



Article

Supplementary Material S1: Questionnaire data

Table S1. Study questionnaire

| | | | | |
|---|------------------|--------------------------|-----------------------|---------------|
| Questionnaire number | | | | |
| Participant's age | | Height | | |
| Partner's age | | Weight | | |
| Ethnicity | | The cause of infertility | | |
| Working conditions | Sitting | Standing | Manual worker | |
| Harmful working conditions, if applicable | High temperature | Low temperature | Pesticides, chemicals | Radiation |
| Other exposure to high temperatures | Sauna | Thermal pools | Excessive sunbathing | Other |
| Physical activity | None | Once a week | 3 times a week | Daily |
| Diet | No limitations | Protein | Vegetarian | Other |
| Drugs: | | | | |
| Alcohol | None | Occasionally | Frequently | Daily |
| Cigarettes | None | Occasionally | 2-5 daily | >6 daily |
| Narcotics | None | Occasionally | Frequently | Daily |
| Coffee | None | Occasionally | 1-2 cups daily | >3 cups daily |
| Fruits and vegetables intake | None | Less than once a week | 1-2 times a week | Daily |

Table S2. History of infertility

| | | |
|---|-----|----|
| Do you have any children? | Yes | No |
| Number of intercourses per week: | | |
| How long for are you trying to conceive? | | |
| Do you have any troubles with erection? | Yes | No |
| Is there any blood in semen? | Yes | No |
| Is ejaculation painful? | Yes | No |
| Did you experience testicles swelling, enlargement or displacement? | Yes | No |
| Did you experience any troubles with ejaculation, premature ejaculation or lack of ejaculate? | Yes | No |
| Does infertility occur in your family (brothers or sisters)? | Yes | No |
| Has your father been treated from any urological condition? | Yes | No |

Table S3. Disease history

| | | |
|---------------------|-----------------|----|
| Autoimmune diseases | Yes | No |
| Thyroid disease | Hyperthyroidism | No |
| | Hypothyroidism | |
| Diabetes | Type 1 | No |
| | Type 2 | |
| Viral hepatitis | Type A | No |

| | | |
|-------------------------------|---|----|
| Urological diseases | Type B | No |
| | Type C | |
| | Nephritis | |
| | Kidney stone disease (urolithiasis) | |
| Genetic diseases in family | Difficulty in urinating | No |
| | Prostate diseases | |
| | Bacterial urinary tract infection | |
| | X-linked (linked with sex) | |
| Rheumatic diseases | Autosomal | No |
| | Rheumatoid arthritis | |
| | Systemic lupus erythematosus | |
| | Sjögren syndrome | |
| Mental disorders | Ankylosing spondylitis | No |
| | Other | |
| | Depression | |
| | Bipolar affective disorder | |
| Cancer | Schizophrenia | No |
| | Anxiety disorders | |
| | Chronic stress | |
| | Malignant | |
| Hypertension | Benign | No |
| | Yes | |
| Sexually transmitted diseases | Syphilis / gonorrhoea / chlamydiosis / AIDS / herpes virus / human papillomavirus / scabies / lice / trichomoniasis / candidiasis / non-gonococcal urethritis | No |

Table S4. History of treatment

| | | |
|--|-----|----|
| Did you take any antibiotics in the last 6 months? | Yes | No |
| Do you take any drugs chronically? If so, please enter their names | Yes | No |

Study questionnaire – results

Table S5. Working conditions

| Parameter (Mean±SD) | Sitting N=48 | Standing N=9 | Manual worker N=18 | p* |
|--|--------------|--------------|--------------------|-------|
| Total sperm cell count (mL) | 91.44±55.21 | 114.01±44.24 | 109.46±79.63 | 0.329 |
| Sum of progressive and non-progressive cells (%) | 43.88±18.04 | 56.11±22.3 | 44.72±17.41 | 0.317 |
| Plasma membrane integrity (%) | 63.36±17.61 | 76.18±20.39 | 67.18±14.97 | 0.154 |
| Viability with Eosin Staining (%) | 65.6±18.11 | 77.2±18.28 | 70.78±13.01 | 0.093 |
| TRAP (mmol/L) | 4.71±2.25 | 6.17±1.87 | 5.29±1.8 | 0.191 |
| Protein (mg/mL) | 23.75±5.46 | 24.81±5.42 | 24±3.41 | 0.570 |

