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Phytoestrogen intake and other dietary risk factors for low motile sperm count and poor sperm morphology

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Abstract

Background: Few potentially modifiable risk factors of male infertility have been identified, and while different diets and food groups have been associated with male infertility, evidence linking dietary factors including phytoestrogens and semen quality is limited and contradictory.

Objectives: To study the associations between phytoestrogen intake and other dietary factors and semen quality.

Materials and Methods: A case-referent study was undertaken of the male partners, of couples attempting conception with unprotected intercourse for 12 months or more without success, recruited from 14 UK assisted reproduction clinics. A total of 1907 participants completed occupational, lifestyle and dietary questionnaires before semen quality (concentration, motility and morphology) were assessed. Food intake was estimated by a 65-item food frequency questionnaire (FFQ) covering the 12 months prior to recruitment. Analyses of dietary risk factors for low motile sperm concentration (MSC: $<4.8 \times 10^6/\text{mL}$) and poor sperm morphology (PM: $<4\%$ normal morphology) used unconditional logistic regression, accounting for clustering of subjects within the clinics, first without, and then with, adjustment for confounders associated with that outcome.

Results: High consumption of daidzein ($\geq 13.74 \mu\text{g/d}$), a phytoestrogen found in soy products, was a protective factor for MSC with an odds ratio (95%CI) of 0.58 (0.42-0.82) after adjustment for clustering and potential confounding. Dietary risk factors for PM after similar adjustment showed that drinking whole milk (OR 0.67, 95%CI 0.47-0.96) and eating red meat were protective with an OR 0.67 (0.46-0.99) for eating red meat >3 times/wk.

Discussion: In this case-referent study of men attending an infertility clinic for fertility diagnosis, we have identified that low MSC is inversely associated with daidzein intake. In contrast, daidzein intake was not associated with PM but eating red milk and drinking whole milk were protective.

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Conclusions: Dietary factors associated with semen quality were identified, suggesting that male fertility might be improved by dietary changes.

KEYWORDS

semen quality, sperm motility, sperm morphology, daidzein, phytoestrogens, diet

1 | INTRODUCTION

Male subfertility is a significant factor in approximately 50% of all couples experiencing a period of infertility.¹ Despite widespread concern about reported but disputed declines in semen quality and more specifically total sperm count,^{2,3} few modifiable factors of semen quality have so far been identified (eg 4-6). The characterization of these factors is of particular interest since they offer a means to improve male fertility and potentially reduce the need for assisted reproductive technologies.

Diet is possibly one such factor, and while the relationship between conventional semen quality parameters (concentration, motility and morphology) and diet patterns and specific dietary components including dietary phytoestrogens has been studied (eg 7-9), the results from these association studies are often inconsistent. Increasing consumption of "Western diets" has been associated with increased sperm concentration,¹⁰ but not in all studies,¹¹⁻¹³ and furthermore has been both positively¹² and negatively¹⁰ associated with sperm morphology. Similarly, increasing intake of diets considered healthy (eg Mediterranean) has been reported to improve sperm concentration or total sperm count in some¹²⁻¹⁴ but not all^{10,15} studies. In contrast, sperm motility has not been associated with diets considered healthy^{10,12,15} or indeed a Western diet,¹⁰⁻¹² though a "prudent" dietary pattern has been positively associated with motility¹¹ but not consistently so.^{13,14} In addition, despite the reported associations between endocrine disrupting chemicals and male fertility,¹⁶ there are few studies that have examined associations between semen quality and oestrogenic phytoestrogens that are found in plant-based foods (especially soy). Soy food intake has been inversely associated with sperm concentration but not sperm motility nor morphology,¹⁷ but a feeding trial of a phytoestrogen supplement did not find any evidence of changes in semen quality despite increases in plasma levels of the isoflavone phytoestrogens, genistein and daidzein.¹⁸

The inconsistency in the reported associations between these different food groups and semen quality may reflect methodological differences in study design (eg subfertile men vs healthy fertile men), study size or study populations (eg Asian vs Western). There are also intrinsic differences in the measurement of food intake and in how food groups were defined from one study to another. As a result, the evidence base linking diet to male subfertility is limited and often contradictory and there are no dietary recommendations currently reported in the UK National Institute for Health and Care Excellence guidelines on fertility problems: assessment and treatment.¹⁹ To improve this evidence base, in this paper we examine associations

between dietary factors (with a particular focus on phytoestrogen intake) and low motile sperm concentration and sperm morphology in a large multi-centre case-referent study (CHAPS-UK).

