

Supplementary Figures

***De novo* reconstruction of a functional *in vivo*-like equine endometrium using collagen-based tissue engineering**

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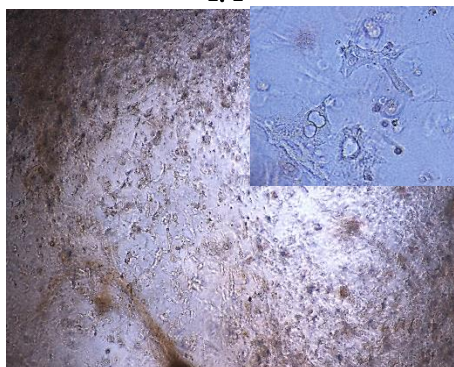
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The preliminary ratio of stromal: epithelial cells in the *de novo* reconstructed 3D-ET

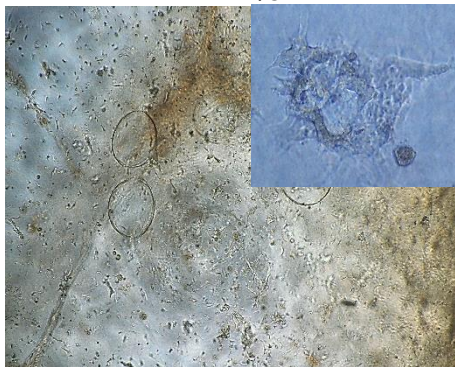
Stromal: Epithelial ratio

A D3 After 3D-ET reconstruction

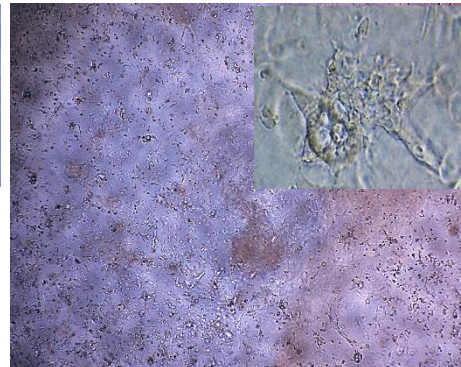
1: 1



1: 5

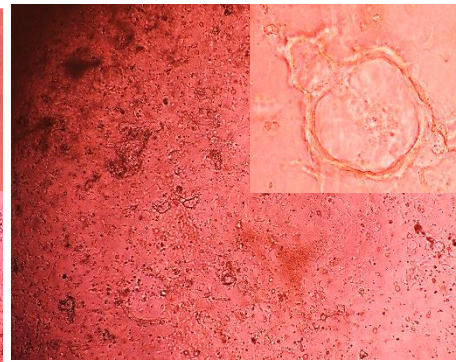
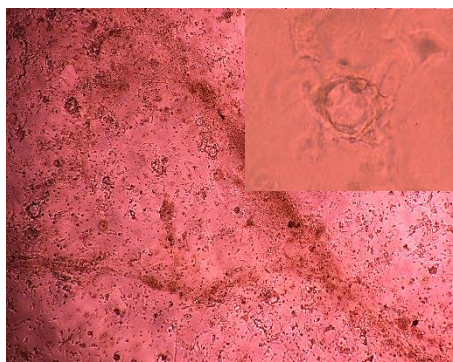
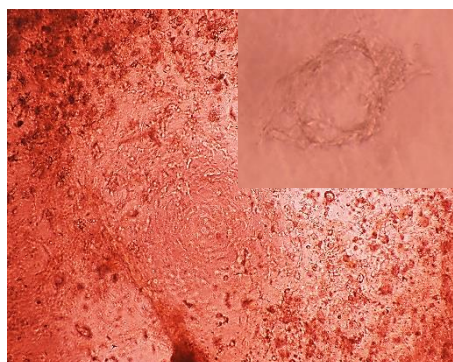


