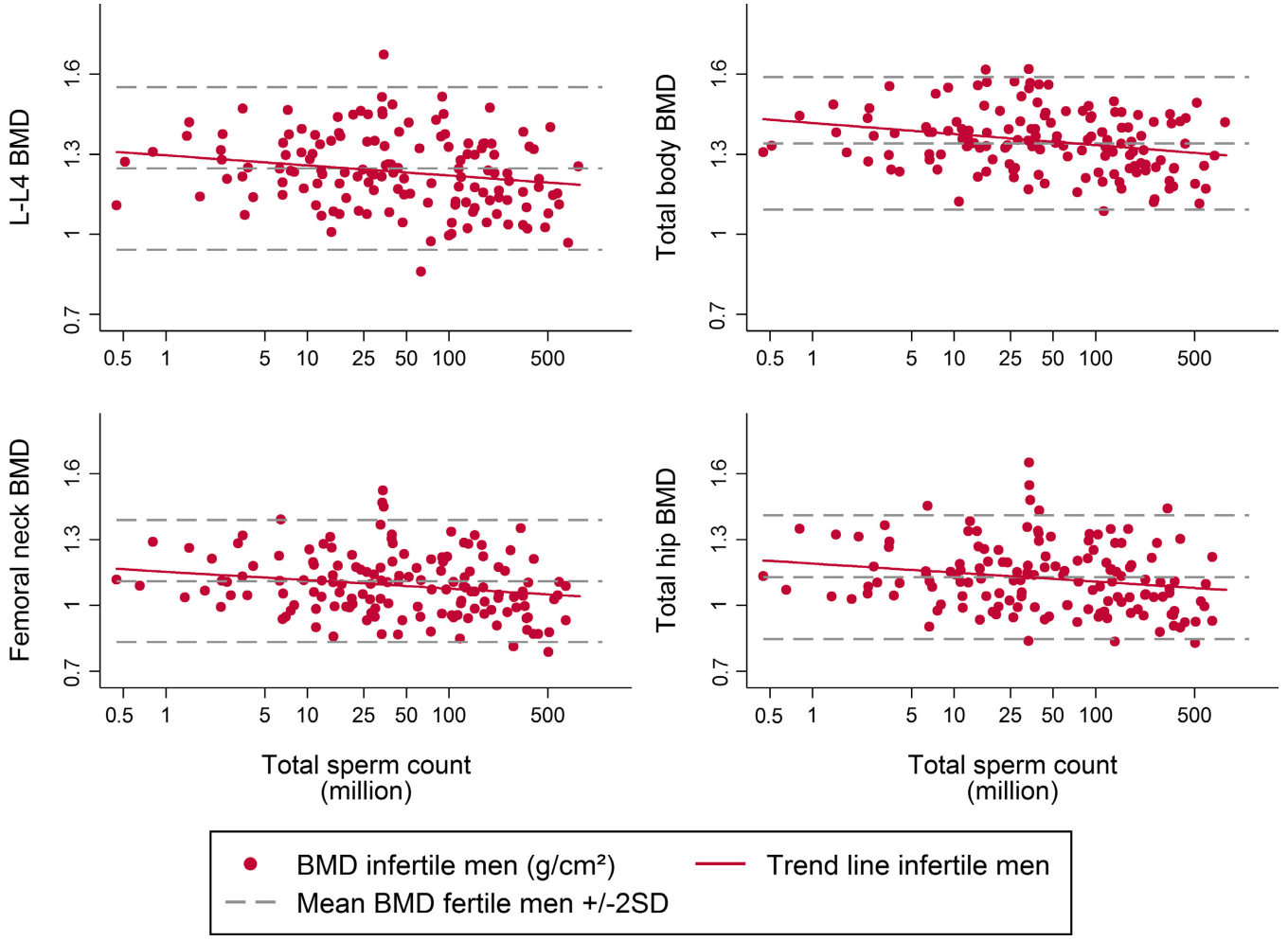
with a range of diseases and use of various medications showed that total hip BMD in infertile men with sperm concentration < 20 million/mL was slightly lower than that of control subjects from the general population after adjusting for age, BMI, and smoking. Additionally, infertile testosterone deficient men had a lower lumbar spine BMD than infertile men with normal testosterone levels (Bobjer *et al.*, 2016).

Different inclusion and exclusion criteria could partly explain the differences between our results and the previously reported data. We did not restrict inclusion to men with oligozoospermia. Also, men with infertility due to low sperm motility and/or a low number of morphologically normal spermatozoa were included. Although not all men had semen parameters below the WHO reference limits, 78% of men had at least one of the three main variables below the WHO cutoffs and all couples required ICSI because of male factor infertility determined after a diagnostic wash trial.

On the other hand, we only included men with unexplained infertility as our focus if these men had a lower BMD, as this could be a contributing factor to the higher morbidity and mortality risk that has been observed in infertile men (Jensen *et al.*, 2009; Eisenberg *et al.*, 2015; Latif *et al.*, 2018). Of note, this association between infertility and a higher mortality and morbidity risk in later life is not only observed among men with semen parameters below WHO cutoff levels, but also far above. Men with a genetic etiology such as Klinefelter syndrome or with a medical history of a condition that can cause testicular damage were excluded. These conditions not only affect spermatogenesis but often have a negative impact on Leydig cell function as well.

