Superhydrophobic soot coatings-regulated cryostorage of human ejaculate enables equal post-thaw sperm survival independent of the freezing rate

Supporting information

Karekin D. Esmeryan[1]* and Todor A. Chaushev[2]

[1]Acoustoelectronics Laboratory, Georgi Nadjakov Institute of Solid State Physics, Bulgarian Academy of Sciences, 72, Tzarigradsko Chaussee Blvd., 1784 Sofia, Bulgaria

[2]Specialized Surgical Hospital "Doctor Malinov", 46, Gotse Delchev blvd., 1860 Sofia, Bulgaria

*Corresponding author: tel. +359 2 979 5811; e-mail: karekin_esmerian@abv.bg

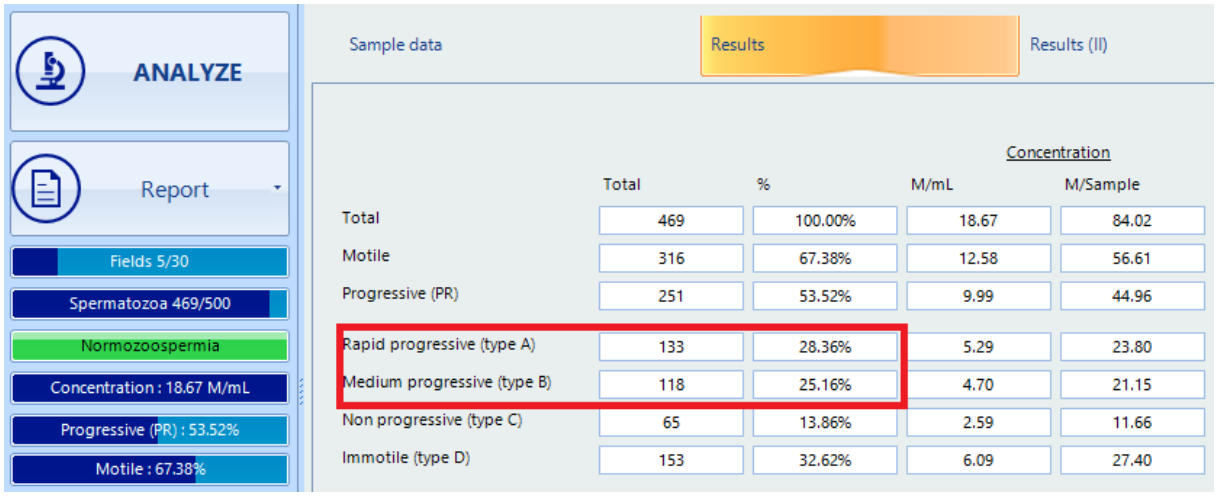


Figure S1: Progressive sperm motility of *Patient 3* prior to slow freezing.

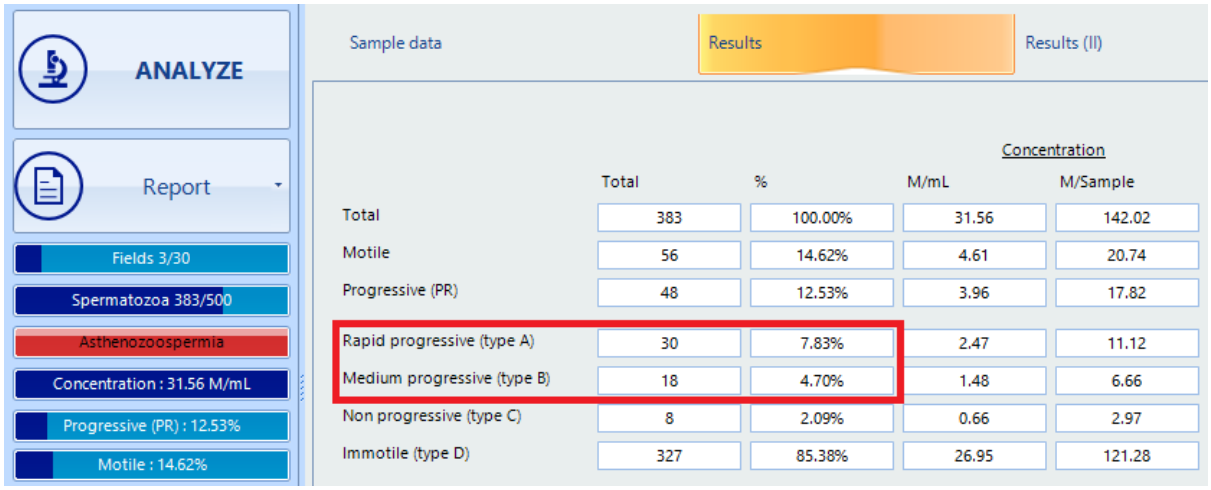


Figure S2: Progressive sperm motility of *Patient 3* after slow freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.

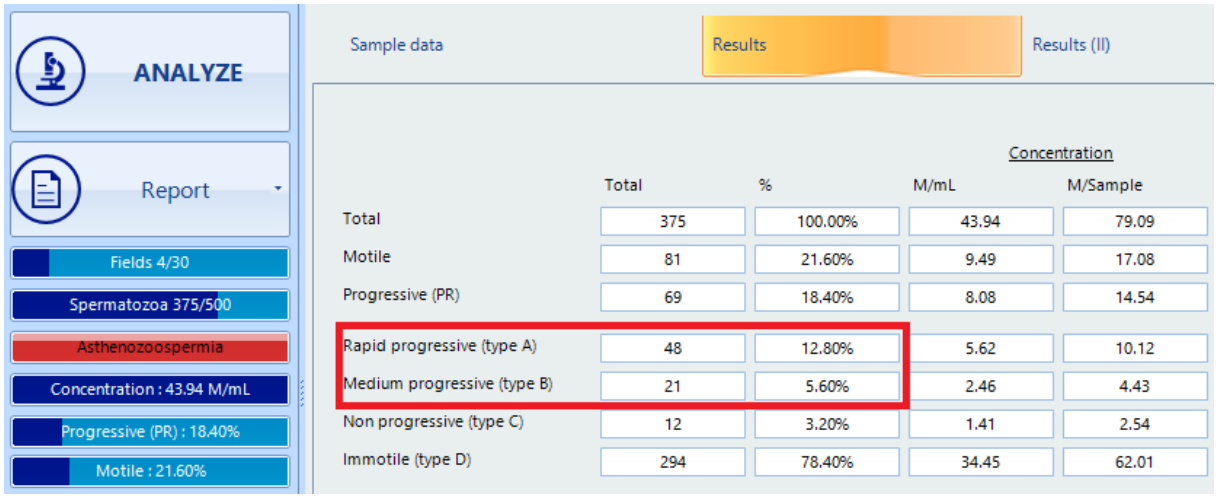
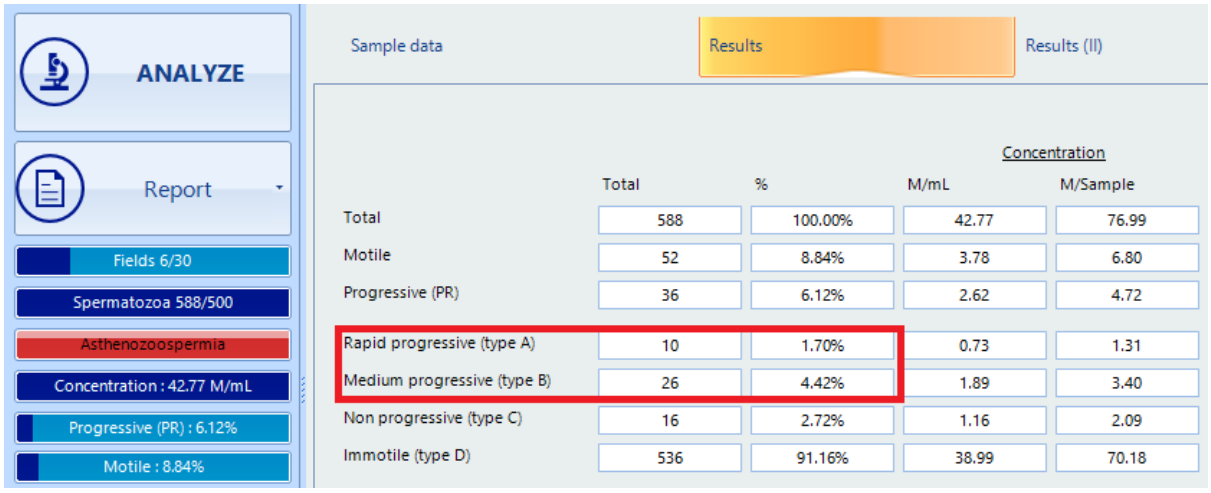
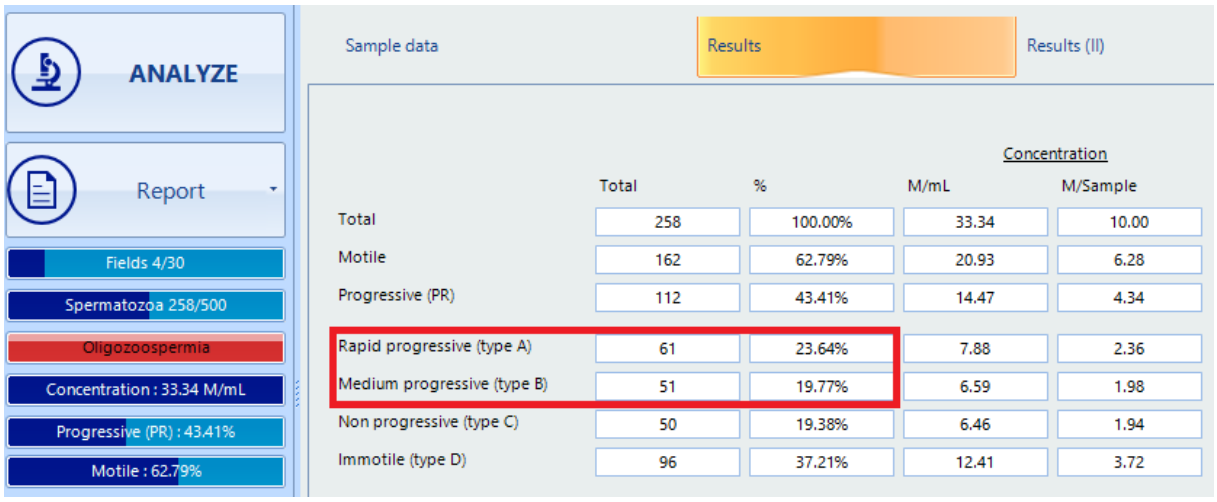


