Int J Fertil Steril
International Journal of Fertility & Sterility
ISSN:2008-076X eISSN:2008-0778

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 (%)	52.2 ± 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 ($\times 10^6$/ml)	55.0 ± 38.3	7.7 – 172.7	44.7 – 65.4
Total sperm number ($\times 10^6$/ejaculate)	154.4 ± 137.3	15.1 – 737.5	117.3 – 191.5
Sperm mucus penetration ($\times 10^6$/ejaculate)	23.7 ± 28.5	1.0 – 167.9	16.0 – 31.3
Vitality (live spermatozoa, %)	57.2 ± 14.0	29.0 – 89.0	57.0 – 63.8
Normal sperm morphology (%)	11.0 ± 7.8	1.0 – 39.0	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 paramaters labelld 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	High motile fraction			Low motile fraction			P value
	Mean ± SD	Range	95% CI	Mean ± SD	Range	95% CI	
VCL (µm/s)							
Rapid	134.4 ± 31.0	100.2 – 237.1	123.8 – 145.1	128.3 ± 30.9	96.8 – 206.2	114.9 – 141.7	0.22
Med	126.1 ± 41.2**	86.5 – 249.7	111.9 – 140.2	103.6 ± 31.0**	65.9 – 229.1	92.4 – 114.8	0.001
Slow	62.4 ± 9.4	51.3 – 92.9	59.2 – 65.7	59.4 ± 8.4	35.2 – 76.4	56.5 – 62.2	0.22
RP	145.6 ± 32.0	111.7 – 246.6	134.6 – 156.5	135.4 ± 29.7	81.5 – 215.4	123.9 – 146.9	0.17
MP	83.9 ± 9.9	72.7 – 110.4	80.5 – 87.3	82.6 ± 9.2	68.3 – 110.7	79.4 – 85.8	0.77
SP	52.4 ± 7.4	42.8 – 73.7	49.9 – 55.0	49.1 ± 6.1	35.2 – 67.2	47.0 – 51.2	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	67.7 – 84.3	0.58
Med	64.8 ± 13.5**	42.4 – 106.0	60.1 – 69.4	55.7 ± 11.9**	31.5 – 95.0	51.4 – 60.0	0.002
Slow	33.9 ± 7.6	21.9 – 50.9	31.3 – 36.5	32.5 ± 6.8	15.7 – 48.8	30.2 – 34.8	0.66
RP	81.0 ± 18.4	47.1 – 128.9	74.7 – 87.3	76.6 ± 27.2	30.8 – 140.4	66.0 – 87.1	0.12
MP	53.6 ± 7.1	36.8 – 65.1	51.2 – 56.1	50.7 ± 9.2	35.8 – 71.0	47.5 – 54.0	0.15
SP	32.4 ± 6.7*	21.8 – 51.5	30.1 – 34.7	28.6 ± 5.9*	15.7 – 39.3	26.6 – 30.6	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	60.8 – 76.9	0.57
Med	45.5 ± 7.7	28.1 – 61.1	42.8 – 48.1	43.3 ± 9.6	18.3 – 68.6	39.9 – 46.8	0.31
Slow	21.5 ± 7.1	10.1 – 41.6	19.1 – 24.0	21.2 ± 7.4	8.3 – 46.8	18.7 – 23.8	0.93
RP	61.9 ± 16.8	37.7 – 97.0	56.1 – 67.6	59.7 ± 24.8	11.6 – 113.2	50.1 – 69.3	0.58
MP	43.8 ± 8.8	27.6 – 59.3	40.7 – 46.8	40.5 ± 11.4	20.6 – 68.9	36.6 – 44.5	0.18
SP	23.8 ± 7.4*	11.3 – 44.5	21.3 – 26.4	20.2 ± 6.9*	8.3 – 33.4	17.8 – 22.5	0.03

Received: 03/June/2021, Accepted: 13/December/2021
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Int J Fertil Steril
International Journal of Fertility & Sterility
ISSN:2008-076X eISSN:2008-0778

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 – 67.6	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 (0.002) and ALH (0.01) kinematics in addition to the slow – progressive VAP (0.01), 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 asteriks in bold were significantly different between the two sperm fractions (*P<0.05 and **P<0.01).

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.