Table S1. Literature review of mouse embryo culture studies using variations from 100% rat serum (RS *) as culture medium

	Paper	Stages cultured	Brief culture period < 18 h?	Culture medium variations			Comparisons of culture success (excluding effects of experimental treatments)
				Serum-free	Rat serum dilution	Non-rat serum	
1.	Miyazawa H., Yamamoto M., Yamaguchi Y., and Miura M. (2018). Mammalian embryos show metabolic plasticity toward the surrounding environment during neural tube closure**.** *Genes Cells* **23:** 794-802.	E7.5; E8.5; E9.5	Yes (1.5 h)		RS 1:1 with OPTI-MEM		No
2.	Ivanovitch K., Temino S., and Torres M. (2017). Live imaging of heart tube development in mouse reveals alternating phases of cardiac differentiation and morphogenesis. *Elife* **6**: e30668.	E7.5	Yes (3-13 h)		RS 1:1 with DMEM + 1% N-2 neuronal growth suppl + 1% B-27 suppl		No
3.	Huang Y., Wilkie R., and Wilson V. (2015). Methods for precisely localized transfer of cells or DNA into early postimplantation mouse embryos**.** *J. Vis. Exp.* **106:** e53295.	E7.5; E8.5; E9.5	No		RS 1:1 or 1:3 with Glasgow (G)MEM, 1% Non-Essential Amino Acids, L-glutamine, Na pyruvate		No
4.	Drakou K. and Georgiades P. (2015). A serum-free and defined medium for the culture of mammalian postimplantation embryos**.** *Biochem. Biophys. Res. Commun.* **468:** 813-819.	E5.5-E7.5	No	N2B27: serum-free medium			Yes - against in vivo embryo staging system

5.	Kimura-Yoshida C., Mochida K., Ellwanger K., Niehrs C., and Matsuo I. (2015). Fate specification of neural plate border by canonical Wnt signaling and Grhl3 is crucial for neural tube closure. *EBioMedicine.* **2:** 513-527.	E8.5-E9.0	Yes (6 h)			FBS 1:4 with DMEM	No
6.	Kalaskar V.K. and Lauderdale J.D. (2014). Mouse embryonic development in a serum-free whole embryo culture system**.** *J. Vis. Exp.* **85:** e50803.	E10.5-E11.5	No	Knockout serum-free medium			Yes - against in vivo developed embryos
7.	Gordon J., Moore B.A., Blackburn C.C., and Manley N.R. (2014). Serum-free culture of mid-gestation mouse embryos: a tool for the study of endoderm-derived organs**.** *Methods Mol. Biol.* **1092:** 183-194.	E10.5-E11.5	No	Knockout serum-free medium			No
8.	Glanville-Jones H.C., Woo N., and Arkell R.M. (2013). Successful whole embryo culture with commercially available reagents**.** *Int. J Dev. Biol.* **57:** 61-67.	E7.5-E8.5; E8.5-E9.5	No		RS (commercial **) 1:1 with DMEM or Ham's F12 with N-2 growth supplement		Yes - between two diluent media
9.	Mahaffey J.P., Grego-Bessa J., Liem K.F., Jr., and Anderson K.V. (2013). Cofilin and Vangl2 cooperate in the initiation of planar cell polarity in the mouse embryo**.** *Development* **140:** 1262-1271.	E7.25-E8.0	No		RS 1:1 with DMEM/F12		No
10.	Zeeb M., Axnick J., Planas-Paz L., Hartmann T., Strilic B., and Lammert E. (2012). Pharmacological manipulation of blood and lymphatic vascularization in ex vivo-cultured mouse embryos. *Nat. Protoc.* **7:** 1970-1982.	E8.0; E8.75; E11.5; E15.5	Yes (12 h, E8.0; 8 h, E8.75; 5 h, E11.5; 0.5 h, E15.5)	M16 + HEPES for E8.0 and E8.75		FBS 1:4 with DMEM for E11.5 and E15.5	No

11.	Trichas G., Smith A.M., White N., Wilkins V., Watanabe T., Moore A., Joyce B., Sugnaseelan J., Rodriguez T.A., Kay D. et al. (2012). Multi-cellular rosettes in the mouse visceral endoderm facilitate the ordered migration of anterior visceral endoderm cells**.** *PLoS. Biol.* **10:** e1001256.	E5.75	Yes (8 h)			MS 1:1 with CMRL containing glutamine	No
12.	Shinohara K., Kawasumi A., Takamatsu A., Yoshiba S., Botilde Y., Motoyama N., Reith W., Durand B., Shiratori H., and Hamada H. (2012). Two rotating cilia in the node cavity are sufficient to break left-right symmetry in the mouse embryo**.** *Nat. Commun.* **3:** 622.	E8.5-9.5	No		RS 3:1 with DMEM		No
13.	Gray J. and Ross M.E. (2011). Neural tube closure in mouse whole embryo culture**.** *J. Vis. Exp.* **56:** e3132.	E8.5-10	No		RS 1:1 with DMEM		No
14.	Tung E.W. and Winn L.M. (2011). Valproic acid increases formation of reactive oxygen species and induces apoptosis in postimplantation embryos: a role for oxidative stress in valproic acid-induced neural tube defects**.** *Mol. Pharmacol.* **80:** 979-987.	E8.5-9.5	No		RS 9:1 with Hanks' balanced salt solution (HBSS)		No
15.	Mao J., McKean D.M., Warrier S., Corbin J.G., Niswander L., and Zohn I.E. (2010). The iron exporter ferroportin 1 is essential for development of the mouse embryo, forebrain patterning and neural tube closure**.** *Development* **137:** 3079-3088.	E7.5-8.5	No		RS 1:1 with DMEM +HEPES, pen/strep		No