Figure S3: Progressive sperm motility of *Patient 3* prior to flash freezing.



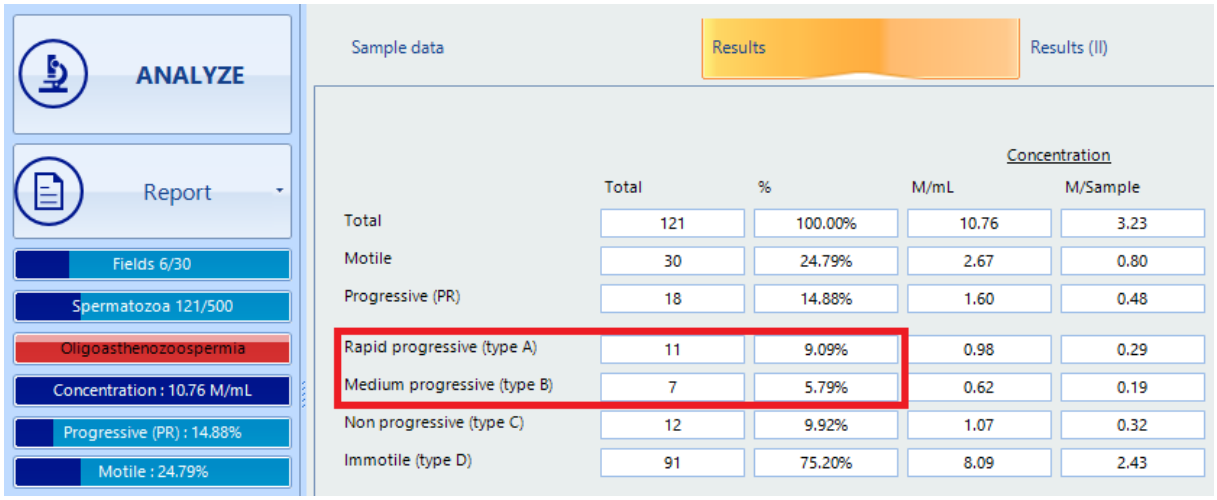
	Total	%	Concentration M/mL	Concentration M/Sample
Total	588	100.00%	42.77	76.99
Motile	52	8.84%	3.78	6.80
Progressive (PR)	36	6.12%	2.62	4.72
Rapid progressive (type A)	10	1.70%	0.73	1.31
Medium progressive (type B)	26	4.42%	1.89	3.40
Non progressive (type C)	16	2.72%	1.16	2.09
Immotile (type D)	536	91.16%	38.99	70.18

Figure S4: Progressive sperm motility of *Patient 3* after flash freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.



	Total	%	Concentration M/mL	Concentration M/Sample
Total	258	100.00%	33.34	10.00
Motile	162	62.79%	20.93	6.28
Progressive (PR)	112	43.41%	14.47	4.34
Rapid progressive (type A)	61	23.64%	7.88	2.36
Medium progressive (type B)	51	19.77%	6.59	1.98
Non progressive (type C)	50	19.38%	6.46	1.94
Immotile (type D)	96	37.21%	12.41	3.72

Figure S5: Progressive sperm motility of *Patient 4* prior to slow freezing.



	Total	%	Concentration M/mL	Concentration M/Sample
Total	121	100.00%	10.76	3.23
Motile	30	24.79%	2.67	0.80
Progressive (PR)	18	14.88%	1.60	0.48
Rapid progressive (type A)	11	9.09%	0.98	0.29
Medium progressive (type B)	7	5.79%	0.62	0.19
Non progressive (type C)	12	9.92%	1.07	0.32
Immotile (type D)	91	75.20%	8.09	2.43

Figure S6: Progressive sperm motility of *Patient 4* after slow freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

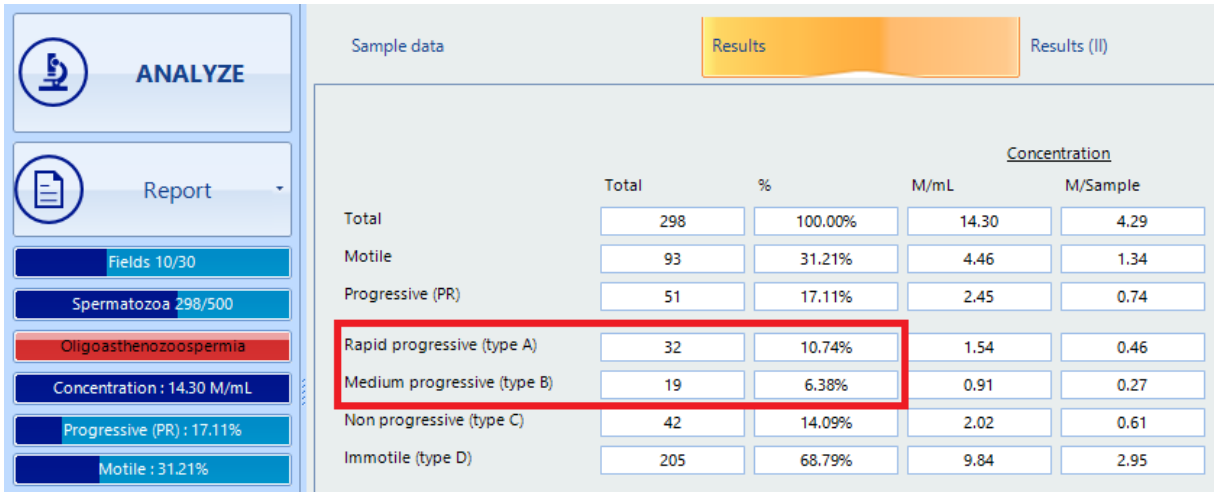


Figure S7: Progressive sperm motility of *Patient 4* after slow freezing using sample S4 accommodating a 50 µL semen-cryoprotectant droplet.