1: 10



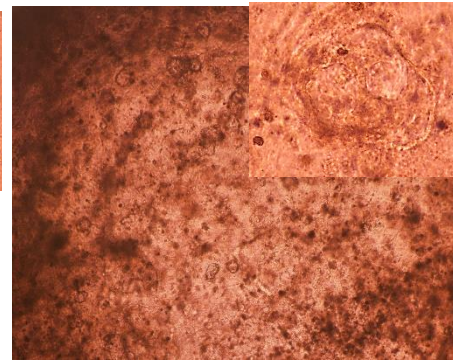
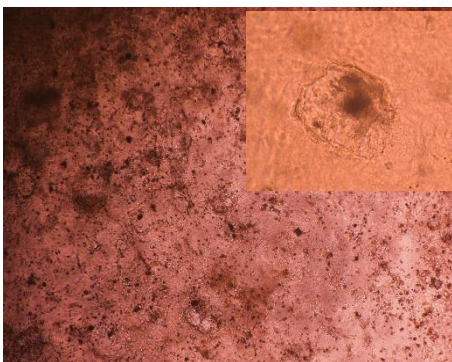
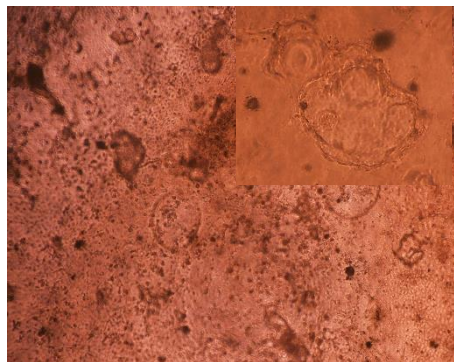
Scale bar 200 μ m

B D5 After 3D-ET reconstruction



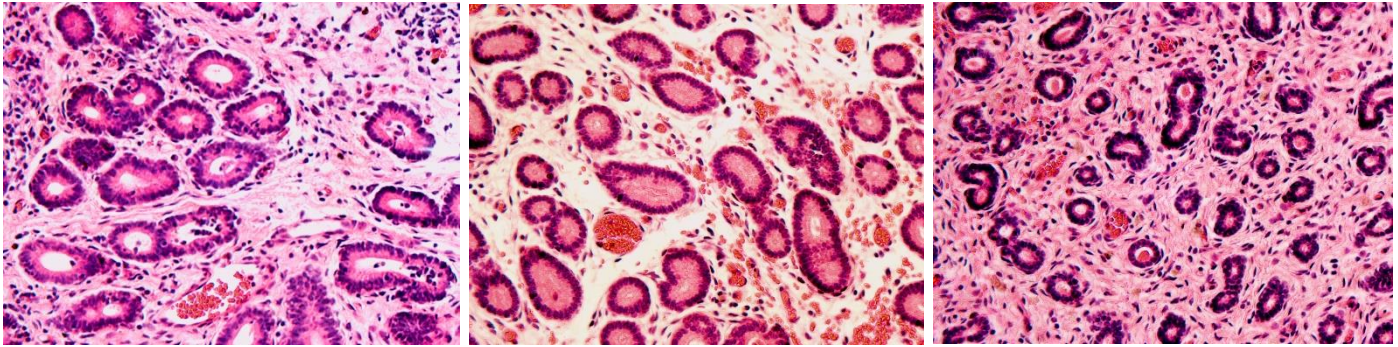
Scale bar 200 μ m

C D10 After 3D-ET reconstruction



Scale bar 200 μ m

Supplementary Figure 1: In the preliminary 3D-ET reconstruction **A)** the ratio between stromal and epithelial cells was observed at D3 after seeding. At this stage, 3d-ETs from all ratios began illustrating gland-like structures. **B)** The gland-like structures exhibited continued growth, eventually developing into hollow structures. **C)** At D10 of culture, all 3D-ET initial cell ratios exhibited pigmentation in the glands, indicating glandular apoptosis. However, at seeding ratios of 1:5 and 1:10, the apoptotic effect was more pronounced than at the 1:1 ratio.



Supplementary Figure 2: displays examples of endometrial tissue post-biopsy specifically selected as exhibiting normal healthy endometrium (grade 1) according to Kenney, 1986. The figures illustrate either a normal endometrium or mild focal inflammation or fibrosis.

interleukin-4 precursor [Equus caballus]

