

Supplementary table 1 Overview of different combinations of 2D culture methodology and signaling factors on best outcomes per study

AUTHORS	DATE	START	CULTURE METHODOLOGY	TIME IN CULTURE	CELL REORGANIZATION	SPERMATOGENESIS	BASAL MEDIUM	SERUM	GONADOTROPHINS	TGFβ-SUPERFAMILY	GROWTH FACTORS	VITAMIN A	OTHER INGREDIENTS
Tung and Fritz	1980	20 dpp rat SCs and PTMCs	Non-coated	90 days	Mounds	N/A	MEM	10% FCS	100 ng/ml FSH	-	-	-	-
Tung and Fritz	1984	20 dpp rat SC	ST biomatrix	3 weeks	Cord-like structures	N/A	MEM	-	-	-	-	-	-
Tung and Fritz	1986	20 dpp rat SCs and PTMCs	Collagen I, IV, LN or fibronectin	16 days	Cord-like structures	N/A	MEM	10% FCS	-	-	-	-	-
Tung and Fritz	1987	20 dpp rat SCs and PTMCs	Non-coated	18 days	Cord-like structures	N/A	MEM	10% FCS	-	-	-	-	-
Schlatt et al.	1996	7 dpp rat SCs and PTMCs	LN	7 days	Mounds	N/A	DMEM	-	200 ng/ml FSH	-	-	-	-
Van der Wee and Hofmann	1999	10 dpp immortalized mouse SCs	GFR-Matrigel	5 days	Tubule-like structures	N/A	DMEM	10% FCS	-	-	-	-	1 mM sodium pyruvate 2 mM glutamine 100 uM NEAA
Mackay et al.	1999	Embryonic mouse SCs	Matrigel	7 days	Cord-like structures	N/A	DMEM	10% FCS	-	-	-	-	-
Willerton et al.	2004	Embryonic mouse SCs	Matrigel	7 days	Cord-like structures	N/A	DMEM	10% FCS	-	-	10 ng/ml FGF9	-	-
El Ramy et al.	2005	20 dpp rat SCs and PTMCs	Non-coated	5 days	Cord-like structures	N/A	DMEM/F12	10% FCS	-	-	50 ng/ml FGF9 50 ng/ml FGF2 50 ng/ml PDGF	-	-
Mincheva et al.	2018	Adult human TCs	Glass	3 weeks	Cord-like structures	N/A	DMEM	-	-	-	-	-	1% NEAA
Von Kopylow et al.	2018	Adult human TCs	Non-coated	3 months	Cord-like structures	ES, SG loss	DMEM	15% KSR	-	100 ng/ml GDNF	40 ng/ml EGF 20 ng/ml FGF1 20 ng/ml FGF2 20 ng/ml FGF9 100 ng/ml GDNF 10 ng/ml IGF	-	0.1 mM 2-Mercaptoethanol 2 mM glutamine 0.1 mM NEAA

dpp; days postpartum; ES, elongates spermatids; GFR, growth factor reduced; LN, laminin; NEAA, non-essential amino-acids; PTMC, peritubular myoid cells; SC, Sertoli cells; SG, spermatogonia; ST, seminiferous tubule; TC, testicular cells