Culture of human ovarian tissue in xeno-free conditions using laminin components of the human ovarian extracellular matrix

ONLINE RESOURSES

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Table 1. Overview of participant characteristics.

Study	Type of sample and donor	Number of donors	Donor age Mean, SD
Laminin expression in ovarian cortex	Ovarian cortical biopsies C-section patients	6	33.50 ±4.13
Laminin expression in mural granulosa cells	Follicular fluid *In vitro* fertilization patients	10	Anonymous
Laminin expression in cumulus granulosa cells	Cumulus cells from denuded oocytes Intracytoplasmic sperm injection patients	10	Anonymous
7-day culture	Ovarian cortical biopsies C-section patients	17	33.59±5.39

Table 2. Antibodies

Target	Host species	Company	Catalogue number	Dilution
LAMA1	Rabbit	Atlas Antibodies	HPA032110	1:200
LAMA2	Rabbit	Assay Biotech	C13065	1:200
LAMA3	Rabbit	Atlas Antibodies	HPA009309	1:200
LAMA5	Mouse	Abnova	MAB7872	1:50
LAMB1	Rabbit	Atlas Antibodies	HPA004132	1:200
LAMB2	Rabbit	Atlas Antibodies	HPA001895	1:200
LAMC1	Rabbit	Atlas Antibodies	HPA001908	1:200
FSHR	Goat	Santa Cruz	sc-7798	1:100
Inhibin-a	Rabbit	Abcam	ab81234	1:200
Ki67	Rabbit	Abcam	Ab92742	1:500
Rabbit IgG	Chicken	Santa Cruz	sc-2963	1:200
Mouse IgG	Chicken	Santa Cruz	sc-2954	1:200

Table 3. Primers

Gene	Target transcript(s)	Forward primer (5'-3')	Reverse primer (5'-3')
LAMA1	NM_005559.3	CAGCCCTCCTGCCAGTTTTA	AACTGCCACCTGGTGTTTGA
LAMA2	NM_000426.3 NM_001079823.1	TTTGCCAAAGCAGTTGGTGG	CCCCAGAAGAACTCCAGTCG
LAMA3	NM_000227.3 NM_001127717.1 NM_001127718.1 NM_198129.1	GGTCATGTCGTCTTGGCTCA	CTTGGGCGGATGCTGAAAAC
LAMA4	NM_001105206.2 NM_001105207.2 NM_001105208.2 NM_001105209.2 NM_002290.4	GAGGGAAGACCGCAGGGAAA	CCTGGCTTCCTTCTCACATTCA
LAMA5	NM_005560.4	GGAGAACGGAGAGATCGTGG	CAGCGGCGAGTAGGAGAAAT
LAMB1	NM_002291.2	CAAAGCAACAGCACAGCCAA	CTATCCAAGGCACCCCGAAT
LAMB2	NM_002292.3	AGACCGTTCACCTCCCCTTA	GCCAGCACGCTTAGCAGTAG
LAMB3	NM_000228.2 NM_001017402.1 NM_001127641.1	CCCATGAATGCCAAAGGTGC	CTGACACCGCTCACAGTTCT
LAMB4	NM_007356.2	GCTAGCGAATGTCGCCCTAT	TGACCGTGAACCATTCCAGG
LAMC1	NM_007356.2	GTCCTGTGGGCTCTCTAAGC	AGGGATCACAAGAGCATGGC
LAMC2	NM_005562.2 NM_018891.2	TCCAGGAGGGAAGTCTGTGA	GTCATTGCAGTTGAGGCAGC
LAMC3	NM_006059.3	GCGACGCTACGGCAAACAG	TGCAGCTTCATTCCTTCCAGAT
RPLP0	NM_001002.3	ACAGGGCGACCTGGAAGTCCAACTA	AGCCCACATTGTCTGCTCCCACA
HPRT1	NM_000194.2	GCCCTGGCGTCGTGATTAG	CGAGCAAGACGTTCAGTCCTGT

Table 4. Statistical values for laminin expression in single-cell data

P-values for differences in laminin gene expression levels within each cell type in the single-cell dataset. Associates to Figure 3. All significant differences are shown.

Cell type	Comparison	P adjusted
Endothelial	LAMB2-LAMA2	4,9E-07
Endothelial	LAMB2-LAMA3	0,00269
Endothelial	LAMB2-LAMA4	0,00046
Endothelial	LAMC3-LAMA4	0,00815
Endothelial	LAMB2-LAMA5	0,00072
Endothelial	LAMC3-LAMA5	0,00167
Endothelial	LAMB2-LAMB1	6,1E-08
Endothelial	LAMC1-LAMB2	6,1E-06
Endothelial	LAMC3-LAMB2	1,5E-07
Endothelial	LAMC3-LAMC1	0,01653
Perivascular	LAMB2-LAMA3	0,01129
Perivascular	LAMB2-LAMA5	0,00079
Stromal	LAMB2-LAMA1	1,1E-06
Stromal	LAMA3-LAMA2	0,0221
Stromal	LAMA5-LAMA2	0,00061
Stromal	LAMB2-LAMA2	3,9E-11
Stromal	LAMB2-LAMA3	3,6E-07
Stromal	LAMB2-LAMA4	1,3E-11
Stromal	LAMB2-LAMA5	1,4E-11
Stromal	LAMB2-LAMB1	6,2E-10
Stromal	LAMC1-LAMB2	1,3E-11

Table 5. Summary of laminin expression analyses

		A1	A2	A3	A4	A5	B1	B2	B3	B4	C1	C2	C3
RNA	Bulk cortex	-	++	+	-	+	+	++	-	-	++	-	-
	Bulk whole ovary	-	++	+	-	-	+	++	-	-	++	-	-
	Bulk mural GC	+	-	+	-	-	+	++	-	-	++	-	-
	Bulk cumulus GC	-	-	++	-	-	-	++	-	-	++	-	-
	Single stromal cells	+	++	+	++	+	+	++	-	-	++	-	-
	Single endothelial cells	+	+	+	+	++	+	++	-	-	++	-	+
	Single perivascular cells	-	+	+	+	++	+	++	-	-	++	-	+
PROTEIN	Stroma and preantral follicles	-	++	+	NA	-	+	++	NA	NA	+	NA	NA
	Small antral follicles	++	++	+	NA	-	++	+	NA	NA	++	NA	NA
	Mural GC	++	++	++	NA	-	+	++	NA	NA	++	NA	NA
	Cumulus GC	++	++	++	NA	-	+	++	NA	NA	++	NA	NA

++ robustly expressed

+ expression detected but either at low level or only in some cells

- no clear expression detected

GC granulosa cells

NA not analyzed