Sequence ID: [NP_001075988.1](#) Length: 137 Number of Matches: 1
[See 5 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Range 1: 1 to 137 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
145 bits(366)	1e-50	Compositional matrix adjust.	85/154(55%)	98/154(63%)	20/154(12%)
Query 1	MGLTSOLLPLFFLLACAGNFVGHKCDITLQEIITLNSLTE--QKTLCTELTVTDIFA	5i			
Sbjct 1	MGLT QL+P L LLAC NF+ G K DITLQEIITLNL+ K C ELTV D FA	6i			
Query 59	ASKNTEKETFCRAATVLRQFYSHHEKDTRLCGATAQQFHRHKQLIR-FLKRLDRNLWGL	1:			
Sbjct 61	KNT KE CRAA VL+Q Y H+ + LI+ L LDRNL G+ GPKNTDGKE-ICRAAKVLQQLYKRHD-----RSLIKECLSGLDRLNKGH	1i			
Query 118	AGLNSCPVKEANQSTLENFLERLKTIMREKYSKC	151			
Sbjct 104	A C V EA +STL++FLERLKTIM+EKYSKC ANGTCCTVNEAKKSTLKDFLERLKTIMKEKYSKC	137			

interferon gamma precursor [Equus caballus]

Sequence ID: [NP_001075418.1](#) Length: 166 Number of Matches: 1
[See 4 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Range 1: 1 to 166 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
224 bits(571)	4e-81	Compositional matrix adjust.	112/166(67%)	130/166(78%)	0/166(0%)
Query 1	MKYTSYLAFQLCIVLGSGLGVCYQDPYVKEAENLKYYFNAGHSDVADNGTLFLGILKNWK	6i			
Sbjct 1	M NYTS+ILAFQLC +LGS YCQ + KE ENLK+YFNA + DV D G LFL ILKNWK MNYTSFILAFQLCAILGSSTYYCQAFFKEIENLKEYFNASNPVGDGGPLFLDILKNWK	6i			
Query 61	EESDRKIMQSQIVSFYFKLFKNFKDDQSQSVETIKEDMNVKFFNSNKKRRDDFEKLTN	1:			
Sbjct 61	E+SD+KI+QSQIVSFYFKLF+N KD+Q IQKS++TIKED+ VKFFNS+ K +DF+KL EDSDKKIISQIVSFYFKLFENLKDQVQIKSMDTIKEDL FVKFFNSSTSKLED FQKLIQ	1:			
Query 121	YSVTDLVORKAIELIQVMAELSPAAGTKRRKRSQMLFRGRRASQ	166			
Sbjct 121	V DL VORKAI ELI+VM +LSP A KRKR SQ FRGRRA Q IPVNDLVORKAISELIKVMNDLSPKANLKRKR SQNPFRRGRALQ	166			

interleukin-10 isoform X1 [Equus caballus]Sequence ID: [XP_023495993.1](#) Length: 263 Number of Matches: 1

Range 1: 72 to 224 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
255 bits(652)	2e-91	Compositional matrix adjust.	124/153(81%)	134/153(87%)	0/153(0%)
Query 1	MHSSALLCCLVLLTGVRASPGGTQSENSCTHFGNLPNMLRDLRDAFSRVKTFQMKDQ				61
Sbjct 72	M S+ALLC LV L GV AS +GTQSENSCTHFP +LP+ML +LR AFSRVKTFQMKDQ				
Query 61	LDNLLKESLLEDKFGYLGCAQALSEMIQFYLEEVMPQAEQDPDIKAHVNSLGENLKT				1
Sbjct 132	LDN+LL SLEDKFGYLGCAQALSEMIQFYLEEVMPQAEQDPDIK HVNSLGE LKTLR				1
Query 121	LRLRRCHRFPCENKSKAVEQVKNFKNLEKQG 153				
Sbjct 192	+RLRRCHRFPCENKSKAVEQVK+AF+K+ G 224				

interleukin-5 precursor [Equus caballus]Sequence ID: [NP_001075968.1](#) Length: 134 Number of Matches: 1

Range 1: 1 to 134 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
199 bits(505)	4e-72	Compositional matrix adjust.	98/134(73%)	108/134(80%)	0/134(0%)
Query 1	MRMLHLHLLALGAAYVYAIPTEIPTSALVKETLALLSTHRTLLIANETLRIPVPHKNH				61
Sbjct 1	MRMLHLH+LALGAAYV A+ E P + LV ETL LLSTHRTLLI + L IP P HKNH				61
Query 61	QLCTEEIFQIGTLESQTVGGTVERLFKNLSLIKIDGQKKCGEERRRVNQFLDYLO				1
Sbjct 61	QLC EE+FGI TL++QTVOG V +LF+NLSLIK YID QKKKCG ER RV QFLDYLO				1
Query 121	EFLGVNTEWIIIES 134				
Sbjct 121	EFLGVNTEWIEG 134				

interleukin-6 precursor [Equus caballus]Sequence ID: [NP_001075965.2](#) Length: 208 Number of Matches: 1