2 | MATERIALS AND METHODS

2.1 | Design and recruitment

The CHAPS-UK study was a multi-site, case-referent study, and the design and methods have been described previously.²⁰ Cases and referents were the male partners of couples attempting conception with unprotected intercourse for 12 months or more without success and were recruited between 1 January 1999 and 31 January 2002. Men were excluded if they had prior knowledge of their own semen quality, were not able to understand English, had had a medical condition (eg cystic fibrosis) or medical treatment (eg chemotherapy) that could have caused their infertility and either they or their partner had been sterilized previously.²⁰ Prior to their first clinic visit following recruitment, participants completed a short questionnaire on work, lifestyle and health factors and further information on these factors was obtained by interview at the clinic. Men were asked to complete a dietary questionnaire at home with their partner in order to obtain the most complete and accurate information.

2.2 | Semen analysis

Men were requested to abstain from ejaculation for a period of 3-5 days (depending on the clinic) prior to providing a semen sample for a diagnostic analysis. This sample was used not only for infertility investigations but also for this study, and it was analysed according to a protocol based upon the techniques outlined by the World Health Organization²¹ as described previously.^{5,20} Sperm concentration was estimated at an andrology laboratory associated with each centre using a haemocytometer. Motility was captured on videotape using a computer outstation commissioned for the study from Hobson Tracker Systems Limited, Sheffield, UK, and the tape was returned to the central laboratory for analysis of sperm motility by computer-assisted sperm analysis (CASA). The proportion of motile sperm was calculated as the % of sperm moving forward at 5 $\mu\text{m/s}$ or greater. Morphology slides were prepared and fixed at each site but analysed at the central laboratory by the Papanicolaou method and 200 spermatozoa assessed using a Computer Aided Sperm Morphometric Assessment system developed by Hobson Tracker

Systems (Sheffield, UK). The machine was programmed to recognize as 'normal' stained sperm heads which fitted the dimensions given in WHO 1999²¹: a length of 4.0-5.0 mm and a width of 2.5-3.5 mm, with a length-to-width ratio between 1.50 and 1.75.

2.3 | Case definitions

Two case definitions were used, reflecting WHO guidelines.²² The first was based on sperm motility, and cases were men with a motile sperm concentration (MSC) of $<4.8 \times 10^6$ /mL: refers to a MSC of $\geq 4.8 \times 10^6$ /mL. The second definition was based on morphology, and cases with poor morphology (PM) were men whose sperm showed $<4\%$ normal morphology; referents had $\geq 4\%$ normal forms.

2.4 | Dietary exposures

Information on dietary habits was collected using a questionnaire (Appendix S1) that included a 65-item food frequency questionnaire (FFQ) covering the 12 months prior to recruitment to assess phytoestrogen intake that was developed from then available information on phytoestrogen levels in food (eg 23-25). The following exposures were assessed:

