

**Longitudinal study on the short-term effects of increased alcohol and cigarette consumption in young men's seminal quality**

Joana Vieira Silva<sup>1+</sup>, Daniel Cruz<sup>1+</sup>, Mariana Gomes<sup>1+</sup>, Bárbara Regadas Correia<sup>1</sup>, Maria João Freitas<sup>1</sup>, Luís Sousa<sup>1</sup>, Vladimiro Silva<sup>2</sup>, Margarida Fardilha<sup>1\*</sup>

<sup>1</sup> Laboratory of Signal Transduction, Department of Medical Sciences, Institute of Biomedicine – iBiMED, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

<sup>2</sup> Ferticentro – Center for Fertility Studies, Praceta Prof. Robalo Cordeiro (Idealmed), Circular Externa de Coimbra, 3020-479 Coimbra, Portugal

+ These authors contributed equally to this work

\* Corresponding author: Margarida Fardilha, Departamento de Ciências Médicas, Agra do Crasto, Campus de Santiago, Universidade de Aveiro, 3810-193 Aveiro, Portugal. T: +351-918143947. E: [mfardilha@ua.pt](mailto:mfardilha@ua.pt)

Supplementary Table S1. Paired difference tests between the study time-points (TPs).

	N	Paired Samples	p value (2-tailed)	Effect size Cohen'd
Sexual abstinence	32	TP1 vs TP2	0.88 <sup>a</sup>	0.0
		TP1 vs TP3	0.25 <sup>a</sup>	0.3
		TP2 vs TP3	0.29 <sup>a</sup>	0.3
Volume	36	TP1 vs TP2	0.02 <sup>a</sup>	0.4
		TP1 vs TP3	0.00 <sup>a</sup>	0.8
		TP2 vs TP3	0.04 <sup>a</sup>	0.3
Concentration	36	TP1 vs TP2	0.21 <sup>a</sup>	0.2
		TP1 vs TP3	0.04 <sup>a</sup>	0.3
		TP2 vs TP3	0.57 <sup>a</sup>	0.1
Total number of spermatozoa	36	TP1 vs TP2	0.05 <sup>a</sup>	0.3
		TP1 vs TP3	0.00 <sup>a</sup>	0.8
		TP2 vs TP3	0.10 <sup>a</sup>	0.3
Progressive motility	36	TP1 vs TP2	0.01 <sup>a</sup>	0.4
		TP1 vs TP3	0.07 <sup>a</sup>	0.4
		TP2 vs TP3	0.52 <sup>a</sup>	0.1
Non-progressive motility	36	TP1 vs TP2	0.04 <sup>a</sup>	0.5
		TP1 vs TP3	0.002 <sup>a</sup>	0.8
		TP2 vs TP3	0.54 <sup>a</sup>	0.1
Immotile	36	TP1 vs TP2	0.05 <sup>a</sup>	0.3
		TP1 vs TP3	0.77 <sup>a</sup>	0.1
		TP2 vs TP3	0.21 <sup>a</sup>	0.2
Normal morphology	36	TP1 vs TP2	0.00 <sup>a</sup>	0.4
		TP1 vs TP3	0.00 <sup>a</sup>	0.5
		TP2 vs TP3	1.00 <sup>a</sup>	0.0
Head defects	36	TP1 vs TP2	0.00 <sup>a</sup>	0.6
		TP1 vs TP3	0.22 <sup>a</sup>	0.1
		TP2 vs TP3	0.001 <sup>a</sup>	0.5
Midpiece defects	36	TP1 vs TP2	0.00 <sup>a</sup>	0.6
		TP1 vs TP3	0.00 <sup>a</sup>	0.5
		TP2 vs TP3	0.20 <sup>a</sup>	0.1
Tail defects	36	TP1 vs TP2	0.28 <sup>a</sup>	0.2
		TP1 vs TP3	0.08 <sup>a</sup>	0.3
		TP2 vs TP3	0.30 <sup>a</sup>	0.1
Alcohol consumption	36	TP1 vs TP2	0.00 <sup>a</sup>	1.1
		TP1 vs TP3	0.48 <sup>a</sup>	0.1
		TP2 vs TP3	0.00 <sup>a</sup>	1.1
Nicotine consumption	36	TP1 vs TP2	0.00 <sup>a</sup>	0.3
		TP1 vs TP3	0.21 <sup>a</sup>	0.1
		TP2 vs TP3	0.08 <sup>a</sup>	0.2
Total antioxidant capacity (TAS)	22	TP1 vs TP2	0.07 <sup>b</sup>	0.3
		TP1 vs TP3	0.21 <sup>a</sup>	0.3
		TP2 vs TP3	1.00 <sup>b</sup>	0.1
3- Nitrotyrosine (3-NT)	24	TP1 vs TP2	0.24 <sup>b</sup>	0.2
		TP1 vs TP3	0.08 <sup>b</sup>	0.1
		TP2 vs TP3	0.01 <sup>b</sup>	0.3
Superoxide dismutase 1 (SOD1)	23	TP1 vs TP2	0.68 <sup>c</sup>	0.1
		TP1 vs TP3	0.68 <sup>c</sup>	0.4
		TP2 vs TP3	1.00 <sup>c</sup>	0.4
Glutathione peroxidase 4 (GPx4)	20	TP1 vs TP2	0.41 <sup>c</sup>	0.2
		TP1 vs TP3	0.84 <sup>c</sup>	0.0
		TP2 vs TP3	0.84 <sup>c</sup>	0.2
Carbonyl groups (CG)	12	TP1 vs TP2	0.39 <sup>a</sup>	0.1
		TP1 vs TP3	0.41 <sup>a</sup>	0.3

		TP2 vs TP3	1.00 <sup>c</sup> .	0.5
Citric acid	12	TP1 vs TP2	0.21 <sup>b</sup> .	0.2
		TP1 vs TP3	0.03 <sup>a</sup> .	1.0
		TP2 vs TP3	0.77 <sup>c</sup> .	0.5
		TP1 vs TP2	0.76 <sup>a</sup> .	0.1
Fructose	12	TP1 vs TP3	0.02 <sup>a</sup> .	0.5
		TP2 vs TP3	0.04 <sup>a</sup> .	0.4
		TP1 vs TP2	0.28 <sup>a</sup> .	0.2
Neutral alfa-glucosidase	12	TP1 vs TP3	0.02 <sup>a</sup> .	0.7
		TP2 vs TP3	0.04 <sup>c</sup> .	0.5

a. T-test; b. Wilcoxon Signed Ranks test (asymp. sig.); c. Sign test (exact sig.) - Binomial distribution used.