16.	Migeotte I., Omelchenko T., Hall A., and Anderson K.V. (2010). Rac1-dependent collective cell migration is required for specification of the anterior-posterior body axis of the mouse**.** *PLoS. Biol.* **8:** e1000442.	E5.5-E6.5	No		RS 1:1 DMEM/F12		No
17.	Pyrgaki C., Trainor P., Hadjantonakis A.K., and Niswander L. (2010). Dynamic imaging of mammalian neural tube closure**.** *Dev. Biol* **344:** 941-947.	E8.5-9.5	No		RS 2:1 DMEM/F12		No
18.	Miura S. and Mishina Y. (2003). Whole-embryo culture of E5.5 mouse embryos: Development to the gastrulation stage**.** *Genesis* **37:** 38-43.	E5.5-E7.5	No		RS 100%, 3:1 or 1:1 with DMEM		Yes - between 50%, 75% and 100% RS
19.	Soleman D., Cornel L., Little S.A., and Mirkes P.E. (2003). Teratogen-induced activation of the mitochondrial apoptotic pathway in the yolk sac of day 9 mouse embryos**.** *Birth Defects Research (Part A)* **67:** 98-107.	E8.5-E9	Yes (5 h and 10 h)		RS 4:1 with HBSS		No
20.	Torchinsky A., Brokhman I., Shepshelovich J., Orenstein H., Savion S., Zaslavsky Z., Koifman M., Dierenfeld H., Fein A., and Toder V. (2003). Increased TNF-alpha expression in cultured mouse embryos exposed to teratogenic concentrations of glucose**.** *Reproduction.* **125:** 527-534.	E8.5-E9.5	No			FBS 3:1 with DMEM + pen/ strep	Yes - against in vivo developed embryos
21.	Moore-Scott B.A., Gordon J., Blackburn C.C., Condie B.G., and Manley N.R. (2003). New serum-free in vitro culture technique for midgestation mouse embryos**.** *Genesis* **35:** 164-168.	E10.5-11.5	No	Knock Out DMEM + 10–20% KSR			Yes - against in vivo developed embryos

22.	Jones E.A., Crotty D., Kulesa P.M., Waters C.W., Baron M.H., Fraser S.E., and Dickinson M.E. (2002). Dynamic in vivo imaging of postimplantation mammalian embryos using whole embryo culture**.** *Genesis* **34:** 228-235.	E8.5-9.5; E9.5-10.5	No		RS 1:1 with DMEM +HEPES, pen/strep		Yes - against in vivo embryos and staging system
23.	Nonaka S., Shiratori H., Saijoh Y., and Hamada H. (2002). Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature* **418***:* 96-99.	E7.5-E9.5	No		RS 1:1 with DMEM + pen/strep		No
24.	Penkov L.I., Platonov E.S., and New D.A. (1995). Prolonged development of normal and parthenogenetic postimplantation mouse embryos in vitro**.** *Int. J Dev. Biol.* **39:** 985-991.	E8.5-E10.5	No		RS 3:1 with DMEM + pen/strep		No
25.	Van Maele-Fabry G., Gofflot F., and Picard J.J. (1995). Whole embryo culture of presomitic mouse embryos**.** *Toxicol. In Vitro* **9:** 671-675.	E7.5-E9.5; E8.5-E10.5	No			MS: RS: HS 1:2:5 (1-24 h) then RS: HS 1:4 (25-48 h)	Yes - between different pre-somite stages at start of culture
26.	Van Maele-Fabry G., Picard J.J., Attenon P., Berthet P., Delhaise F., Govers M.J., Peters P.W., Piersma A.H., Schmid B.P., Stadler J. et al. (1991). Interlaboratory evaluation of three culture media for postimplantation rodent embryos**.** *Reprod. Toxicol.* **5:** 417-426.	E8.5-E10.5	No			RS, HS or RS:HS 1:4	Yes - between the 3 serum-based media
27.	Hunter E.S., III, Balkan W., and Sadler T.W. (1988). Improved growth and development of presomite mouse embryos in whole embryo culture**.** *J Exp. Zool.* **245:** 264-269.	E7.5-9.5	No			MS: RS: Tyrode's 3:0:1, 2:1:1, 1:2:1, 0:3:1 (1-72 h) +/-	Yes - between the 4 serum-based media