- Type of diet (Q3.1; Appendix S1) coded into three categories: meat \pm fish eater, fish eater, vegan or vegetarian.
- Frequency of red meat consumption (Q1.3; Appendix S1) recoded into three categories; ≤ 1 /wk, $>1 \leq 3$ times/wk, and >3 times/wk.
- Frequency of poultry consumption (Q1.2; Appendix S1) recoded into three categories ≤ 1 /wk, $>1 \leq 3$ times/wk, and >3 times/wk.
- Frequency of fish consumption (Q2.2; Appendix S1) recoded into three categories ≤ 1 /wk, $>1 \leq 3$ times/wk, and >3 times/wk.
- Number of portions of vegetables/d; (product of use and frequency summed over 28 questions (excluding potatoes) in the FFQ (Appendix S1) and grouped for those answering at least 60 of the FF questions into the following quartiles: low consumption (0-1.55); below average consumption (1.55-2.53); above average consumption (2.53-3.67); and high consumption (≥ 3.67).
- Number of portions of fruit/d; (product of use and frequency summed over 7 questions in the FFQ (Appendix S1) and grouped for those answering at least 60 of the FFQ into the following quartiles: low consumption (0-0.30); below average consumption (0.30-0.86); above average consumption (0.86-1.65); and high consumption (≥ 1.65).
- Type of milk used (Q4.1 Appendix S1) recoded into full fat, semi-skim milk, skim milk, other (including soya milk), and none.
- Quantity of butter/margarine spread on bread (Q5.4 Appendix S1) categorized as thick spread, medium spread, scrape, and none at all.

In addition, soy intake was estimated from 10 soy-based food questions (FFQ: Appendix S1). Soy intake (number of portions per

day) was categorized into none, below median intake (<0.0506) and above median intake (>0.0506). The amount of daidzein and genistein consumed was estimated as described in Appendix S2 for those answering at least 60 FFQ questions (Appendix S1). For daidzein, the amount consumed was grouped into the following quartiles: low intake (0-1.47 $\mu\text{g/d}$); below average intake (1.47-3.72 $\mu\text{g/d}$); above average intake (3.72-13.74 $\mu\text{g/d}$); and high intake (≥ 13.74 $\mu\text{g/d}$). For genistein, the following quartiles were used: low intake (0-5.30 $\mu\text{g/d}$); below average intake (5.30-9.62 $\mu\text{g/d}$); above average intake (9.62-21.93 $\mu\text{g/d}$); and high intake (≥ 21.93 $\mu\text{g/d}$).

2.5 | Assessment of confounders

Confounders found to be significantly related to outcome in earlier analysis of these data^{4,5,20} were re-examined here for those completing the dietary questionnaire (see Appendices S3 and S4) and all relating to outcome retained. Age was not related to motility or morphology. The factors retained for motility were ethnic group, testes surgery, manual work, wearing boxer shorts and abstinence (Appendix S3), and for morphology were BMI, cannabis use, season and abstinence (Appendix S4).

2.6 | Statistical methods

Analyses of the two outcomes (low motile sperm count and poor morphology) used unconditional logistic regression, accounting for clustering of subjects within the 14 fertility clinics, first without, and then with, adjustment for confounders associated with that outcome. Where more than one dietary factor was associated with the outcome ($P < .10$), the contribution of each factor was assessed using a Wald statistic. All analyses were carried out using the generalized linear latent and mixed models (gllamm) command within STATA 14.2 (Stata Corporation; Statcorp).

2.7 | Ethics

Ethical approval was given by the Multi-Centre Ethics Committee for the North West (ref. no. MREC 98/8/73) with subsequent approval given by the Local Research Ethics Committee at each site.

3 | RESULTS

3.1 | Study population

A total of 2249 men were recruited and provided a semen sample and 1907 of these (84.8%) returned the dietary questionnaire. Sperm motility was determined for all 1907 men, and 440 were cases (23.1%) with a low MSC ($<4.8 \times 10^6$ /mL). Sperm morphology was determined