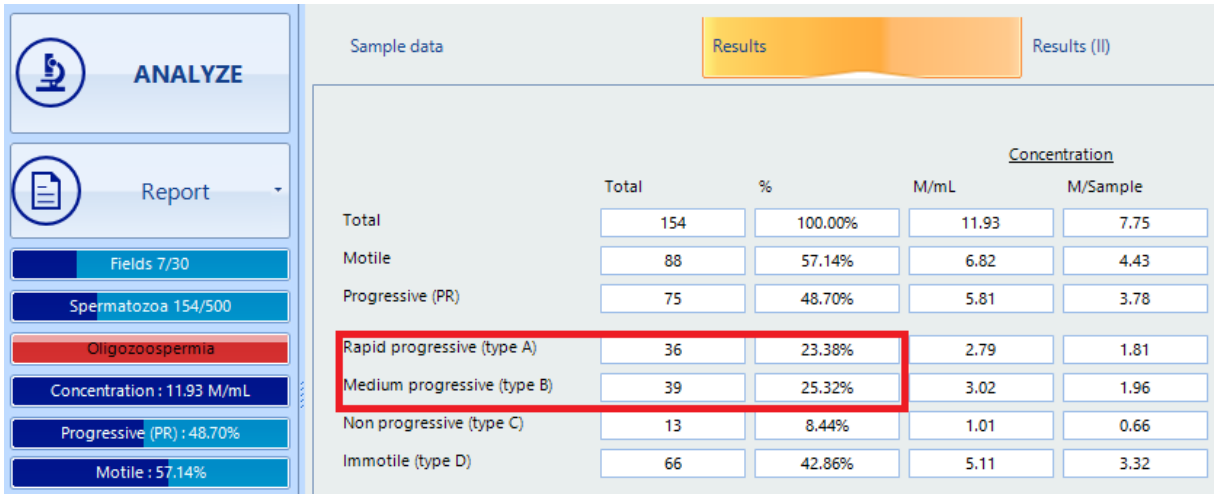


Figure S8: Progressive sperm motility of *Patient 4* prior to flash freezing.

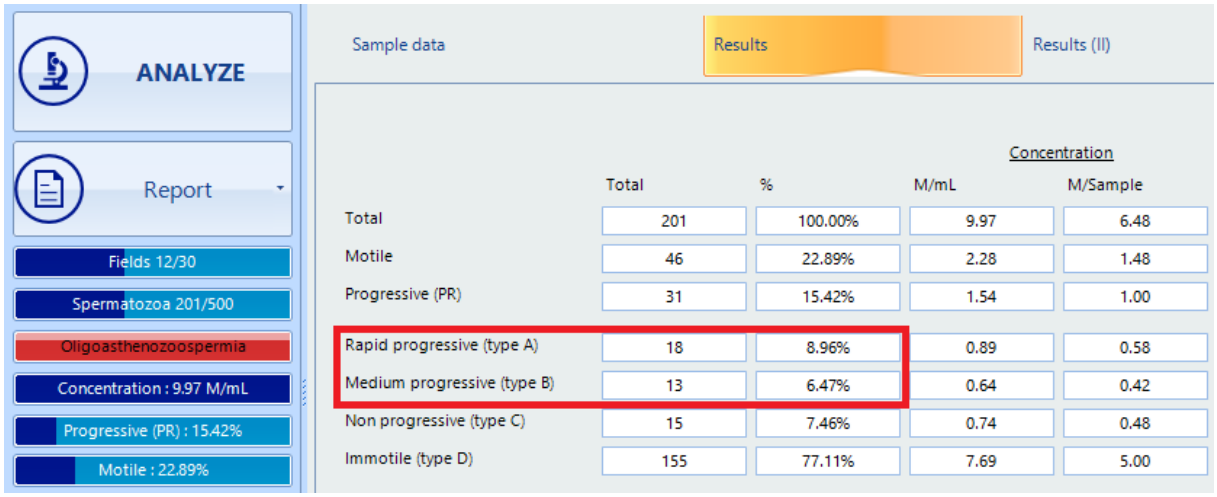


Figure S9: Progressive sperm motility of *Patient 4* after flash freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

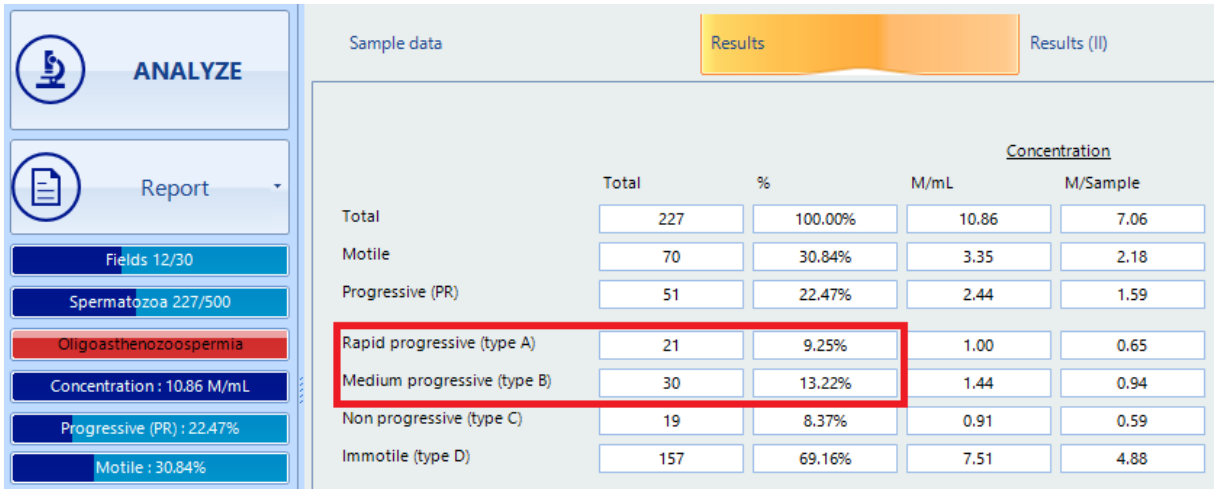


Figure S10: Progressive sperm motility of *Patient 4* after flash freezing using sample S4 accommodating a 50 µL semen-cryoprotectant droplet.

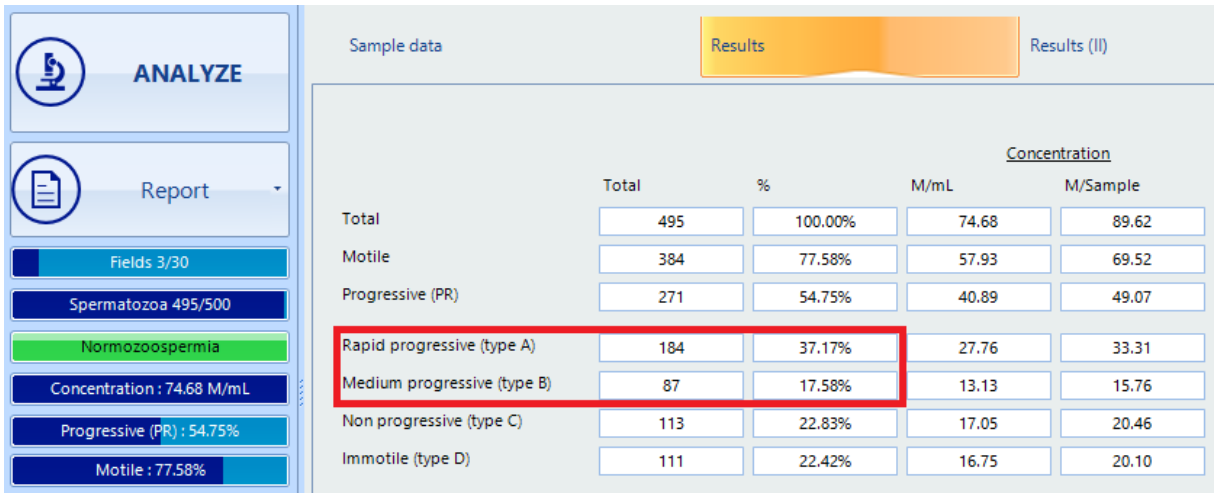


Figure S11: Progressive sperm motility of *Patient 5* prior to slow freezing.

