Men who recruited to the study were asked a questionnaire to determine any lifestyle factors that could influence the quality of their semen. Table T1 – T6 contain details the responses from the men used the ‘proton’ MRS and ‘carbon-13’ MRS component of the study. Table T7 shows the ethnicity of men taking part in the study. Tables T8 – 9 show the semen parameters measured by the Andrology clinic based at the Jessop Wing, Sheffield.

Statistical test were either: unpaired t-test – age, height, weight, BMI; all others – Fisher’s exact test. For unpaired t-test either a Students t-test (stt) or Wilcoxon ranksum (wil) was applied depending on the outcome of a D'Agostino-Pearson normality test. p-values: \* < 0.05, n.s. not significant.

Table TI: Patient data for men diagnosed with normozoospermia or asthenozoospermia used in the proton component of the study. Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis).

|  |  |  |
| --- | --- | --- |
| Parameter | Normozoospermia  (n =45) | Asthenozoospermia  (n =15) |
| Age (years) | 34 (24–51) | 31 (25–55) |
| Height, m | 1.80 (1.63 – 2.00) | 1.80 (1.70 – 1.92) |
| Weight, Kg | 88 ( 66.7 – 117.5) | 79.4 (63.5 – 98.4) |
| BMI (Kg/m2) | 26.9 (20.1 – 36.3) | 25.4 (20.0 – 30.8) |
| Conceived previously | 36% (16) | 20% (3) |
| Blood diseases | 0% | 0% |
| Cancer treatment | 0% | 0% |
| STI | 2% (1) | 0% |
| Alcohol: units,  number of drinkers | 4 (0 – 33),  40 | 5 (0 – 21),  11 |
| Smokes: cigarettes/week, number of smokers | 0 (0 –20),  10 | 0 (0 – 13),  6 |
| Use bicycle | 22% (10) | 13% (2) |
| Tight underwear | 40% (18) | 60% (9) |
| Use laptop | 36% (16) | 33% (5) |
| Use hot tub | 7% (3) | 13% (2) |
| Hot environment | 22% (10) | 13% (2) |
| Has fever | 2% (1) | 0% |
| Warm groin | 29% (13) | 53% (8) |
| Use glues | 11% (5) | 13% (2) |
| Exposed to lead | 4% (2) | 7% (1) |
| Night shifts | 13% (6) | 27% (4) |

Table TII: Patient data for men diagnosed with normozoospermia (n = 126) or asthenozoospermia (n = 36) used in the carbon-13 component of the study. Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis).

|  |  |  |
| --- | --- | --- |
| Parameter | Normozoospermia | Asthenozoospermia |
| Age (years) | 33 (21 – 59) | 33.5 (23 – 62) |
| Height, m | 1.80 (1.67 – 1.96) | 1.80 (1.57 – 2.00) |
| Weight, Kg | 83 (60.3 – 147.9) | 81 (57.2 – 97.1) |
| BMI (Kg/m2) | 25.8 (17.6 – 41.8) \* | 25.0 (17.7 – 30.9)\* |
| Conceived previously | 25% (32) | 36% (13) |
| Blood diseases | 2% (3) | 0% |
| Cancer treatment | 0% | 0% |
| STI | 1%(1) | 0% |
| Alcohol: units,  number of drinkers | 5 (0 –40)  103 | 5 (0 –24)  27 |
| Smokes: cigarettes/week, number of smokers | 0 (0 –25)  15 | 0 (0 –20)  7 |
| Use bicycle | 20% (25) | 17% (6) |
| Tight underwear | 40% (50) | 39% (14) |
| Use laptop | 35% (44) | 50% (18) |
| Use hot tub | 11% (14) | 11% (4) |
| Hot environment | 11% (14) | 11% (4) |
| Has fever | 1% (1) | 3% (1) |
| Warm groin | 35% (44) | 31% (11) |
| Use glues | 16% (20) | 19% (7) |
| Exposed to lead | 2% (2) | 8% (3) |
| Night shifts | 17% (21) | 22% (8) |

\* p < 0.05, Wilcoxon

Table TIII: Patient data for men diagnosed with normozoospermia (n = 31) or asthenozoospermia (n = 12) used in the **13C-glucose** component of the study. Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis).