TABLE 1 Dietary factors for low motile sperm count

	Case	Referent		
	N (%)	N (%)	OR (95% CI) ^a	OR _{adj} (95%CI _{adj}) ^b
Type of diet				
Meat (\pm fish)	426 (97.0)	1415 (96.5)	1	1
Fish only	7 (1.6)	27 (1.8)	0.88 (0.38-2.05)	0.87 (0.36-2.06)
Vegetarian or vegan	6 (1.4)	24 (1.6)	0.84 (0.34-2.07)	0.99 (0.39-2.50)
Frequency of red meat consumption				
≤ 1 /wk	98 (22.7)	326 (22.7)	1	1
$>1 \leq 3$ times/wk	185 (42.9)	703 (49.0)	0.87 (0.66-1.16)	0.84 (0.63-1.12)
>3 times/wk	148 (34.3)	407 (28.3)	1.20 (0.89-1.62)	1.13 (0.83-1.53)
Frequency of poultry consumption				
≤ 1 /wk	93 (21.6)	306 (21.3)	1	1
$>1 \leq 3$ times/wk	232 (53.8)	810 (56.3)	0.93 (0.71-1.23)	0.92 (0.70-1.23)
>3 times/wk	106 (24.6)	320 (22.3)	1.08 (0.78-1.49)	1.06 (0.76-1.47)
Frequency of fish consumption				
≤ 1 /wk	301 (70.0)	1031 (71.6)	1	1
$>1 \leq 3$ times/wk	110 (25.6)	341 (23.7)	1.12 (0.87-1.45)	1.09 (0.84-1.41)
>3 times/wk	19 (4.4)	68 (4.7)	0.97 (0.57-1.64)	0.96 (0.55-1.65)
Portions of vegetables/d				
Low (0-1.55)	102 (26.0)	327 (24.3)	1	1
Below average (1.55-2.53)	118 (30.1)	321 (23.9)	1.18 (0.87-1.60)	1.24 (0.90-1.70)
Above average (2.53-3.67)	91 (23.2)	343 (25.5)	0.86 (0.62-1.19)	0.86 (0.61-1.20)
High (≥ 3.67)	81 (20.7)	354 (26.3)	0.75 (0.54-1.04)	0.78 (0.55-1.09)
Portions of fruit/d				
Low (0-0.30)	101 (25.8)	328 (24.4)	1	1
Below average (0.30-0.86)	95 (24.2)	329 (25.2)	0.90 (0.65-1.24)	0.90 (0.65-1.25)
Above average (0.86-1.65)	95 (24.2)	345 (25.7)	0.89 (0.65-1.23)	0.96 (0.69-1.33)
High (≥ 1.65)	101 (25.3)	333 (24.8)	0.97 (0.71-1.34)	0.99 (0.72-1.38)
Type of milk used				
Full fat	108 (24.7)	288 (19.7)	1	1
Semi-skim milk	256 (58.4)	945 (64.7)	0.72 (0.55-0.93)	0.77 (0.58-1.01)
Skim milk	53 (12.1)	179 (12.3)	0.81 (0.55-1.18)	0.89 (0.60-1.33)
Other	4 (0.9)	19 (1.3)	0.59 (0.19-1.78)	0.79 (0.26-2.44)
None	17 (3.9)	29 (2.0)	1.58 (0.83-3.00)	1.71 (0.88-3.31)
Quantity of butter/margarine used on bread				
Thick spread	25 (5.7)	91 (6.3)	1	1
Medium spread	236 (54.3)	836 (57.5)	1.01 (0.63-1.61)	1.02 (0.63-1.65)
Scrape	130 (29.9)	452 (31.1)	1.02 (0.63-1.67)	1.02 (0.62-1.68)
None	44 (10.1)	74 (5.1)	2.16 (1.21-3.87)	2.32 (1.28-4.23)

^aAdjusted for clustering within centre.^bAdjusted for clustering and ethnicity, surgery, work, boxer shorts and abstinence

in 1673 men who had returned a dietary questionnaire, and of these, 274 men were cases (16.4%) who had poor sperm morphology ($<4\%$ normal forms). There was no relation between case status and likelihood of returning the questionnaire but those that did so were more likely to be older and of white ethnicity (Appendix S5).