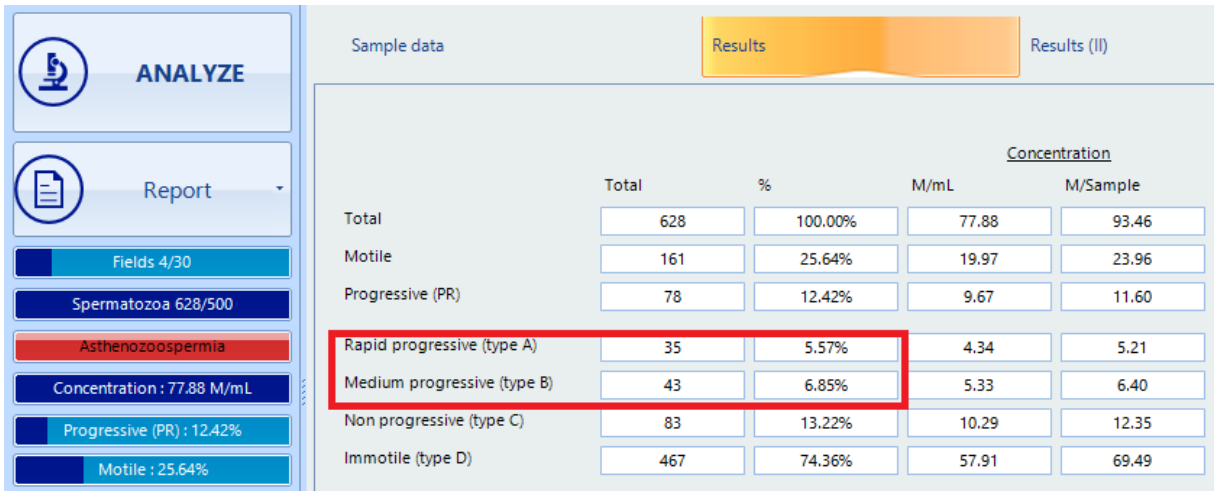


Figure S12: Progressive sperm motility of *Patient 5* after slow freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

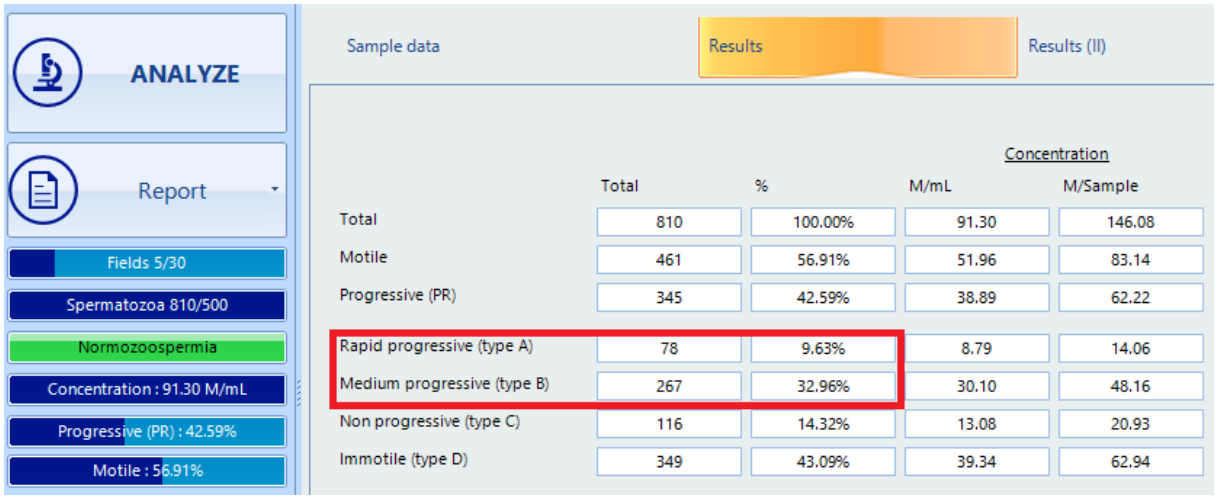


Figure S13: Progressive sperm motility of *Patient 5* prior to flash freezing.

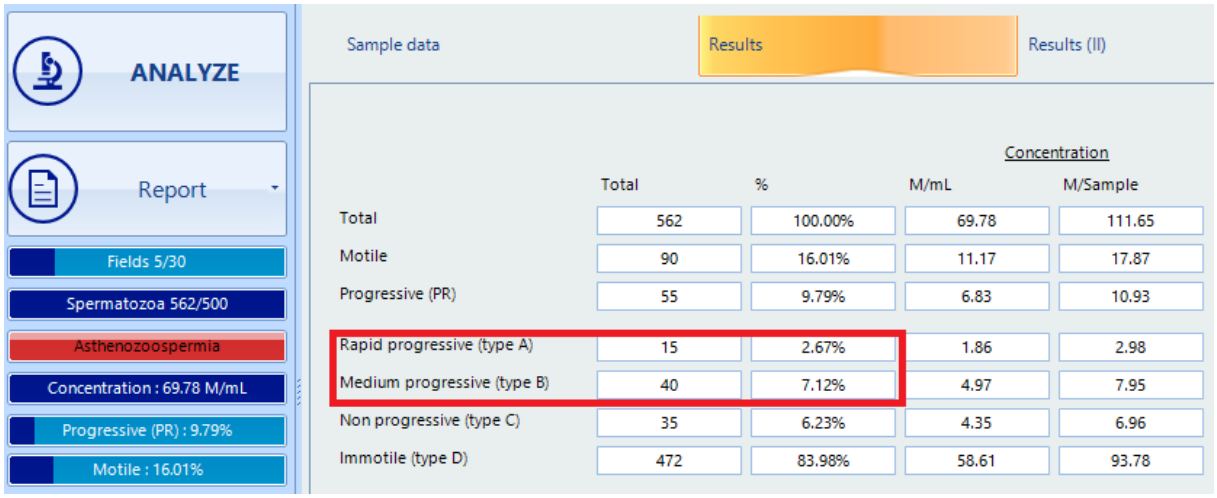


Figure S14: Progressive sperm motility of *Patient 5* after flash freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

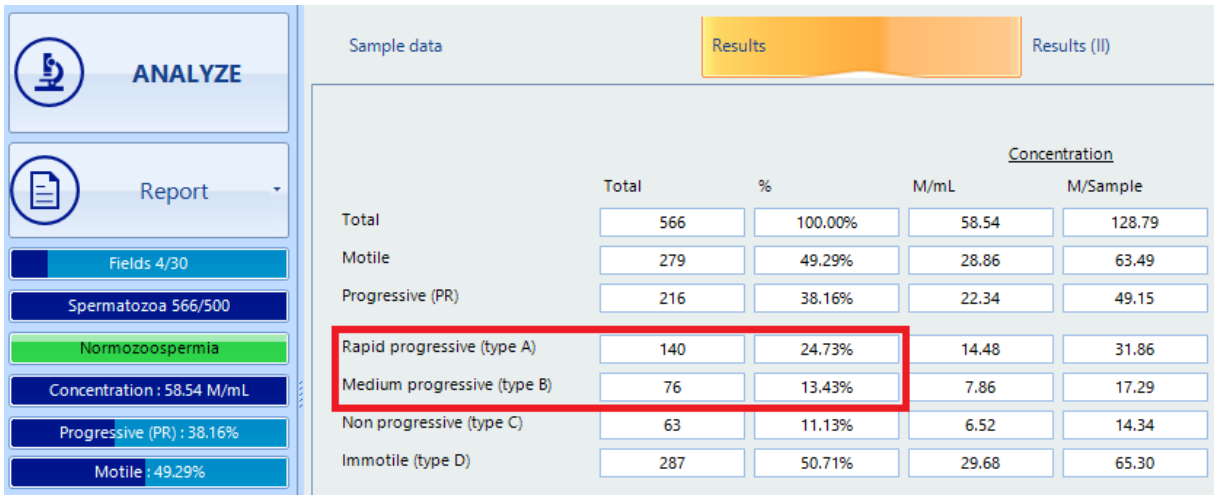


Figure S15: Progressive sperm motility of *Patient 7* prior to slow freezing.