|  |  |  |
| --- | --- | --- |
| Parameter | Normozoospermia | Asthenozoospermia |
| Age (years) | 33 (22 – 49) | 35.5 (24 – 40) |
| Height, m | 1.80 (1.67 – 1.96) | 1.81 (1.73 – 1.88) |
| Weight, Kg | 78 (62 – 121) | 81 (70 – 92) |
| BMI (Kg/m2) | 25.1 (19.5 – 41.8) | 25.9 (19.8 – 28.1) |
| Conceived previously | 19% (6) | 33% (4) |
| Blood diseases | 3% (1) | 0% |
| Cancer treatment | 0% | 0% |
| STI | 0% | 0% |
| Alcohol: units,  number of drinkers | 4 (0 – 20)  24 | 5 (0 – 20)  10 |
| Smokes: cigarettes/week, number of smokers | 0 (0 – 6)  3 | 0 (0 – 15)  3 |
| Use bicycle | 16% (5) | 17% (2) |
| Tight underwear | 36% (11) | 67% (8) |
| Use laptop | 32% (10) | 50% (6) |
| Use hot tub | 16% (5) | 17% (2) |
| Hot environment | 19% (6) | 0% |
| Has fever | 0% | 0% |
| Warm groin | 26% (8) | 33% (4) |
| Use glues | 13% (4) | 17% (2) |
| Exposed to lead | 3% (1) | 8% (1) |
| Night shifts | 19% (6) | 8% (1) |

Table TIV: Patient data for men diagnosed with normozoospermia (n = 32) or asthenozoospermia (n = 12) used in the **13C-fructose** component of the study. Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis).

|  |  |  |
| --- | --- | --- |
| Parameter | Normozoospermia | Asthenozoospermia |
| Age (years) | 34 (26 – 59) | 33.5 (23 – 43) |
| Height, m | 1.80 (1.68 – 1.93) | 1.80 (1.68 – 2.00) |
| Weight, Kg | 83.5 (70 – 121) | 85 (57 – 97) |
| BMI (Kg/m2) | 26.6 (21.9 – 33.1) | 26.4 (17.7 – 29.8) |
| Conceived previously | 25% (8) | 25% (3) |
| Blood diseases | 6% (2) | 0% |
| Cancer treatment | 0% | 0% |
| STI | 0% | 0% |
| Alcohol: units,  number of drinkers | 5.5 (0 – 20)  28 | 4 (0 – 12)  9 |
| Smokes: cigarettes/week, number of smokers | 0% (0 – 15)  2 | 0% (0 – 13)  2 |
| Use bicycle | 36% (12) | 8% (1) |
| Tight underwear | 41% (13) | 25% (3) |
| Use laptop | 25% (8) | 58% (7) |
| Use hot tub | 12.5% (4) | 17% (2) |
| Hot environment | 19% (6) | 25% (3) |
| Has fever | 0% | 0% |
| Warm groin | 44% (14) | 33% (4) |
| Use glues | 16% (5) | 33% (4) |
| Exposed to lead | 3% (1) | 17% (2) |
| Night shifts | 19% (6) | 42% (5) |

Table TV: Patient data for men diagnosed with normozoospermia (n = 32) or asthenozoospermia (n = 12) used in the **13C1-pyruvate** component of the study. Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis).

|  |  |  |
| --- | --- | --- |
| Parameter | Normozoospermia | Asthenozoospermia |
| Age (years) | 34.5 (22 – 42) | 30.5 (23 – 62) |
| Height, m | 1.80 (1.68 – 1.91) | 1.83 (1.57 – 1.88) |
| Weight, Kg | 85 (60 – 127) | 76 (64 – 86)\*, STT |
| BMI (Kg/m2) | 27.1 (17.6 – 40.1) | 23.4 (18.0 – 30.9)\*, STT |
| Conceived previously | 25% (8) | 50% (6) |
| Blood diseases | 0% | 0% |
| Cancer treatment | 0% | 0% |
| STI | 3% (1)1 | 0% |
| Alcohol: units,  number of drinkers | 8 (0 – 40)  26 | 5.5 (0 – 24)  8 |
| Smokes: cigarettes/week, number of smokers | 0 (0 – 20)  6 | 0 (0 – 20)  2 |
| Use bicycle | 13% (4) | 25% (3) |
| Tight underwear | 45% (14) | 25% (3) |
| Use laptop | 39% (12) | 42% (5) |
| Use hot tub | 13% (4) | 0% |
| Hot environment | 6.5% (2) | 8% (1) |
| Has fever | 3% (1) | 8% (1) |
| Warm groin | 39% (12) | 25% (3) |
| Use glues | 13% (4) | 8% (1) |
| Exposed to lead | 0% | 0% |
| Night shifts | 26% (8) | 17% (2) |

