



Computer assisted motility analysis (SpermVision) of epididymal spermatozoa from domestic cat

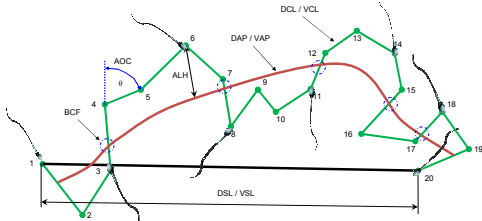
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Computer assisted sperm motility analysis (CASA)

is an essential tool to generate objective data on sperm cell movement (percentage of motile cells, velocity, movement pattern).



DCL – Distance: Curve Line [µm] = Distance that the sperm head travels along its actual path
 DAP – Distance: Average Path [µm] = Distance that the sperm head traveled along its average trajectory
 DSL – Distance: Straight Line [µm] = Distance that the sperm head traveled along a straight line from its first position to its last position
 VCL – Velocity: Curve Line [µm/s] = Time average velocity of the sperm head along its actual path
 VAP – Velocity: Average Path [µm/s] = Time average velocity of the sperm head along its average trajectory
 VSL – Velocity: Straight Line [µm/s] = Time average velocity of the sperm head along a straight line from its first position to its last position
 LIN – Linearity = VSL / VCL = Linearity of the curvilinear trajectory
 STR – Straightness = VSL / VAP = Straightness of the average path
 WOB – Wobble = VAP / VCL = Degree of oscillation of the actual sperm-head trajectory about its average path
 BCF – Beat Cross Frequency [Hz] = Time average rate that the actual sperm trajectory crosses the average path trajectory
 ALH – Amplitude of Lateral Head Displacement [µm] = Maximum amplitude of variation of the actual sperm head trajectory about its average trajectory
 AOC – Average Orientation Change [°] = Degree average of the change in orientation of the sperm head

Why we have to establish a customized method to analyse feline spermatozoa?

- Feline spermatozoa have comparatively small and round heads and pose a challenge to CASA systems because it is hard to discriminate the sperm head from other semen contaminants or egg yolk particles.
- Furthermore spermatozoa have species-specific requirements concerning the geometry of the measuring chamber, the incubation time and culture media.

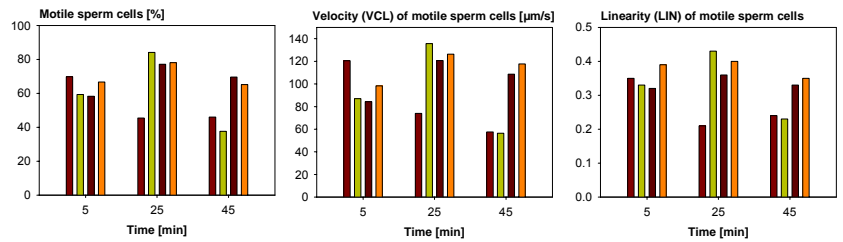
Optimisation of the settings

The available settings of the SpermVision system (Minitüb GmbH, Germany) were successfully adapted to enable the detection and tracking of cat spermatozoa.

1. Level of classification	Settings for selection	Color
Immotile objects	AOC < 4.5, A _{max} > 18, DSL < 10 AOC < 10, DCL < 7.5, A _{max} > 18, A _{max} < 70	Maroon
Locally motile objects	DSL < 7, VCL < 20, VCL > 50 DSL < 7, VCL < 20, BCF < 32 DCL/DAP > 2, DAP < 30, WOB < 0.5, DSL < 10 LIN ≤ 0.1, DAP ≥ 2.2, AOC > 6.5, AOC < 25	Yellow
Progressively motile objects		
2. Level of classification		
Particles in locally motile objects	AOC ≥ 10, BCF = 0 A _{max} < 23 A _{max} > 71	Sky blue
Linear in progressively motile objects	STR > 0.9 & LIN > 0.5	Lime
Non-linear in progressively motile objects	STR ≤ 0.9 & LIN ≤ 0.5	Green
Curve-linear in progressively motile objects	DAP/r ≥ 3 & LIN ≤ 0.5	Olive
Particles	AOC > 4.5, BCF = 0 DCL < 10 A _{max} < 32 A _{max} > 76	Blue
Low speed	VAP < 80	Money green
High speed	VAP > 120	Aqua
Non-classified		Lime

Definition of the measuring conditions

Epididymal spermatozoa were collected after castration of domestic cats and sperm motility was measured in different chambers and at different incubation times (at 38°C) after release of the sperm cells from cauda epididymis into M199. Data of one male are shown.