						then RS: Tyrode’s 3:1 (25-72 h)	
28.	Balkan W., Rooman R.P., Hurst-Evans A., Phillips L.S., Goldstein S., Du Caju M.V.L., and Sadler T.W. (1988). Somatomedin inhibitors from human serum produce abnormalities in mouse embryos in culture**.** *Teratology.* **38:** 79-86.	E8.5-E9.5; E9.5-E10.5	No		RS 3:1 with Tyrode's		No
29.	Sadler T.W., Phillips L.S., Balkan W., and Goldstein S. (1986). Somatomedin inhibitors from diabetic rat serum alter growth and development of mouse embryos in culture**.** *Diabetes* **35:** 861-865.	E8.5-E9.5; E9.5-E10.5	No		RS 3:1 with Tyrode's		No
30.	Priscott P.K. and Ford J.R. (1985). An in vitro model of acetaldehyde metabolism by rodent conceptuses. *In Vitro Cell Dev. Biol*. **21:** 88-92.	E10-E11	No		RS 1:3 with DMEM + pen/strep		No
31.	Fisher D.L. and Martinez de Villareal, L. (1982). Effects of hormones on postimplantation mouse embryos in vitro. II. Progesterone and estrogen**.** *J Exp. Zool.* **224:** 205-210.	E9.5-E11; E11.5-E13	No			FBS 1:1 with Waymouth's + insulin	No
32.	Sadler T.W. and New D.A.T. (1981). Culture of mouse embryos during neurulation**.** *J. Embryol. Exp. Morphol.* **66:** 109-116.	E8.5-E10.5	No		RS 100% or RS 1:1 with DMEM or Waymouth's		Yes - against in vivo developed embryos

33.	Tam P.P. and Snow M.H. (1980). The in vitro culture of primitive-streak-stage mouse embryos. *J Embryol. Exp. Morphol.* **59:** 131-143.	E6.5-E8.5; E7.5-E8.5	No		RS 100% or RS 1:1 with DMEM	FBS, NCS, MS, RS; 100% or 1:1 with DMEM	Yes - against in vivo developed embryos, against other sera, and between RS with/without 50% dilution by DMEM
34.	Galloway S.M., Perry P.E., Meneses J., Nebert D.W., and Pedersen R.A. (1980). Cultured mouse embryos metabolize benzo[a]pyrene during early gestation: genetic differences detectable by sister chromatid exchange**.** *Proc. Natl. Acad. Sci U. S. A* **77:** 3524-3528.	E7.5-E9; E8.5-E10	No			FBS: NCS: Eagle's 1:1:18	No
35.	Sadler T.W. (1979). Culture of early somite mouse embryos during organogenesis**.** *J. Embryol. Exp. Morphol.* **49:** 17-25.	E8.5-E10.5	No			RS, FBS or FBS 1:1 with Weymouth's	Yes - against in vivo developed and FBS-cultured embryos
36.	Agnish N.D. and Kochhar D.M. (1976). Direct exposure of postimplantation mouse embryos to 5-bromodeoxyuridine in vitro and its effect on subsequent chondrogenesis in the limbs**.** *J Embryol. Exp. Morphol.* **36:** 623-638.	E10.5-E11.5	No			FBS 1:1 with Waymouth's	No
37.	Kochhar, D.M., 1975. The use of in vitro procedures in teratology. *Teratology.* **11,** 273-287.	E11.5-E12.5; E12.5-E13.5	No			FBS 1:1 with Weymouth's	No
38.	Clarkson S.G., Doering J.V., and Runner M.N. (1969). Growth of postimplantation mouse embryos cultured in a serum-supplemented, chemically defined medium**.** *Teratology.* **2:** 181-185.	E8.5-E9.5	No		RS (commercial) 1:4 or 1:9 with Waymouth's		Yes - between dilutions of RS and against in vivo developed embryos

* Abbreviations: CMRL, Connaught Medical Research Laboratories; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; HBSS, Hank's Balanced Salt Solution; HS, human serum; KSR, Knock-out Serum Replacement; MS, mouse serum; NCS, newborn calf serum; pen/strep, penicillin and streptomycin; RS, rat serum.

** Unless otherwise stated, rat serum was produced specifically for embryo culture in the research laboratory. 'Commercial' rat serum was purchased from a supplier.

Table S2. Comparison of the amino-acid compositions of DMEM and GMEM + Defined Supplements (DS), as used to dilute rat serum for whole embryo culture

		GMEM + DS (mg/L)	DMEM (mg/L)
Essential amino-acids	L-Histidine HCl-H_2O	21.0	42.0
	L-Isoleucine	52.4	105.0
	L-Leucine	52.4	105.0
	L-Lysine HCl	73.1	146.0
	L-Methionine	15.0	30.0
	L-Phenylalanine	33.0	66.0
	L-Threonine	47.6	95.0
	L-Tryptophan	8.0	16.0
	L-Valine	46.8	94.0
Non-essential amino-acids	L-Alanine	8.5	-
	L-Arginine HCl	42.0	84.0
	L-Asparagine	13.2	-
	L-Aspartic acid	13.3	-
	L-Cystine 2HCl	31.3	63.0
	L-Glutamine	712.0	580.0
	Glycine	15.0	30.0
	L-Proline	11.5	-
	L-Serine	21.0	42.0
	L-Tyrosine	36.0	72.0