3.2 | Risk factors for low MSC

In the fully adjusted model (adjusting for clustering and ethnicity, surgery, work, boxer shorts and abstinence: Appendix S3), low MSC was not positively associated with any dietary factor other than a

report of no spread used on bread. Before adjustment for clustering and confounding, there was a trend ($P = .017$) towards lower risk with the consumption of more vegetables but this was no longer significant after adjustment (Table 1). After adjusting for confounders and clustering within centres, low MSC was inversely associated with above median intake of soy (OR = 0.75, 95%CI 0.56-1.00), high daidzein intake (OR 0.58, 95%CI (0.42-0.82) and high genistein intake (OR 0.60 95%CI 0.43-0.83; Table 2). In a multivariable regression, multilevel consumption of soy ($P = .85$) and genistein ($P = .73$) did not add significantly to a model including daidzein. In a combined model of daidzein and spread, the OR (95%CI) for high daidzein intake (>13.74) was 0.57 (0.41-0.81) and that of no spread 2.39 (1.56-3.67).

3.3 | Risk factors for PM

After adjustment for clustering, body mass index, cannabis use, abstinence and season (see Appendix S4), poor sperm morphology was positively associated with use of semi-skimmed milk and inversely associated with consumption of red meat more than three times per week (Table 3). When all other milk types and no milk (with similar odds ratios in Table 3) were grouped together and contrasted with whole milk consumption, whole full fat milk was found to be protective (OR = 0.67 95%CI 0.47-0.96; Table 4) and associated with good morphology. When red meat and whole full fat milk were entered into the same model, both retained their protective effect but this was reduced slightly for each factor individually as the two were correlated: those who ate red meat 3 or more times a week were also more likely to drink whole milk. The interaction between them was not significant in a fully adjusted model (last column, Table 4).

Consumption of soy, genistein and daidzein was not associated with poor morphology (Table 5).

4 | DISCUSSION

Dietary risk factors for male subfertility are potentially modifiable and hence of special interest. In this case-referent study of a male population attending an infertility clinic for fertility diagnosis, we have identified that being a case with a low MSC is inversely associated with daidzein intake. In contrast, daidzein intake was not associated with poor morphology but eating red milk and drinking whole milk were protective. These results suggest that dietary risk factors for low MSC and PM differ and that changes to a diet at an individual level, depending upon their type of subfertility, might improve semen quality.

The results of this study are indicative of a protective effect of daidzein (or genistein) on motile sperm count (but not morphology). This effect could potentially be attributed to their interactions with oestrogen receptors²⁶ but the potential mode of action is difficult to identify as isoflavones have a wider range of cellular effects that can be considered either protective, for example, antioxidant activity²⁷ or increased androgen levels²⁸ or detrimental, for example, increasing capacitation²⁹ and altering neonatal testicular development.³⁰ Our results initially appear to contradict previous smaller studies that have reported phytoestrogen intake reduces semen quality (eg 17). However, certain Asian populations consume high levels of soy products with little apparent effect on male fertility³¹ and soy food intake has not been associated with IVF treatment outcomes.³² Consistent with our results, daily consumption of a soy product reportedly increased semen quality in a man with oligozoospermia³³ but a larger trial, over 6 months, of 14

TABLE 2 Soy and phytoestrogen intake with low motile sperm count

	Case	Referent		
	N (%)	N (%)	OR (95% CI) ^a	OR _{adj} (95%CI _{adj}) ^b
Soy (Intake/d)				
None	201 (51.3)	617 (45.9)	1	1
Below median (<0.0506)	99 (25.3)	354 (26.3)	0.87 (0.66-1.15)	0.92 (0.69-1.22)
Above median (\geq 0.0506)	92 (23.5)	374 (27.8)	0.76 (0.58-1.01)	0.75 (0.56-1.00)
Estimated daidzein Intake (μ g/d)				
Low (0-1.47)	119 (30.4)	315 (23.4)	1	1
Below average (1.47-3.72)	98 (25.0)	336 (25.0)	0.79 (0.58-1.07)	0.80 (0.58-1.11)
Above average (3.72-13.74)	97 (24.7)	338 (25.1)	0.78 (0.57-1.06)	0.81 (0.59-1.12)
High (\geq 13.74)	78 (19.9)	356 (26.5)	0.59 (0.43-0.82)	0.58 (0.42-0.82)
Estimated genistein Intake (μ g/d)				
Low (0-5.30)	120 (30.6)	314 (23.3)	1	1
Below average (5.30-9.62)	98 (25.0)	336 (25.0)	0.76 (0.56-1.04)	0.82 (0.60-1.13)
Above average (9.62-21.93)	93 (23.7)	342 (25.4)	0.71 (0.52-0.97)	0.75 (0.50-1.03)
High (\geq 21.93)	81 (20.7)	353 (26.2)	0.60 (0.44-0.83)	0.60 (0.43-0.83)