\* p < 0.05, Student’s t-test

Table TVI: Patient data for men with normozoospermic ejaculates incubated with a pair of 13C-substrates either: **13Cu-glucose/13C1-pyrvuate** (n = 16), or **13Cu-fructose/13C1-pyrvuate** (n = 16). Values quoted either as median (range) or percentage number of cases (absolute number in parenthesis). No asthenozoospermic ejaculate were present in this component of the study.

|  |  |  |
| --- | --- | --- |
| Parameter | 13Cu-glucose/13C1-pyrvuate | 13Cu-fructose/13C1-pyrvuate |
| Age (years) | 32.5 (27 –46) | 34.5 (27 – 51) |
| Height, m | 1.78 (1.70 – 1.96) | 1.80 (1.70 – 1.96) |
| Weight, Kg | 73 (65 – 148) | 89 (70 – 140) |
| BMI (Kg/m2) | 24.2 (20.6 – 38.5) | 29.0 (21.6 – 36.4) |
| Conceived previously | 38% (6) | 25% (4) |
| Blood diseases | 0% | 0% |
| Cancer treatment | 0% | 0% |
| STI | 0% | 0% |
| Alcohol: units,  number of drinkers | 4.5 (0 – 30)  12 | 6.5 (0 – 28)  13 |
| Smokes: cigarettes/week, number of smokers | 0 (0 – 1)  1 | 0 (0 – 25)  3 |
| Use bicycle | 6% (1) | 13% (2) |
| Tight underwear | 31% (5) | 44% (7) |
| Use laptop | 44% (7) | 44% (7) |
| Use hot tub | 6% (1) | 0% |
| Hot environment | 0% | 0% |
| Has fever | 0% | 0% |
| Warm groin | 31% (5) | 31% (5) |
| Use glues | 31% (5) | 13% (2) |
| Exposed to lead | 0% | 0% |
| Night shifts | 6% (1) | 0% |

Table TVII: Ethnicity breakdown self-reported by men in the 1H and 13C MRS cohorts. Normo – Men producing normozoospermic ejaculates; Astheno – Men producing asthenozoospermic ejaculates. E & W Avg: ‘Ethnic diversity and national identity in England & Wales’ obtained from census data for 2011 ([www.ons.gov.uk/peoplepopulationandcommunity/culturalidentity/ethnicity/articles/ethnicityandnationalidentityinenglandandwales/2012-12-11](https://www.ons.gov.uk/peoplepopulationandcommunity/culturalidentity/ethnicity/articles/ethnicityandnationalidentityinenglandandwales/2012-12-11)).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort | N | Asian, Asian/British | Black,Black/British | Chinese, Chinese/British | Mixed | Other | white | p-value† |
| 1H MRS  Normo | 45 | 4.0% (n=2) | 2.0% (n=1) | 0 | 0 | 2.0% (n=1) | 91.0% (n=41) | 0.26 |
| 1H MRS  Astheno | 15 | 20.0% (n=3) | 0 | 0 | 7.0% (n=1) | 7.0% (n=1) | 67.0% (n=10) | <0.0001 |
| 13C MRS  Normo | 126 | 6.0% (n=7) | 1.0% (n=1) | 1.0% (n=1) | 2.0% (n=2) | 3.0% (n=4) | 88.0% (n=111) | 0.73 |
| 13C MRS  Astheno | 36 | 14.0% (n=5) | 8.0% (n=3) | 0 | 0 | 0 | 78.0% (n=28) | <0.0001 |
| E & W Avg | – | 6.8% | 3.3% | 0.7% | 2.20% | 1.0% | 86.0% | – |

