New Approaches to Define The Functional Competency of Human Sperm Subpopulations and Its Relationship to Semen Quality

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Table S1: Standard semen analysis parameters of the donor semen samples used in this investigation (n=55)

| Semen characteristic         | Mean ± SD | Range       | 95% CI       |
|------------------------------|-----------|-------------|--------------|
| Volume (ml)                  | 2.8 ± 1.5 | 1.0 – 6.5   | 2.4 – 3.1    |
| pH#                         | 7.7 ± 0.8 | 6.8 – 8.7   | 7.5 – 7.8    |
| Viscosity (cP)               | 9.0 ± 7.7 | 3.4 – 42.1  | 6.9 – 11.1   |
| Total motility (%)           | 52.1 ± 15.5 | 25.1 – 79.1 | 48.0 – 56.4  |
| Progressive motility (%)     | 27.1 ± 16.9 | 3.6 – 84.8  | 22.6 – 31.7  |
| Sperm concentration (×10⁶/ml) | 55.0 ± 38.3 | 7.7 – 172.7 | 44.7 – 65.4  |
| Total sperm number (×10⁶/ejaculate) | 154.4 ± 137.3 | 15.1 – 737.5 | 117.3 – 191.5 |
| Sperm mucus penetration (×10⁶/ejaculate) | 23.7 ± 28.5 | 1.0 – 167.9 | 11.0 – 191.5 |
| Vitality (live spermatozoa, %) | 57.2 ± 14.0 | 29.0 – 89.0 | 57.0 – 63.8  |
| Normal sperm morphology (%)  | 11.0 ± 7.8 | 3.6 – 84.8  | 8.8 – 13.1   |
| TZI                          | 1.6 ± 0.3 | 1.2 – 2.5   | 1.6 – 1.7    |
| DI                           | 2.2 ± 0.4 | 1.2 – 3.1   | 2.0 – 2.3    |

*; Semen parameters labeled with a hashtag had a sample size of n=35, CI; Confidence interval, cP; Centipoise, DI; Deformity index, and TZI; Teratozoospermic index.

Table S2: Comparison of the different speed and progressive speed group kinematics of the high motile (HM) and low motile (LM) sperm fractions (n=35)

| Kinematic parameter | Mean ± SD | High motile fraction | Mean ± SD | Low motile fraction | P value |
|---------------------|-----------|----------------------|-----------|---------------------|---------|
|                     | Rapid     | Med                  | Slow      | RP                  | MP      | SP      |
| VCL (μm/s)          |           |                      |           |                     |         |         |
| Rapid               | 134.4 ± 31.0 | 100.2 – 237.1        | 123.8 – 145.1 | 128.3 ± 30.9        | 69.8 – 206.2 | 0.22    |
| Med                 | 126.1 ± 41.2 | 86.5 – 249.7         | 111.9 – 140.2 | 103.6 ± 31.0        | 65.9 – 229.1 | 0.001   |
| Slow                | 62.4 ± 9.4  | 51.3 – 92.9          | 59.2 – 65.7 | 59.4 ± 8.4          | 35.2 – 76.4 | 0.22    |
| RP                  | 145.6 ± 32.0 | 111.7 – 246.6        | 134.6 – 156.5 | 135.4 ± 29.7        | 81.5 – 215.4 | 0.17    |
| MP                  | 83.9 ± 9.9  | 72.7 – 110.4         | 80.5 – 87.3 | 82.6 ± 9.2          | 68.3 – 110.7 | 0.77    |
| SP                  | 52.4 ± 7.4  | 42.8 – 73.7          | 49.9 ± 55.0 | 49.1 ± 6.1          | 35.2 – 67.2 | 0.06    |
| VAP (μm/s)          |           |                      |           |                     |         |         |
| Rapid               | 78.6 ± 17.0 | 52.2 – 121.7         | 72.8 – 84.4 | 76.0 ± 19.1         | 52.9 – 115.7 | 0.58    |
| Med                 | 64.8 ± 13.5 | 42.4 – 106.0         | 60.1 – 69.4 | 55.7 ± 11.9**       | 31.5 – 95.0 | 0.002   |
| Slow                | 33.9 ± 7.6  | 21.9 – 50.9          | 31.3 – 36.5 | 32.5 ± 6.8          | 15.7 – 48.8 | 0.30    |
| RP                  | 81.0 ± 18.4 | 47.1 – 128.9         | 74.7 – 87.3 | 76.6 ± 27.2         | 30.8 – 140.4 | 0.12    |
| MP                  | 53.6 ± 7.1  | 36.8 – 65.1          | 51.2 – 56.1 | 50.7 ± 9.2          | 35.8 – 71.0 | 0.15    |
| SP                  | 32.4 ± 6.7  | 21.8 – 51.5          | 30.1 – 34.7 | 28.6 ± 5.9*         | 15.7 – 39.3 | 0.01    |
| VSL (μm/s)          |           |                      |           |                     |         |         |
| Rapid               | 71.4 ± 15.7 | 47.6 – 111.8         | 66.0 – 76.8 | 68.8 ± 18.6         | 45.4 – 109.1 | 0.57    |
| Med                 | 45.5 ± 7.7  | 28.1 – 61.1          | 42.8 – 48.1 | 43.3 ± 9.6          | 18.3 – 68.6 | 0.31    |
| Slow                | 21.5 ± 7.1  | 10.1 – 41.6          | 19.1 – 24.0 | 21.2 ± 7.4          | 8.3 – 46.8 | 0.93    |
| RP                  | 61.9 ± 16.8 | 37.7 – 97.0          | 56.1 – 67.6 | 59.7 ± 24.8         | 11.6 – 113.2 | 0.58    |
| MP                  | 43.8 ± 8.8  | 27.6 – 59.3          | 40.7 – 46.8 | 40.5 ± 11.4         | 20.6 – 68.9 | 0.18    |
| SP                  | 23.8 ± 7.4  | 11.3 – 44.5          | 21.3 – 26.4 | 20.2 ± 6.9*         | 8.3 – 33.4 | 0.03    |
**Table S2: Continued**