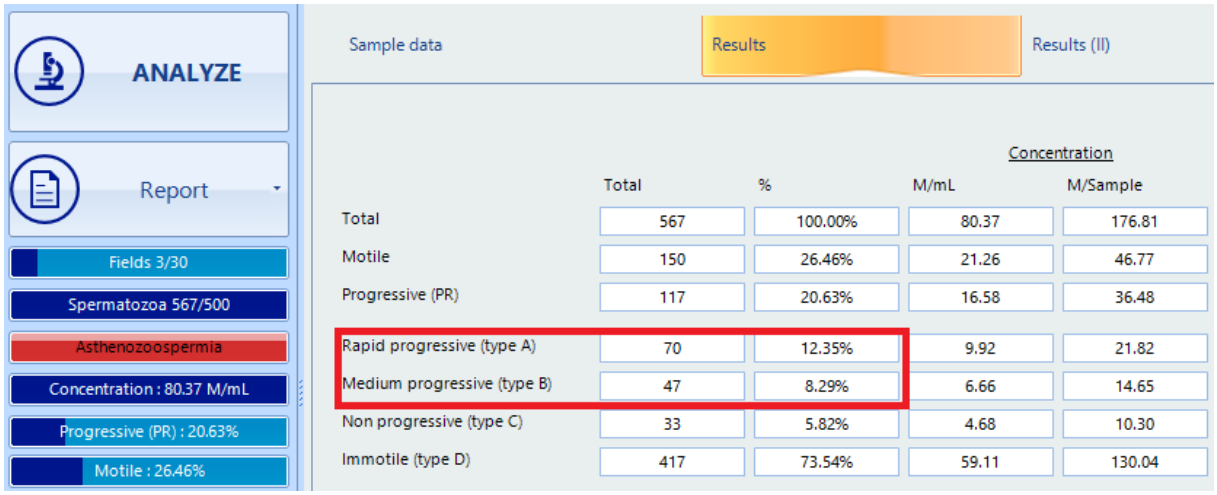


Figure S16: Progressive sperm motility of *Patient 7* after slow freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

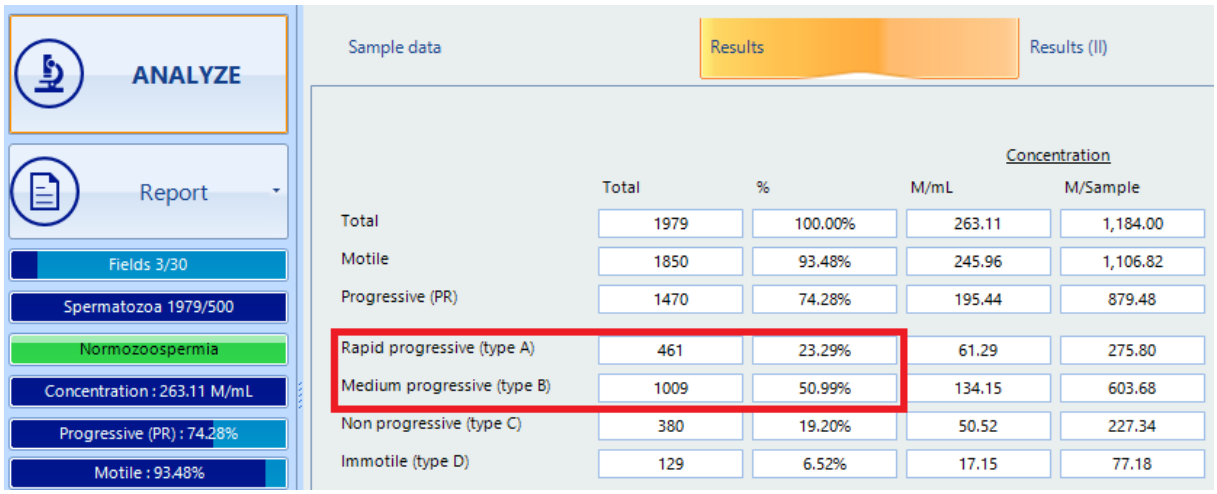


Figure S17: Progressive sperm motility of *Patient 7* prior to flash freezing.

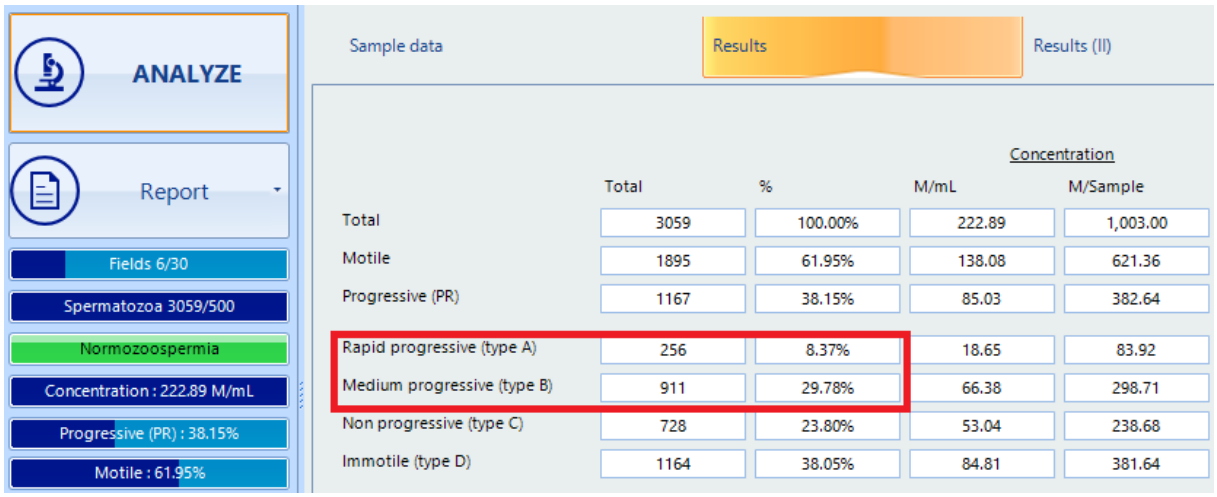


Figure S18: Progressive sperm motility of *Patient 7* after flash freezing using a cryovial filled with 400 µL semen-cryoprotectant suspension.

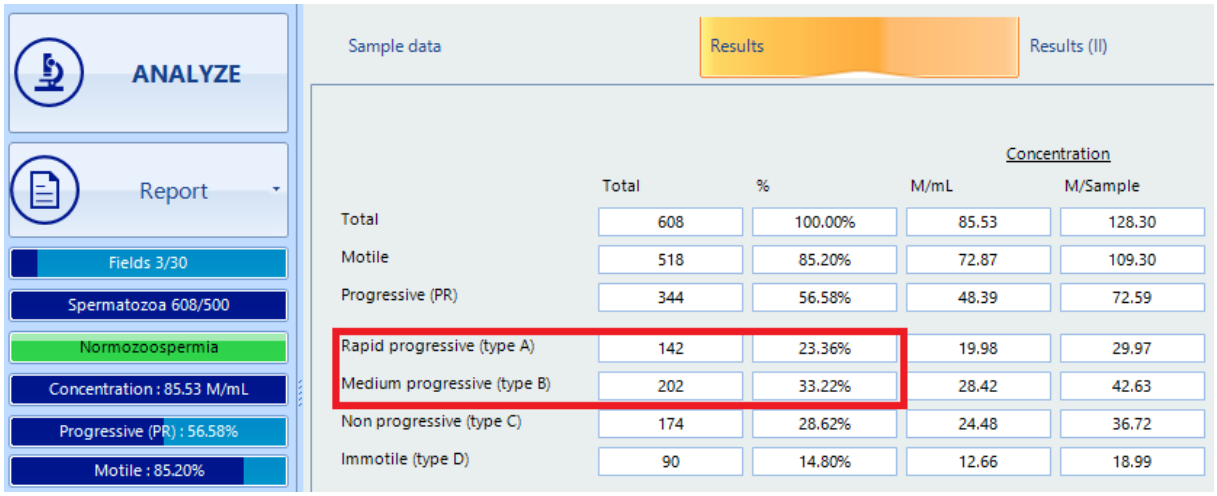


Figure S19: Progressive sperm motility of *Patient 8* prior to slow freezing.