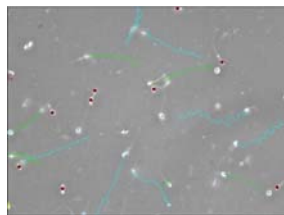
The highest and most stable amounts of motile spermatozoa with the highest velocity and linearity were revealed in the Makler chamber (Sefi-Medical Instr. Ltd, Israel; 10 µm) compared to Leja chamber slides (Leja Products B. V., The Netherlands; 20, 12, 10 µm). After 45 min at 38°C, a significant decline of several motility parameters was observed.

The established method is suited to investigate the influence of media and media components for culture or preservation on sperm motion characteristics.

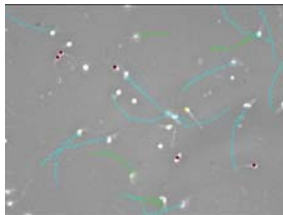
Means (bold) and standard deviations of motility parameters in different media (n = 5 males):

Makler ch. t = 5 min	MOI [%]	PROG [%]	DCL [µm]	DAP [µm]	DSL [µm]	VCL [mm/s]	VAP [µm/s]	VSL [µm/s]	ALH [µm]	BCF [Hz]	SIR	WOB	LIN	AOC [°]	Radius [µm]	A _{average} [µm ²]	A _{min} [µm ²]	A _{max} [µm ²]
TALP _{Cat}	60.3 14.8	46.9 17.1	64.2 10.2	50.8 10.5	45.1 10.3	151.8 24.8	120.5 24.2	107.8 23.7	2.15 0.47	42.8 2.4	0.87 0.04	0.79 0.05	0.70 0.06	36.7 6.1	1497 2454	43.5 7.2	22.2 7.2	53.5 8.1
M199	61.6 18.3	43.2 20.7	66.4 8.4	49.3 5.2	44.1 4.4	150.8 22.6	111.8 13.8	100.3 11.5	2.46 0.43	42.8 2.5	0.88 0.04	0.75 0.05	0.67 0.05	37.3 4.6	206 56	39.6 5.1	20.2 3.6	47.9 6.8
Test(G)	53.2 10.2	34.6 11.5	63.8 11.1	35.7 6.3	28.0 5.5	140.7 25.5	79.2 15.0	62.5 13.3	3.03 0.36	33.4 4.4	0.78 0.04	0.57 0.03	0.44 0.02	36.4 8.1	142 39	33.8 3.6	19.5 6.4	43.1 3.9
TestG	16.2 10.4	2.9 3.5	31.7 2.2	18.7 1.3	16.4 2.5	66.1 5.3	38.9 3.1	34.2 5.6	1.32 0.39	38.3 8.5	0.87 0.09	0.59 0.05	0.52 0.10	15.2 6.2	235 302	30.0 4.1	25.4 4.9	34.6 3.6

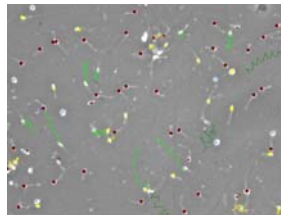
TALP_{Cat}



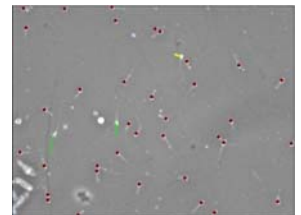
M199



Test(G) (TestG without glycerol)



TestG (contains 5% glycerol)



The best motility was detected in the TALP_{Cat} medium. The addition of glycerol to the Test freezing medium dramatically impaired the sperm motility, reflecting its severe toxicity to cat spermatozoa at higher temperature.