| Kinematic parameter | High motile fraction | Low motile fraction | P value |
|---------------------|----------------------|---------------------|---------|
|                     | Mean ± SD | Range    | 95% CI       | Mean ± SD | Range    | 95% CI       |
| STR (%)             |           |          |              |           |          |              |
| Rapid               | 90.4 ± 2.1 | 84.7 – 94.4 | 89.7 – 91.1 | 89.9 ± 3.1 | 83.3 – 94.9 | 88.6 – 91.3 | 0.53  |
| Med                 | 71.0 ± 9.5* | 55.4 – 91.3 | 67.8 – 74.3 | 78.3 ± 13.0* | 40.4 – 96.6 | 73.6 – 83.0 | 0.01  |
| Slow                | 60.3 ± 9.6 | 42.8 – 77.5 | 57.0 – 63.6 | 61.1 ± 11.6 | 42.4 – 95.9 | 57.1 – 65.0 | 0.76  |
| RP                  | 71.2 ± 9.7 | 38.3 – 89.0 | 67.8 – 74.5 | 70.8 ± 17.4 | 18.8 – 94.2 | 64.1 – 77.6 | 0.41  |
| MP                  | 77.1 ± 8.6 | 59.6 – 92.4 | 74.2 – 80.1 | 74.8 ± 11.7 | 51.4 – 97.6 | 70.8 – 78.9 | 0.35  |
| SP                  | 64.4 ± 13.0 | 23.7 – 84.1 | 59.9 – 68.9 | 62.0 ± 12.6 | 37.3 – 83.8 | 57.6 – 66.3 | 0.43  |
| LIN (%)             |           |          |              |           |          |              |
| Rapid               | 54.6 ± 6.5 | 40.6 – 67.2 | 52.4 – 56.8 | 54.4 ± 8.8 | 41.2 – 78.1 | 50.6 – 58.2 | 0.92  |
| Med                 | 42.3 ± 10.3 | 23.9 – 67.2 | 38.8 – 45.8 | 46.9 ± 14.0 | 14.7 – 73.6 | 41.8 – 51.9 | 0.13  |
| Slow                | 34.5 ± 8.9 | 18.5 – 53.6 | 31.5 – 37.6 | 35.7 ± 11.7 | 21.3 – 81.1 | 31.7 – 39.7 | 0.87  |
| RP                  | 44.2 ± 8.7 | 26.8 – 76.1 | 41.2 – 47.2 | 42.5 ± 13.4 | 6.9 – 71.3 | 37.3 – 47.7 | 0.97  |
| MP                  | 52.1 ± 9.5 | 33.1 – 67.2 | 48.9 – 55.4 | 48.6 ± 12.3 | 28.5 – 78.1 | 44.3 – 52.9 | 0.18  |
| SP                  | 44.8 ± 9.9* | 24.5 – 63.6 | 41.4 – 48.2 | 39.0 ± 12.1* | 20.2 – 65.1 | 34.9 – 43.2 | 0.03  |
| WOB (%)             |           |          |              |           |          |              |
| Rapid               | 59.7 ± 6.2 | 46.6 – 72.1 | 57.6 – 61.9 | 60.2 ± 8.4 | 48.1 – 82.3 | 56.5 – 63.8 | 0.82  |
| Med                 | 55.9 ± 7.4 | 42.6 – 73.4 | 53.4 – 58.5 | 57.2 ± 10.6 | 25.3 – 76.2 | 53.4 – 61.1 | 0.56  |
| Slow                | 54.1 ± 6.8 | 39.3 – 64.4 | 51.8 – 56.4 | 54.9 ± 8.7 | 42.2 – 84.7 | 51.9 – 57.9 | 0.98  |
| RP                  | 56.3 ± 5.6 | 43.7 – 66.5 | 54.4 – 58.2 | 55.3 ± 12.0 | 18.6 – 79.9 | 50.6 – 60.0 | 0.83  |
| MP                  | 63.9 ± 6.9 | 44.5 – 75.1 | 61.6 – 66.3 | 61.1 ± 9.2 | 43.6 – 79.7 | 57.9 – 64.3 | 0.15  |
| SP                  | 59.5 ± 7.8* | 38.7 – 72.5 | 56.8 – 62.1 | 56.2 ± 10.2* | 32.1 – 77.8 | 52.7 – 59.7 | 0.03  |
| ALH (μm)            |           |          |              |           |          |              |
| Rapid               | 3.4 ± 0.9  | 2.0 – 6.4  | 3.1 – 3.7  | 3.3 ± 0.9  | 1.8 – 5.6  | 2.9 – 3.7  | 0.92  |
| Med                 | 3.6 ± 1.3* | 2.0 – 7.3  | 3.1 – 4.0  | 2.9 ± 1.1* | 1.0 – 7.0  | 2.5 – 3.3  | 0.01  |
| RP                  | 3.6 ± 0.8  | 2.5 – 6.1  | 3.3 – 3.9  | 3.3 ± 0.9  | 1.2 – 5.6  | 2.9 – 3.6  | 0.28  |
| MP                  | 2.1 ± 0.4  | 1.6 – 3.3  | 2.0 – 2.2  | 2.2 ± 0.5  | 1.4 – 3.4  | 2.0 – 2.3  | 0.35  |
| SP                  | 1.5 ± 0.2  | 1.0 – 2.1  | 1.4 – 1.5  | 1.4 ± 0.3  | 0.9 – 2.1  | 1.3 – 1.5  | 0.89  |
| BCF (Hz)            |           |          |              |           |          |              |
| Rapid               | 25.0 ± 6.6 | 17.0 – 58.0 | 22.7 – 27.3 | 22.2 ± 3.6 | 15.6 – 27.5 | 20.7 – 23.8 | 0.07  |
| Med                 | 20.3 ± 3.4 | 13.4 – 29.3 | 19.2 – 21.5 | 21.6 ± 5.0 | 13.6 – 37.5 | 19.8 – 23.4 | 0.46  |
| RP                  | 17.8 ± 4.2 | 11.1 – 31.0 | 16.4 – 19.3 | 16.2 ± 5.5 | 6.3 – 27.0 | 14.1 – 18.4 | 0.26  |
| MP                  | 19.2 ± 4.0 | 13.2 – 29.0 | 17.8 – 20.6 | 18.0 ± 6.6 | 7.2 – 37.5 | 15.7 – 20.3 | 0.21  |
| SP                  | 14.9 ± 4.0 | 7.3 – 25.1  | 13.5 – 16.2 | 13.9 ± 4.8 | 5.8 – 21.5 | 12.3 – 15.6 | 0.48  |