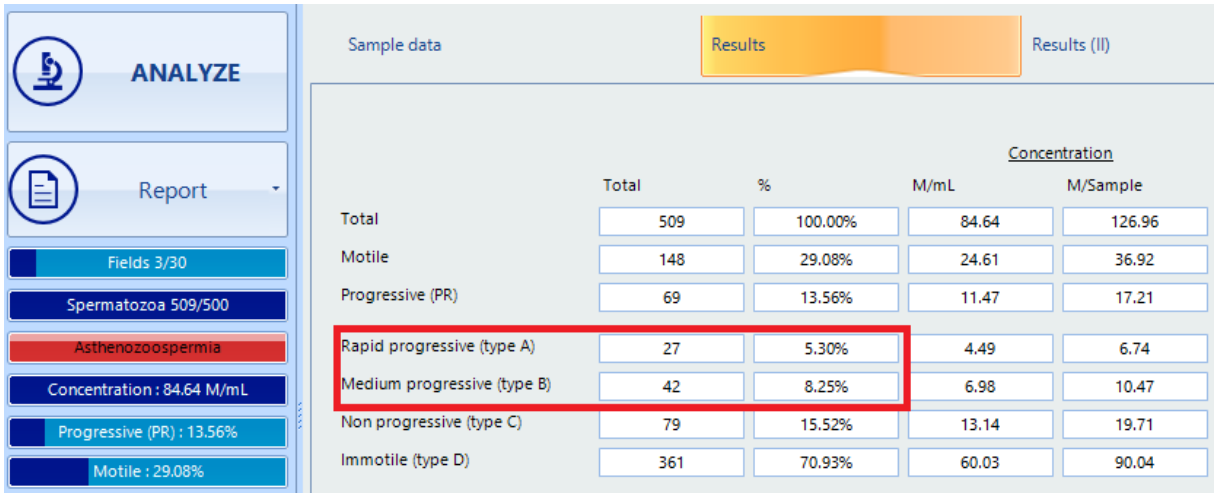


Figure S20: Progressive sperm motility of *Patient 8* after slow freezing using sample S1 accommodating a 50 µL semen-cryoprotectant droplet.

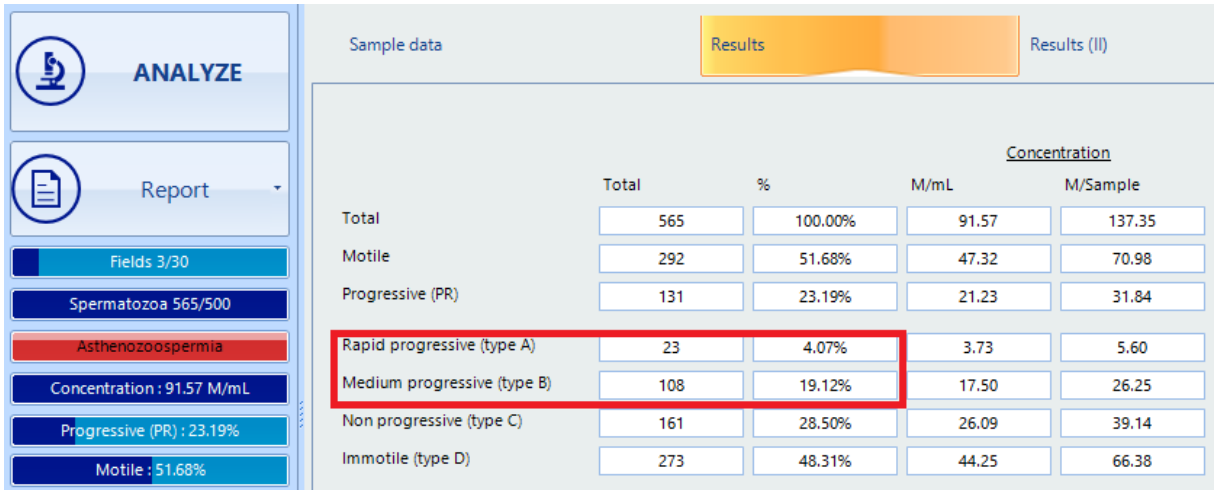


Figure S21: Progressive sperm motility of *Patient 8* after slow freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.

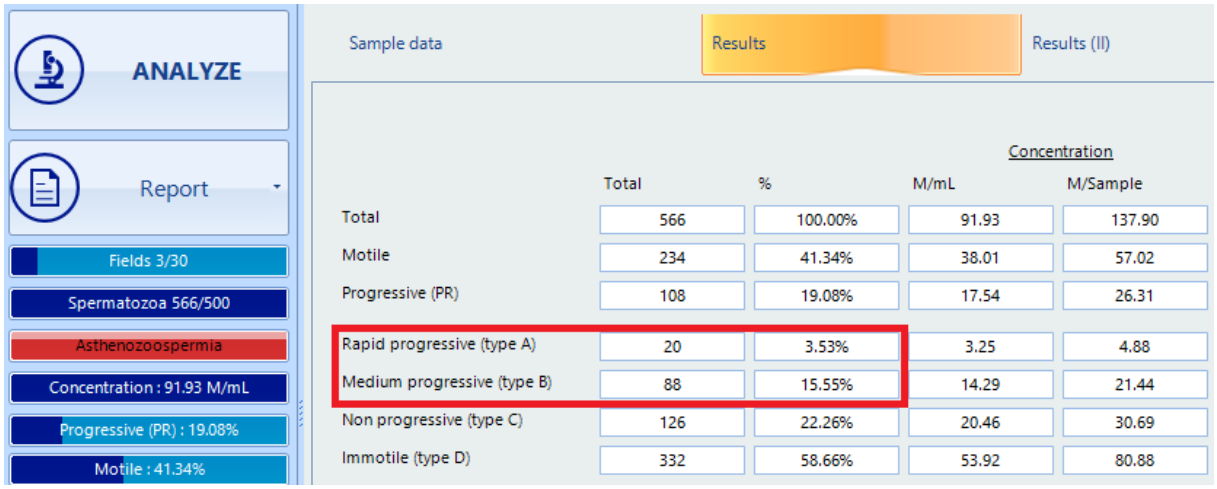


Figure S22: Progressive sperm motility of *Patient 8* after slow freezing using sample S4 accommodating a 50 µL semen-cryoprotectant droplet.

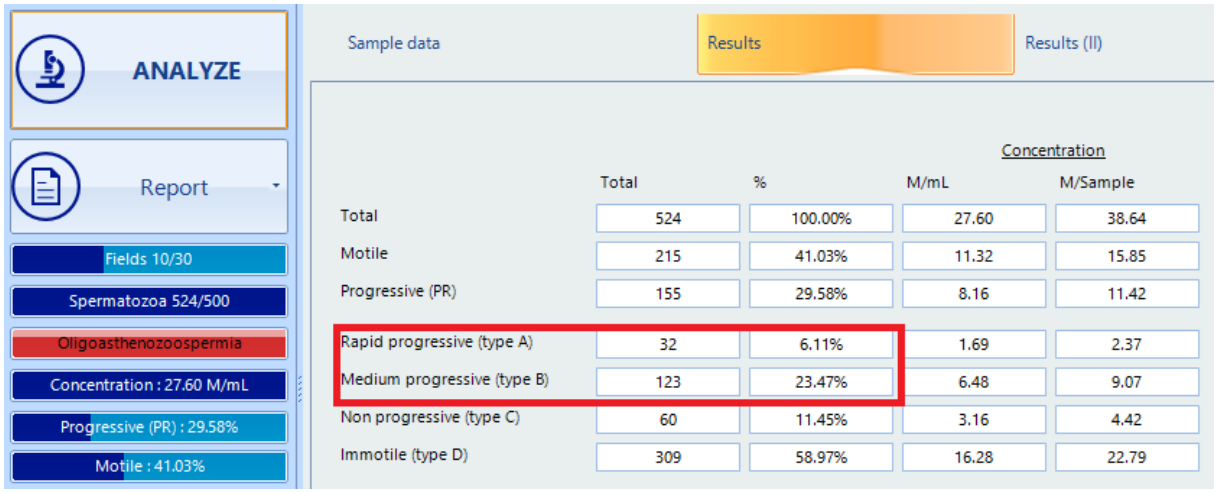


Figure S23: Progressive sperm motility of *Patient 8* prior to flash freezing.