^aAdjusted for clustering within centre.

^bAdjusted for clustering and ethnicity, surgery, work, boxer shorts and abstinence.

TABLE 3 Dietary factors for poor morphology

Dietary factor	Case	Referent	OR (95% CI) ^a	OR _{adj} (95% CI _{adj}) ^b
	N (%)	N (%)		
Type of diet				
Meat (± fish)	263 (6.0)	1350 (96.6)	1	1
Fish only	6 (2.2)	25 (1.8)	1.25 (0.50-3.10)	0.96 (0.38-2.43)
Vegetarian or vegan	5 (1.8)	23 (1.6)	1.07 (0.40-2.86)	1.09 (0.40-2.96)
Frequency of red meat consumption				
≤1/wk	70 (25.8)	300 (21.9)	1	1
>1 ≤ 3 times/wk	133 (49.1)	652 (47.5)	0.89 (0.64-1.23)	0.87 (0.63-1.22)
>3 times/wk	68 (25.1)	420 (30.6)	0.71 (0.49-1.03)	0.67 (0.46-0.99)
Frequency of poultry consumption				
≤1/wk	73 (26.9)	282 (20.6)	1	1
>1 ≤ 3 times/wk	142 (52.4)	771 (56.2)	0.71 (0.52-0.97)	0.75 (0.54-1.03)
>3 times/wk	56 (20.7)	319 (23.3)	0.68 (0.46-0.99)	0.73 (0.49-1.08)
Frequency of fish consumption				
≤1/wk	184 (68.4)	980 (71.4)	1	1
>1 ≤ 3 times/wk	74 (27.5)	328 (23.9)	1.19 (0.89-1.61)	1.14 (0.84-1.54)
>3 times/wk	11 (4.1)	65 (4.7)	0.90 (0.46-1.74)	0.85 (0.43-1.67)
Portions of vegetables/d				
Low (0-1.55)	66 (24.9)	305 (24.0)	1	1
Below average (1.55-2.53)	70 (27.2)	317 (25.0)	1.05 (0.72-1.52)	1.04 (0.71-1.52)
Above average (2.53-3.67)	58 (22.6)	331 (26.1)	0.82 (0.55-1.21)	0.77 (0.52-1.15)
High (≥3.67)	65 (25.3)	317 (25.0)	0.96 (0.65-1.41)	0.90 (0.61-1.33)
Portions of fruit/d				
Low (0-0.30)	68 (26.5)	312 (24.6)	1	1
Below average (0.30-0.86)	64 (24.9)	317 (25.0)	0.93 (0.64-1.35)	0.91 (0.62-1.33)
Above average (0.86-1.65)	55 (21.4)	331 (26.1)	0.76 (0.52-1.12)	0.76 (0.51-1.13)
High (≥1.65)	70 (27.2)	310 (24.4)	1.03 (0.71-1.49)	1.02 (0.70-1.49)
Type of milk used				
Full fat	45 (16.4)	290 (20.8)	1	1
Semi-skim milk	181 (66.1)	889 (63.9)	1.34 (0.94-1.90)	1.45 (1.01-2.09)
Skim milk	37 (13.5)	165 (11.9)	1.48 (0.91-2.39)	1.61 (0.98-2.63)
Other	3 (1.1)	15 (1.1)	1.28 (0.35-4.65)	1.40 (0.38-5.24)
None	8 (2.9)	32 (2.3)	1.65 (0.71-3.82)	1.85 (0.78-4.38)
Quantity of margarine/butter used on bread				
Thick spread	13 (4.8)	84 (6.1)	1	1
Medium spread	153 (58.0)	795 (57.4)	1.20 (0.65-2.22)	1.18 (0.64-2.20)
Scrape	91 (33.3)	418 (30.2)	1.36 (0.72-2.55)	1.37 (0.72-2.60)
None	16 (5.9)	88 (6.4)	1.10 (0.49-2.44)	1.03 (0.46-2.32)