The HM sperm fraction had significantly higher values for the medium speed VCL (0.001), VAP (0.001) and ALH (0.001) kinematics in addition to the slow – progressive VAP (0.001), VSL (0.03), LIN (0.03) and WOB (0.03) kinematic parameters. The LM fraction had significantly higher values for the medium speed STR (0.01) kinematic parameter compared to the HM fraction. ALH: Amplitude of lateral head displacement, BCF: Beat cross frequency, CI: Confidence interval, HM: High motile fraction, LIN: linearity, LM: Low motile fraction, Med: Medium, MP: Medium – progressive, RP: Rapid – progressive, SP: Slow – progressive, STR: Straightness, VAP: Average path velocity, VCL: Curvilinear velocity, VSL: Straight – line velocity, and WOB: Wobble. Values in the same row labelled with an asterisk in bold were significantly different between the two sperm fractions (**P<0.01 and *P<0.05).
Table S3: Multivariate visualisation

| Variables             | Mean ± SD | Range    |
|-----------------------|-----------|----------|
| Vitality (%)          | 55.3 ± 15.1 | 27.0 – 83.0 |
| ARIC (%)              | 42.9 ± 21.4 | 2.0 – 78.6  |
| ROS pos (%)           | 43.0 ± 13.7 | 11.0 – 67.9 |
| MMP intact (%)        | 56.0 ± 17.1 | 19.0 – 86.4   |
| Intact chromatin (%)  | 75.9 ± 9.2  | 54.0 – 93.0    |
| Mature spermatozoa (%)| 74.0 ± 13.1 | 46.1 – 98.0    |

Andrews plot data input key for individual semen samples (n=40) high and low motile sperm fraction parameters. Data variables included in the Andrews plot are the percentage vitality (%), acrosome reaction after calcium – ionophore challenge scores (%), positive reactive oxygen species (%), intact mitochondrial membrane potential (%), intact chromatin (%) and mature spermatozoa (%). The plot has been standardised according to the minimum and maximum values. ARIC; Acrosome reaction after calcium – ionophore challenge scores, MMP; Mitochondrial membrane potential, and ROS pos.; Positive reactive oxygen species.