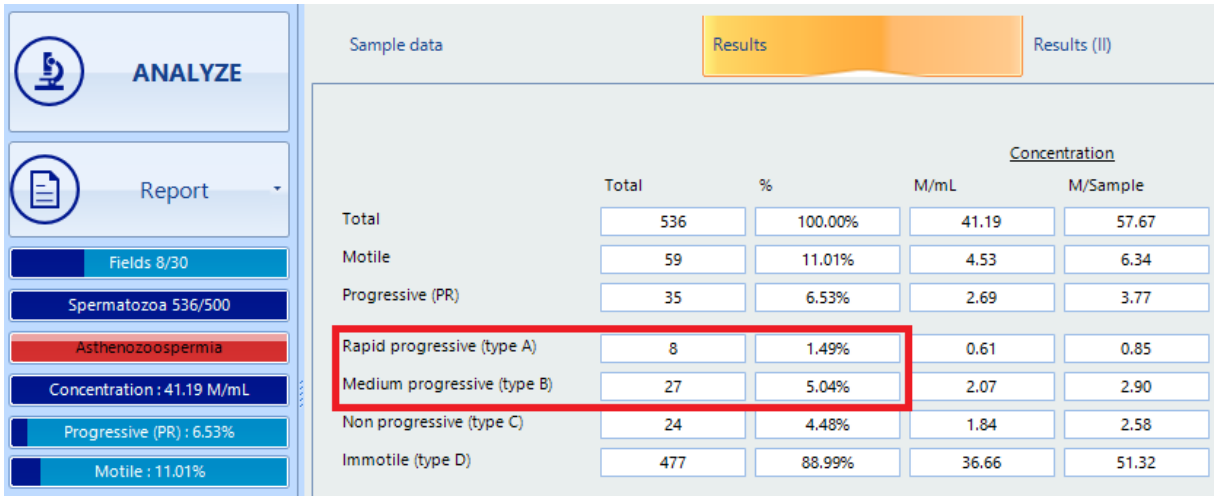


Figure S24: Progressive sperm motility of *Patient 8* after flash freezing using sample S1 accommodating a 50 µL semen-cryoprotectant droplet.

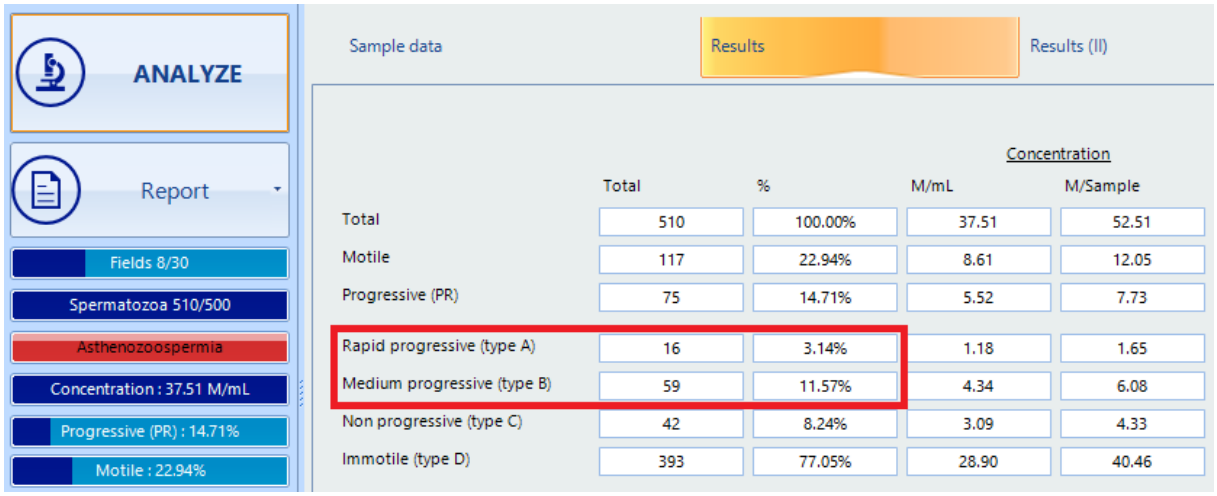


Figure S25: Progressive sperm motility of *Patient 8* after flash freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.

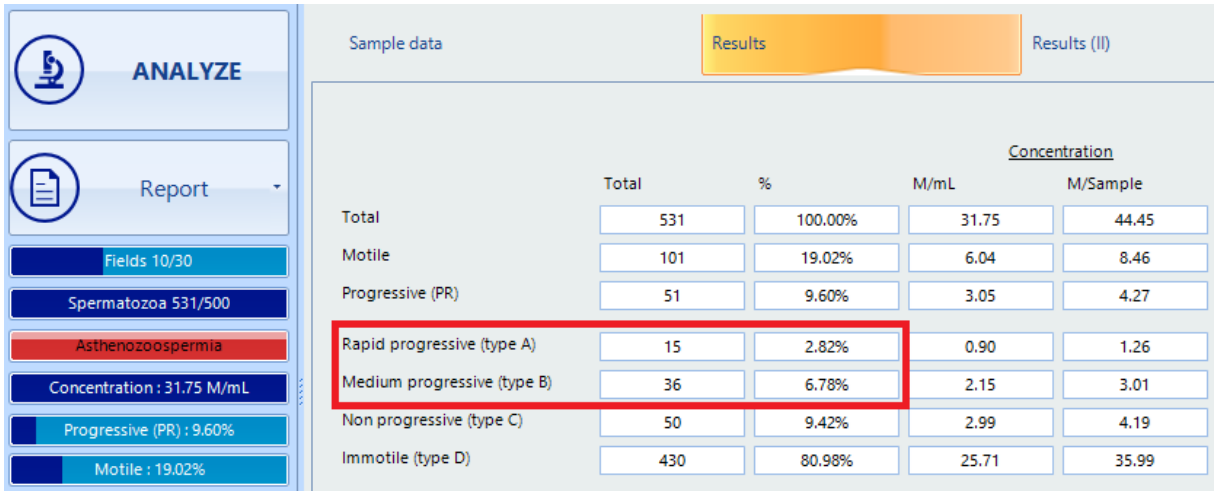


Figure S26: Progressive sperm motility of *Patient 8* after flash freezing using sample S4 accommodating a 50 µL semen-cryoprotectant droplet.