^aAdjusted for clustering with centres.

^bAdjusted for clustering and body mass index, cannabis use, abstinence and season

men with normal semen quality reported no alterations in semen quality although plasma daidzein and genistein levels increased.¹⁸

Dietary recommendations for red and processed meat consumption are controversial.³⁴ In this study, a protective effect of red meat consumption on sperm morphology was observed, a result consistent with reports that diets characterized by high meat intake are indeed associated with an improvement in certain aspects of semen quality,

for example sperm concentration¹⁵ and possibly sperm morphology.³⁵ However, previous hypotheses have suggested that meat consumption would increase risk of adverse semen quality due to increased consumption of, for example, saturated fat, xenobiotics including xenoestrogens and potentially hormone residues.³⁶ However, the evidence for such effects is limited with few studies reporting any negative associations between red meat consumption and semen quality.^{11,15,35,37-40}

TABLE 4 Relation of red meat, full fat milk and their interaction to poor morphology

	OR (95% CI)		
	Unadjusted	Model 1 ^a	Model 2 ^a
Red meat			
<3 times/wk	1	1	1
≥3 times/wk	0.74 (0.54-1.00)	0.71 (0.49-1.01)	0.69 (0.49-0.95)
Type of milk use			
All other	1	1	1
Full fat	0.67 (0.45-0.95)	0.75 (0.55-1.02)	0.62 (0.40-0.96)
Interaction between red meat and full fat milk			
None	–	–	1
Both	–	–	1.50 (0.71-3.19)

^aAdjusted for confounders and clusters within centre**TABLE 5** Soy and phytoestrogen intake with poor morphology

	Case	Referent	OR (95% CI) ^a	OR _{adj} (95%CI _{adj}) ^b
	N (%)	N (%)		
Soy (Intake/d)				
None	121 (47.1)	596 (46.9)	1	1
Below medium (<0.0506)	68 (26.5)	336 (26.5)	1.01 (0.73-1.40)	0.98 (0.70-1.37)
Above medium (≥0.0506)	68 (26.5)	338 (26.6)	1.00 (0.72-1.39)	0.91 (0.65-1.28)
Estimated daidzein Intake (µg/d)				
Low (0-1.47)	70 (27.2)	300 (23.6)	1	1
Below average (1.47-3.72)	60 (23.3)	327 (25.7)	0.77 (0.53-1.13)	0.77 (0.53-1.14)
Above average (3.72-13.74)	57 (22.2)	337 (26.5)	0.72 (0.49-1.06)	0.73 (0.49-1.08)
High (≥13.74)	70 (27.2)	306 (24.1)	0.98 (0.67-1.41)	0.90 (0.62-1.32)
Estimated genistein Intake (µg/d)				
Low (0-5.30)	65 (25.3)	308 (24.3)	1	1
Below average (5.30-9.62)	64 (24.9)	318 (25.0)	0.95 (0.65-1.40)	0.94 (0.64-1.38)
Above average (9.62-21.93)	59 (23.0)	338 (26.6)	0.83 (0.56-1.21)	0.81 (0.54-1.19)
High (≥21.93)	69 (26.9)	306 (24.1)	1.07 (0.73-1.56)	0.97 (0.66-1.43)

^aAdjusted for clustering with centres.^bAdjusted for clustering and body mass index, cannabis use, abstinence and season.