It is well-known that testosterone deficiency is a risk factor for bone loss and osteoporosis in men (Rochira *et al.*, 2018). Low testosterone levels are associated with increased bone resorption, though the effect of testosterone on bone is largely mediated by its conversion to estradiol (Rochira *et al.*, 2006). In two of the previously reported studies, differences in BMD were indeed more pronounced in men with low testosterone levels (Yang *et al.*, 2012; Bobjer *et al.*, 2016). We could not, however, detect any differences in BMD measurements when comparing infertile men with testosterone levels below 10.5 nmol/L to fertile men with testosterone above 10.5 nmol/L (300 ng/dL). Even with a lower cutoff of 7 or 8 nmol/L, infertile men had similar BMD values as fertile men with total testosterone > 10.5 nmol/L (data not shown). Observational and intervention studies have shown that bone loss occurs when testosterone levels drop below 6.9 nmol/L (200 ng/dL) (Snyder *et al.*, 1999; Fink *et al.*, 2006; Finkelstein *et al.*, 2016). Most men with idiopathic infertility have testosterone levels above this threshold (Andersson *et al.*, 2004) as was also the case in our study population.

Figure 2 Scatter plot of total sperm count and bone mineral density measurements in infertile men. The dashed lines represent mean and ± 2 SD of fertile men as a reference range. Beta coefficients and confidence intervals of these associations are shown in Table S1.



From animal models and from observational studies in postmenopausal women, an association between high serum FSH levels and a decrease in BMD has been reported, but the underlying mechanism is still unclear (Zaidi *et al.*, 2018). Although the group of infertile men had higher FSH levels compared with the fertile men, BMD was similar in the two groups. In our study, serum FSH does thus not seem to affect BMD in infertile men at the time of infertility workup. However, longitudinal follow-up of BMD in men with a history of infertility and high FSH levels for a prolonged period of time is needed to further investigate this potential association.

Body weight, waist circumference, and BMI were higher in the group of infertile men. There is a positive correlation between body weight and BMD (Evans *et al.*, 2015). Furthermore, the aromatase enzyme that converts testosterone to estradiol is highly expressed in adipose tissue. Although neither total testosterone nor estradiol levels were different between the two groups, the infertile men did have a lower T/E2 ratio, indicating a higher aromatization of testosterone to estradiol. A positive association between high aromatase activity and BMD has been reported as well (Aguirre *et al.*, 2015). However, also after adjusting for BMI, BMD was not different between infertile and fertile men.

Our study has several strengths. It is the first study to compare infertile men to a well-characterized group of fertile men from couples with an ongoing pregnancy. Both groups were evaluated according to the same study protocol. As we did not select men from the general population as control group, but only included fertile men, this should have increased the likelihood of detecting a difference in BMD between the two groups. The narrow 95% confidence intervals indicate that the number of subjects in both groups is sufficiently large to detect a clinically relevant difference between the two groups. Furthermore, inclusion was not limited to men with low sperm count or low sperm concentration, as men with idiopathic infertility based on a decrease in sperm motility and/or morphology were also invited to participate.

There are nevertheless some limitations to consider. The study participants only provided one semen sample each. However, the infertile men had at least two previous semen samples analyzed prior to offering them fertility treatment. Due to the cross-sectional design, we could only assess bone health at the time when the infertile men were referred for andrological evaluation. Therefore, we cannot exclude that infertile men are at higher risk of developing bone loss in the future or that the 'physiological' age-related bone loss will occur more rapidly than in normal

fertile men. Furthermore, we only included men with unexplained infertility, and most of them had testosterone levels in the normal range. It is very likely that infertile men with concomitant testosterone deficiency have a lower bone density. However, in this study, we wanted to evaluate whether men with idiopathic infertility and no other health problems are at risk of having low bone mass and require long-term follow-up, as these men are usually not followed up after the fertility treatment has ended.

Also of note, only a limited number of men—both fertile and infertile—had osteopenia, and only very few men had lumbar osteoporosis. As bone mass reaches its maximum between 25 and 30 years of age, our findings indicate that peak bone mass acquisition is not a point of concern in men with idiopathic infertility.

CONCLUSION

Men with idiopathic infertility did not suffer from poor bone health at the time of infertility workup. However, longitudinal studies are needed to investigate whether men with a history of idiopathic infertility are at higher risk of developing osteopenia or osteoporosis later in life.

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CONFLICT OF INTEREST

None of the authors have anything to disclose.

AUTHORS' CONTRIBUTIONS

Research design and data acquisition: LP, LN, AKB, TKJ, NES, NJ. Data analysis and interpretation, drafting of the manuscript: LA, LP, JHP, DV, NJ. All authors critically reviewed the manuscript and approved the final version.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Scatter plot of age and bone mineral density measurements in infertile men.

Figure S2. Scatter plot of percentage of progressively motile sperm and bone mineral density measurements in infertile men.

Figure S3. Scatter plot of percentage morphologically normal sperm and bone mineral density measurements in infertile men.

Figure S4. Leydig cell function in fertile and infertile men illustrated by total testosterone/LH and free testosterone/LH bivariate plots.

Table S1. Linear regression analysis for sperm parameters and BMD measurements.

Table S2. Comparison of infertile men with lowest semen quality vs. infertile men with better semen quality.

Table S3. Bone mineral density in fertile men with normal total testosterone concentrations compared to infertile men with total testosterone < 10.5 nmol/L.