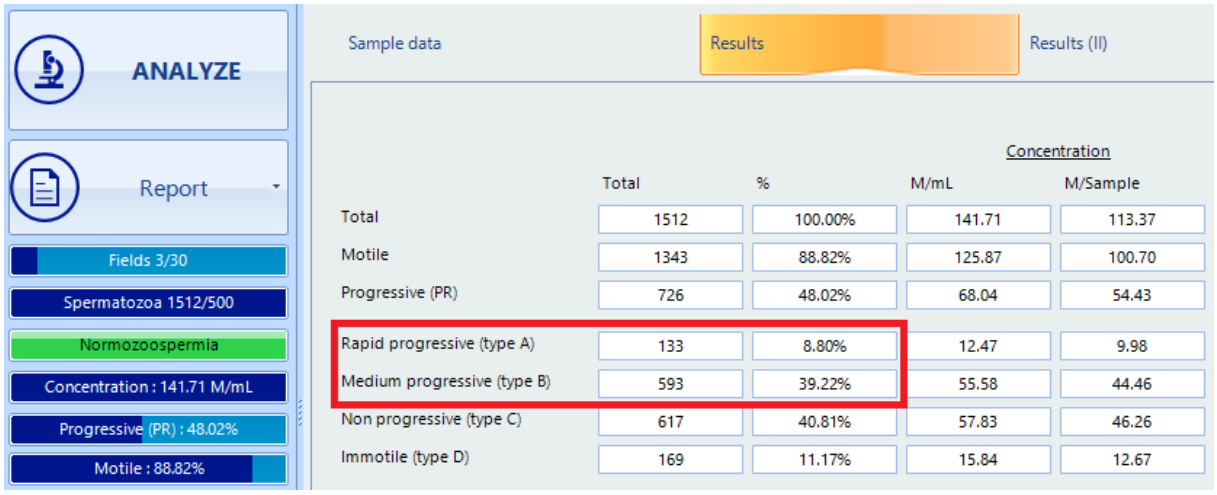


Figure S27: Progressive sperm motility of *Patient 9* prior to slow freezing.

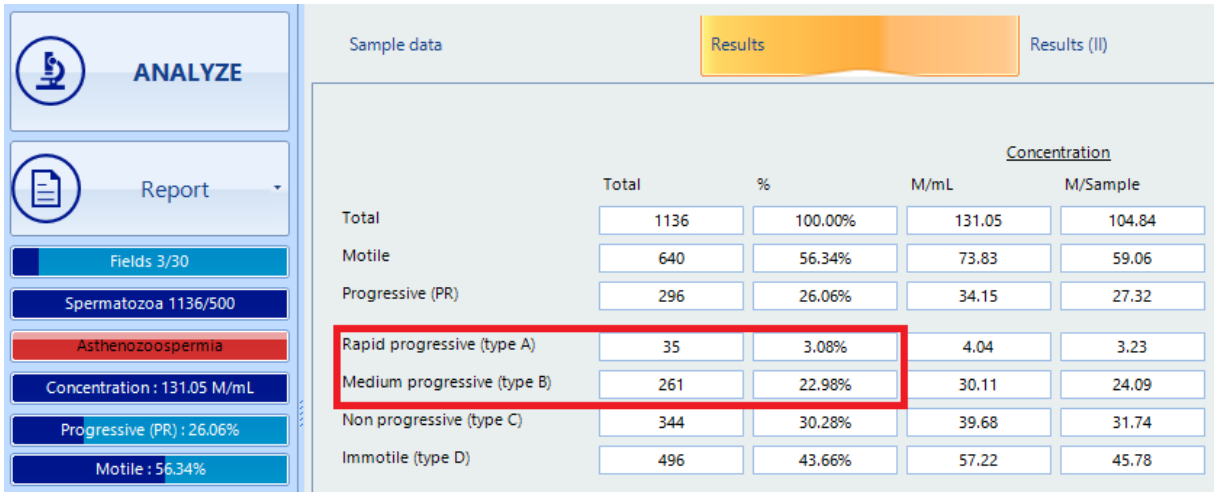


Figure S28: Progressive sperm motility of *Patient 9* after slow freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.

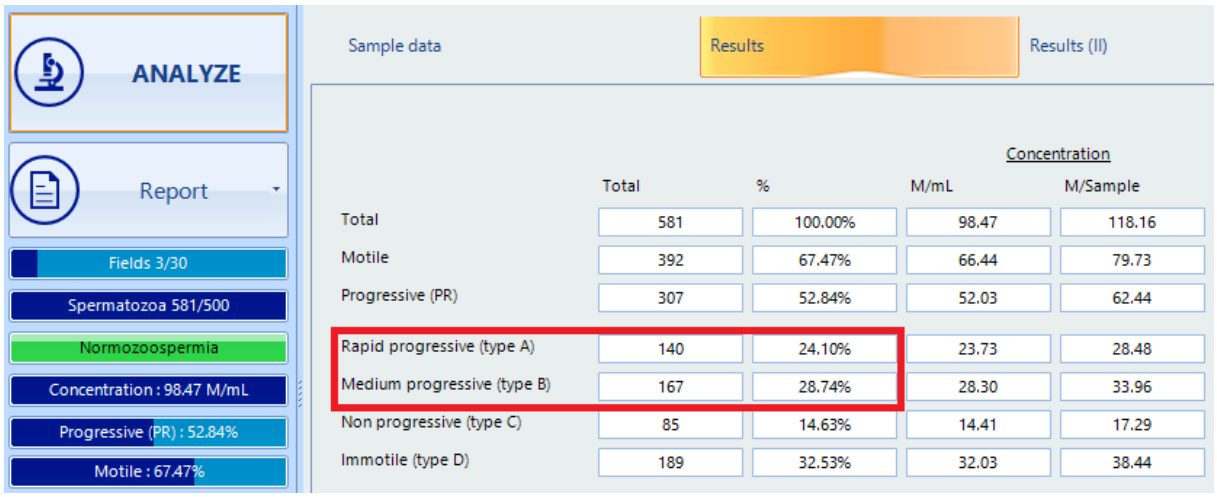


Figure S29: Progressive sperm motility of *Patient 9* prior to flash freezing.

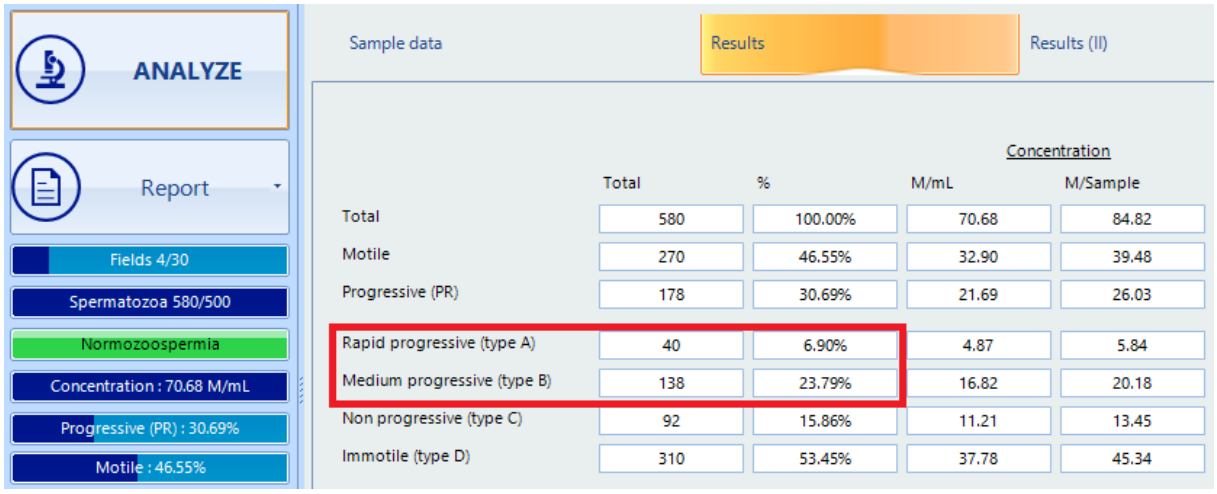


Figure S30: Progressive sperm motility of *Patient 9* after flash freezing using sample S3 accommodating a 50 µL semen-cryoprotectant droplet.

AN IMPORTANT NOTE: The reproductive health status of Patient 3 and Patient 8 differ substantially before the slow and flash freezing assays (i.e. Patient 3 –

normozoospermia/asthenozoospermia; Patient 8 – normozoospermia/oligoasthenozoospemia). This is not an inherent error nor scientific fraud! Simply, the sperm parameters are not constant and change on a daily basis (see Figure 1 in B.J.M. Mayorga-Torres, M. Camargo, A. Agarwal, S.S. du Plessis, A.P. Cadavid, W.D.C. Maya Influence of ejaculation frequency on seminal parameters Reprod. Biol. Endocrinol., 13 (2015), p. 47 - https://doi.org/10.1186/s12958-015-0045-9), and it should be mentioned that the two experimental sets are carried out within the period of two and a half years.