† Chi-Square Goodness of Fit Test

Table TVIII: Semen baseline parameters measured by the Andrology unit at the Jessop Wing separated by 13C-substrate and motility category. a, abstinence – where >2 days was reported this was set to 2 days; b, analysis delay represents the time elapsed between semen ejaculation and commencing Andrology analysis; c, viscosity was graded as either normal or high; d, agglutination presented as number of cases where some degree of agglutination (was noted: isolated, moderate, widespread).Number are presented as either mean and standard error of mean or percentage number of cases (absolute number in parenthesis).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | | Normozoospermia | Asthenozoospermia | p-value |
| 13Cu-glucose MRS cohort, n = 31(N), n =12(A) | | | | |
| abstinencea, days | | 4.6 ± 0.3 | 6.5 ± 1.4 | 0.25 |
| analysis delayb, min | | 23.4 ± 1.5 | 29.5 ± 3.4 | 0.06 |
| volume, ml | | 4.3 ± 1.6 | 4.4 ± 1.7 | 0.78 |
| pH (semen) | | 8.06 ± 0.04 | 8.21 ± 0.10 | 0.21 |
| concentration, 106/ml | | 82.0 ± 10.1 | 53.2 ± 13.7 | 0.09 |
| Morphology, % | | 5.3 ± 0.9 | 7.5 ± 2.2 | 0.42 |
| Motility, %  (range) | TM | 62.2 ± 1.5 | 28.1 ± 1.6 | < 0.0001, stt |
| PR | 59.7 ± 1.6 | 23.2 ± 2.0 | < 0.0001, stt |
| Viscosityc | | 87% (27) | 75% (9) | 0.38 |
| Agglutinationd | | 6% (2) | 0% | - |
| 13Cu-fructose MRS cohort, n = 32(N), n = 12(A) | | | | |
| abstinencea, days | | 4.8 ± 0.3 | 7.3 ± 2.2 | 0.49 |
| analysis delayb, min | | 25.2 ± 1.8 | 24.3 ± 2.5 | 0.80 |
| volume, ml | | 4.4 ± 0.3 | 4.3 ± 0.6 | 0.94 |
| pH (semen) | | 8.06 ± 0.03 | 8.13 ± 0.07 | 0.41 |
| concentration, 106/ml | | 75.6 ± 10.0 | 34.2 ± 8.3 | 0.02, wil |
| Morphology, % | | 7.4 ± 1.1 | 3.4 ± 1.2 | 0.03, wil |
| Motility, %  (range) | TM | 62.2 ± 1.8 | 30.2 ± 2.0 | < 0.0001 wil |
| PR | 59.2 ± 1.8 | 25.6 ± 2.0 | < 0.0001, wil |
| Viscosityc | | 72% (23) | 50% (6) | 0.28 |
| Agglutinationd | | 3% (1) | 0% | - |
| 13C1-pyruvate MRS cohort, n = 31(N), n = 12(A) | | | | |
| abstinencea, days | | 5.0 ± 0.3 | 5.9 ± 1.2 | 0.29 |
| analysis delayb, min | | 24.8 ± 1.6 | 26.8 ± 3.2 | 0.54 |
| volume, ml | | 4.1 ± 0.3 | 4.2 ± 0.3 | 0.89 |
| pH (semen) | | 8.15 ± 0.04 | 8.13 ± 0.07 | 0.81 |
| concentration, 106/ml | | 89.0 ± 11.6 | 44.9 ± 11.1 | 0.02, wil |
| Morphology, % | | 8.9 ± 1.3 | 6.6 ± 2.1 | 0.14 |
| Motility, %  (range) | TM | 64.6 ± 2.0 | 32.4 ± 2.0 | < 0.0001, stt |
| PR | 62.2 ± 2.2 | 25.3 ± 2.5 | < 0.0001, wil |
| Viscosityc | | 77% (24) | 75% (9) | 1.0 |
| Agglutinationd | | 3% (1) | 17% (2) | 0.12 |

Table TIX: Semen baseline parameters measured by the Andrology unit at the Jessop Wing for combination incubations 13Cu-glucose\13C1-pyruvate (n= 16) and 13Cu-fructose\13C1-pyruvate (n= 16). a, abstinence – where >2 days was reported this was set to 2 days; b, analysis delay represents the time elapsed between semen ejaculation and commencing Andrology analysis; c, viscosity was graded as either normal or high; d, agglutination presented as number of cases where some degree of agglutination (was noted: isolated, moderate, widespread).Number are presented as either mean and standard error of mean or percentage number of cases (absolute number in parenthesis).