In this study, poor sperm morphology was associated with the lack of milk consumption and also the intake of skimmed or semi-skimmed milk, suggesting that whole milk is protective of semen quality. Previously, a high intake of omega-3 polyunsaturated fats has been reported to be positively associated with sperm morphology⁴¹ and fish oil supplements with semen volume and total sperm count.⁴² Such results are consistent with the presence, in whole milk, of numerous fat-soluble vitamins and other components that might affect semen quality, for example, by providing protection against oxidative stress.⁴³ These would be lost upon processing to semi skimmed and skimmed milk.^{44,45} Other studies, of smaller populations, have reported positive associations between consumption of low-fat milk and semen quality.^{38,39}

There are a number of strengths associated with this study. Firstly, the number of men providing dietary information via a FFQ is much larger than many other previously published studies resulting

in a study of sufficient power (80%) to detect an OR of 2 assuming 1 in 40 controls would be exposed, and a 2:1 ratio of referents to cases. The second strength of the study is that those men who knew the results of the assessment of their semen quality were excluded from the study. Such prior knowledge of their results could have biased their answers to specific questions or even modified behaviours prior to recruitment. Furthermore, semen analysis was carried out according to WHO protocols, with CASA being undertaken at each recruitment site but analysed centrally to ensure consistency in semen analysis. Case definitions were also as used previously^{5,20} and defined a priori according to WHO definitions whereas other studies have used a wide variety of different measures of semen quality of unknown clinical relevance.

However, there are certain limitations to this work. Firstly, the study population was men attending infertility clinics as part of a couple and so potentially they might not be representative of all

men. In addition, not all eligible men were recruited to the study and the reasons why they did not want to participate are not known. It is possible that they did want to be asked about lifestyle factors (including diet). In addition to the recognized limitations of using FFQs to assess food consumption, which in this study was over a 12-month period because of the sustained period of infertility required to be eligible for the study, there are potential issues regarding the questionnaire coverage of phytoestrogen containing foods and also the accuracy of the levels of phytoestrogens in the covered dietary items.⁴⁶ The food items and phytoestrogen values used here are not those of more recent studies (eg 47-49). These issues are likely to be independent of case status and so result in non-differential bias, which is normally associated with a reduced estimate of effect size ('bias towards the null'). Assessment of phytoestrogen intake may be improved by urinary measurement of specific phytoestrogen metabolites; however, inconsistent results linking urinary daidzein and semen quality have also been reported.^{50,51} Participants were also not asked about the intake of dietary supplements, and while there is little evidence that soy-based [18] or protein-based⁵² supplements may alter male semen quality, other dietary supplements may improve male semen quality⁵³ and potentially interact with soy-based products to alter semen quality. Semen quality measures do not fully correlate with male fertility, and these can explain why dietary food groups associated with semen quality are not necessarily associated with time to pregnancy or other measures of reproductive success. Furthermore, urinary daidzein levels in men were not associated with time to pregnancy.⁵⁴ It has also been suggested that pre-pubertal exposures may be important but we were not able to study them.

In conclusion, this large multi-centre case-control study identified different risk factors for low motile sperm count and poor sperm morphology. Further work is required to confirm these associations but suggests that semen quality may be improved by targeted interventions.

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CONFLICT OF INTEREST

Professor JE Cade is a Director of the University of Leeds company, Dietary Assessment Limited.

AUTHOR CONTRIBUTIONS

All members of the co-ordinating group contributed to the collection of data for the study and discussions on the design, conduct and interpretation of the results. Data management was done by J-AC, HB, NC and HM. ACP, NC, JC and AAP drafted the manuscript, and NC and ACP discussed and performed the statistical analysis. JC oversaw the design and analysis of the dietary questionnaire. All authors critically revised the paper and approved the submitted version. J-AC and HB co-ordinated the study and took care of communication and distribution of study materials to group members.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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