| | | | | |
|----------------------------|-------------------|--------------------|-----------------|-------|
| GSH ($\mu\text{mol/L}$) | 73.17 \pm 39.43 | 107.52 \pm 52.79 | 81.2 \pm 41.3 | 0.120 |
| MDA ($\mu\text{mol/mL}$) | 5.39 \pm 1.06 | 4.81 \pm 0.8 | 4.98 \pm 1.17 | 0.123 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S6. High temperature

| Parameter (Mean \pm SD) | Yes N=25 | No N=50 | p* |
|--|-------------------|-------------------|-------|
| Total sperm cell count (mL) | 78.7 \pm 50.22 | 108.36 \pm 63.6 | 0.056 |
| Sum of progressive and non-progressive cells (%) | 44.16 \pm 20.27 | 46.24 \pm 17.87 | 0.438 |
| Plasma membrane integrity (%) | 62.94 \pm 17.51 | 67.26 \pm 17.69 | 0.208 |
| Viability with Eosin Staining (%) | 66.08 \pm 17.01 | 69.31 \pm 17.5 | 0.186 |
| TRAP (mmol/L) | 4.16 \pm 2.14 | 5.46 \pm 2.02 | 0.013 |
| Protein (mg/mL) | 23.28 \pm 5.26 | 24.27 \pm 4.87 | 0.094 |
| GSH ($\mu\text{mol/L}$) | 71.21 \pm 42.56 | 83.23 \pm 42.26 | 0.206 |
| MDA ($\mu\text{mol/mL}$) | 5.46 \pm 1.11 | 5.1 \pm 1.05 | 0.129 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S7. Low temperature

| Parameter (Mean \pm SD) | Yes N=10 | No N=65 | p* |
|--|-------------------|-------------------|-------|
| Total sperm cell count (mL) | 89.04 \pm 63.86 | 99.92 \pm 60.68 | 0.533 |
| Sum of progressive and non-progressive cells (%) | 38.4 \pm 15.76 | 46.65 \pm 18.86 | 0.190 |
| Plasma membrane integrity (%) | 62.16 \pm 16.57 | 66.38 \pm 17.84 | 0.346 |
| Viability with Eosin Staining (%) | 64.05 \pm 15.29 | 68.88 \pm 17.6 | 0.268 |
| TRAP (mmol/L) | 3.93 \pm 2.11 | 5.2 \pm 2.11 | 0.089 |
| Protein (mg/mL) | 23.1 \pm 3.37 | 24.07 \pm 5.2 | 0.876 |
| GSH ($\mu\text{mol/L}$) | 67.45 \pm 40.9 | 81.03 \pm 42.71 | 0.400 |
| MDA ($\mu\text{mol/mL}$) | 5.58 \pm 1.22 | 5.17 \pm 1.05 | 0.268 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S8. Any harmful working condition

| Parameter (Mean \pm SD) | Yes N=31 | No N=44 | p* |
|--------------------------------|-------------------|--------------------|-------|
| Total sperm cell count (mL) | 81.57 \pm 55.17 | 110.38 \pm 62.32 | 0.037 |

| | | | |
|--|-------------|-------------|-------|
| Sum of progressive and non-progressive cells (%) | 43.71±19.93 | 46.84±17.71 | 0.398 |
| Plasma membrane integrity (%) | 63.77±17.27 | 67.26±17.94 | 0.256 |
| Viability with Eosin Staining (%) | 66.83±16.29 | 69.23±18.09 | 0.307 |
| TRAP (mmol/L) | 4.28±2.11 | 5.55±2.02 | 0.013 |
| Protein (mg/mL) | 23.16±4.78 | 24.48±5.11 | 0.079 |
| GSH (μmol/L) | 72.39±43.36 | 84.03±41.64 | 0.188 |
| MDA (μmol/mL) | 5.39±1.14 | 5.1±1.02 | 0.198 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S9. Physical activity