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | | 13Cu-glucose & 13C1-pyruvate | 13Cu-fructose & 13C1-pyruvate |
| abstinencea, days | | 4.8 ± 0.3 | 4.8 ± 0.4 |
| analysis delayb, min | | 22.8 ± 2.8 | 29.4 ± 2.4 |
| volume, ml | | 4.8 ± 0.4 | 4.4 ± 0.5 |
| pH (semen) | | 8.03 ± 0.03 | 8.09 ± 0.05 |
| concentration, 106/ml | | 87.1 ± 13.9 | 94.5 ± 19.3 |
| Morphology, % | | 9.2 ± 1.8 | 6.8 ± 1.0 |
| Motility, %  (range) | TM | 60.8 ± 1.9 | 62.6 ± 2.6 |
| PR | 57.9 ± 2.0 | 59.9 ± 2.9 |
| Viscosityc | | 88% (14) | 63% (10) |
| Agglutinationd | | 0% | 6% (1) |

Table TX: Bin integrals, mean ± S.E., from1H MR spectra of washed sperm that have been separated into higher motility (‘80%’) and lower motility (‘40%’) fraction obtained from normozoospermic (N) and asthenozoospermic (A) ejaculates (grouped by color in columns 2 & 3).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Bin, | Comparison | | Mean ± SE, x107 | |  |
| ppm | Group 1 | Group 2 | Group 1 | Group 2 | p |
| 3.91 | 80N | 40N | 1.04 ± 0.12 | 1.93 ± 0.20 | 0.0030 |
| 3.67 | 80N | 40N | 1.20 ± 0.11 | 2.04 ± 0.18 | 0.016 |
| 3.27 | 80N | 40N | 3.49 ± 0.43 | 5.20 ± 0.49 | <0.0001 |
| 3.27 | 40N | 80A | 5.21 ± 0.49 | 2.81 ± 0.56 | <0.0001 |
| 3.27 | 80A | 40A | 2.81 ± 0.56 | 4.55 ± 1.21 | <0.0001 |
| 3.23 | 80N | 40N | 8.25 ± 0.80 | 11.59 ± 0.97 | <0.0001 |
| 3.23 | 80N | 80A | 8.25 ± 0.80 | 6.72 ± 1.15 | <0.0001 |
| 3.23 | 80N | 40A | 8.25 ± 0.80 | 11.25 ± 2.07 | <0.0001 |
| 3.23 | 80A | 40N | 6.72 ± 1.14 | 11.59 ± 0.97 | <0.0001 |
| 3.23 | 80A | 40A | 6.72 ± 1.14 | 11.25 ± 2.07 | <0.0001 |
| 3.19 | 80N | 40N | 5.42 ± 0.58 | 6.46 ± 0.54 | <0.0001 |
| 3.19 | 80N | 80A | 5.42 ± 0.58 | 3.71 ± 0.54 | <0.0001 |
| 3.19 | 80N | 40A | 5.42 ± 0.58 | 6.73 ± 1.07 | <0.001 |
| 3.19 | 40N | 80A | 6.46 ± 0.55 | 3.71 ± 0.54 | <0.0001 |
| 3.19 | 80A | 40A | 3.71 ± 0.54 | 6.73 ± 1.07 | <0.0001 |
| 1.34 | 80N | 40N | 0.68 ± 0.08 | 2.29 ± 0.39 | <0.0001 |
| 1.34 | 80N | 40A | 0.68 ± 0.08 | 2.48 ± 0.64 | <0.0001 |
| 1.34 | 80A | 40N | 0.98 ± 0.21 | 2.29 ± 0.39 | <0.001 |
| 1.34 | 80A | 40A | 0.98 ± 0.21 | 2.48 ± 0.64 | 0.007 |
| 1.30 | 80N | 40N | 0.97 ± 0.12 | 3.74 ± 0.49 | <0.0001 |
| 1.30 | 80N | 40A | 0.97 ± 0.12 | 4.44 ± 1.18 | <0.0001 |
| 1.30 | 80A | 40N | 1.67 ± 0.38 | 3.74 ± 0.49 | <0.0001 |
| 1.30 | 80A | 40A | 1.67 ± 0.38 | 4.44 ± 1.18 | <0.0001 |
| 1.26 | 80N | 40N | 0.84 ± 0.10 | 3.18 ± 0.41 | <0.0001 |
| 1.26 | 80N | 40A | 0.84 ± 0.10 | 4.10 ± 1.21 | <0.0001 |
| 1.26 | 80A | 40N | 1.30 ± 0.28 | 3.18 ± 0.41 | <0.0001 |
| 1.26 | 80A | 40A | 1.30 ± 0.28 | 4.10 ± 1.21 | <0.0001 |
| 1.22 | 80N | 40N | 0.50 ± 0.05 | 1.33 ± 0.17 | 0.026 |
| 1.22 | 80N | 40A | 0.50 ± 0.05 | 1.74 ± 0.49 | 0.0044 |