| Parameter (Mean±SD) | Never (A) N=13 | 1-3 times a week (B) N=48 | Every day (C) N=14 | p* |
|--|-------------------|---------------------------------|-----------------------|----------------|
| Total sperm cell count (mL) | 78.68±90.89 | 100.48±55.59 | 109.96±41.02 | 0.045 (C>A) |
| Sum of progressive and non-progressive cells (%) | 38±23.56 | 46.56±17.52 | 49.07±16.48 | 0.155 |
| Plasma membrane integrity (%) | 58.38±13.61 | 66.35±18.19 | 70.88±17.84 | 0.148 |
| Viability with Eosin Staining (%) | 64.46±14.64 | 67.94±18.15 | 72.76±16.65 | 0.412 |
| TRAP (mmol/L) | 3.47±2.01 | 5.36±2.09 | 5.31±1.89 | 0.019 (B>A) |
| Protein (mg/mL) | 22.29±5.39 | 23.98±4.66 | 25.34±5.62 | 0.036 (C>A) |
| GSH (μmol/L) | 66.88±35.16 | 80.84±42.52 | 85.11±48.85 | 0.552 |
| MDA (μmol/mL) | 5.73±1.23 | 5.08±0.93 | 5.22±1.29 | 0.267 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S10. Drinking coffee

| Parameter (Mean±SD) | Never/ occasionally N=47 | Every day N=28 | p* |
|--|-----------------------------|-------------------|-------|
| Total sperm cell count (mL) | 98.26±49.72 | 98.83±76.9 | 0.607 |
| Sum of progressive and non-progressive cells (%) | 46.51±14.73 | 43.93±23.95 | 0.188 |
| Plasma membrane integrity (%) | 66.64±18.36 | 64.44±16.58 | 0.573 |
| Viability with Eosin Staining (%) | 68.55±18.43 | 67.71±15.52 | 0.603 |

| | | | |
|---------------------------|------------------|------------------|-------|
| TRAP (mmol/L) | 5.54±1.99 | 4.17±2.14 | 0.007 |
| Protein (mg/mL) | 23.1±2.76 | 25.34±7.21 | 0.948 |
| TRAP (mmol/mg of protein) | 243.18 ±90.13 | 169.93 ±99.14 | 0.003 |
| GSH (µmol/L) | 87.32±46.74 | 65.62±30.23 | 0.072 |
| MDA (µmol/mL) | 5.06±1.09 | 5.49±1 | 0.036 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S11. Fruits and vegetables intake

| Parameter (Mean±SD) | Less than every day N=33 | Every day N=42 | p* |
|--|-----------------------------|-------------------|-------|
| Total sperm cell count (mL) | 81.3±53.14 | 111.97±63.57 | 0.021 |
| Sum of progressive and non-progressive cells (%) | 43.55±21.81 | 47.12±15.72 | 0.132 |
| Plasma membrane integrity (%) | 61.87±17.26 | 68.92±17.5 | 0.114 |
| Viability with Eosin Staining (%) | 64.24±16.95 | 71.38±17.11 | 0.115 |
| TRAP (mmol/L) | 4.12±2.09 | 5.74±1.92 | 0.001 |
| Protein (mg/mL) | 23.37±4.53 | 24.39±5.33 | 0.191 |
| GSH (µmol/L) | 67.3±38.68 | 88.58±43.39 | 0.059 |
| MDA (µmol/mL) | 5.57±1.03 | 4.95±1.04 | 0.006 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test

Table S12. Having offspring

| Parameter (Mean±SD) | Yes N=41 | No N=34 | p* |
|--|--------------|-------------|--------|
| Total sperm cell count (mL) | 100.79±51.23 | 95.67±71.34 | 0.44 |
| Sum of progressive and non-progressive cells (%) | 54.78±17.56 | 34.41±12.95 | <0.001 |
| Plasma membrane integrity (%) | 73±18.17 | 57.16±12.48 | <0.001 |
| Viability with Eosin Staining (%) | 73.85±18.96 | 61.46±12.18 | 0.002 |
| TRAP (mmol/L) | 5.72±2.08 | 4.19±1.92 | 0.002 |
| Protein (mg/mL) | 23.49±4 | 24.48±5.99 | 0.463 |
| GSH (µmol/L) | 100.76±44.79 | 53.25±18.45 | <0.001 |
| MDA (µmol/mL) | 4.88±0.93 | 5.63±1.09 | 0.001 |

* lack of normality of distribution, non-parametric analysis, Kruskal-Wallis test