Range 1: 75 to 208 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
194 bits(492)	6e-69	Compositional matrix adjust.	91/135(67%)	113/135(83%)	1/135(0%)
Query 2	CESSKEALAENNLNLPKMAEKDGCFOGNEETCLVKIITGLLEFVEYLEYLQNRFSSE				6
Sbjct 75	CE+SKE LAENNLNLPKMAEKDGCFOGFGN+ETCL+KI TGL EF++YLEYLQN F+ +				1
Query 62	EQARAVQMSKTVLIQFLQKAKNLAITTPDPTNASLLTKLQAQNWQDMITHILRS				1
Sbjct 135	E + +Q+STKVL+Q L +K KN + +TTPDPT +SLL KL +QN+WL++ TTHILRS				1
Query 122	FKEFLQSSLRALRQM 136				
Sbjct 194	++FLQ SLRA+R M LEDFLQSLRAVRIM 208				

tumor necrosis factor isoform X1 [Equus caballus]Sequence ID: [XP_005603547.1](#) Length: 252 Number of Matches: 1

See 1 more title(s) [See all Identical Proteins \(IPG\)](#)

Range 1: 20 to 252 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
396 bits(1017)	2e-146	Compositional matrix adjust.	203/233(87%)	215/233(92%)	0/233(0%)
Query 1	MSTESMIRDVELAEALPKKTGGPGGSRRLFLSLFSLIVAGATTLFCLLHFGVIGPQR				61
Sbjct 20	MSTESMIRDVELAE L KK GGPGGSRRL LSLFSL+VAGATTLFCLLHFGVIGPQR				71
Query 61	EEFPRDLISLPLAQAARSSSRTPSDKPVAVHVANPOAEGQLNRRANALLANGVELR				1
Sbjct 80	EE P I+PLAQ +RSSSRTPSDKPVAVHVANPOAEGQLN+ RANALLANGV+L				1
Query 121	DNQLVVPSEGLYLIYSQVLFKGGGCPSTHVLTTHTISRIVSYOTKNLLSAIKSPCORE				1
Sbjct 140	DNQLVVP +GLYLIYSQVLFKGGGCPSTHVLTTHTISR+AVSY +KNVLLSAIKSPC E				1
Query 181	TPEGAEPWYEPYIYLGQVQLEKGDRLSAEINRPDYLDFAESGQVYFGIALL				233
Sbjct 200	+PE AEAKPWYEPYIYLGQVQLEKGD+LSAEIN+P+YLDFAESGQVYFGIALL				252

interleukin-13 precursor [Equus caballus]Sequence ID: [NP_001137263.1](#) Length: 133 Number of Matches: 1

See 4 more title(s) [See all Identical Proteins \(IPG\)](#)

Range 1: 1 to 129 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
182 bits(461)	3e-65	Compositional matrix adjust.	90/130(69%)	104/130(80%)	1/130(0%)
Query 15	MALLTTVIALTCLGGFASPGVPVPSSTALRIEELVNITQNKAPLNGSMHVSINLTA				7
Sbjct 1	MAL LT VIAL CLGG ASP P+P S AL+ELI+ELVNITQNK APLNGSMHVSINLTA				5
Query 75	GMYCAALESINVSQCSAIEKTRQMLSGFCPHKVSAGQFSSLHVROTKIEVAQFVKDLLL				1
Sbjct 60	YC ALES L NVS CSAI+ T++ML+ CPH++SAGQ SS RDTKIEV VKDLL				1
Query 135	HLKKLFREGQ 144				
Sbjct 120	+L+K+F G+ NLRKIFHGK 129				

interleukin-2 precursor [Equus caballus]Sequence ID: [NP_001078902.1](#) Length: 149 Number of Matches: 1

See 6 more title(s) [See all Identical Proteins \(IPG\)](#)

Range 1: 1 to 149 [GenPept](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
190 bits(482)	5e-68	Compositional matrix adjust.	99/154(64%)	120/154(77%)	6/154(3%)
Query 1	MYRMQLLSIALSLALVTNSAPTSSSKTKTQLQLEHLLDLQMLINGINNYKNPKLTRML				60
Sbjct 1	MY+MQLL+IAL+LA++ NSAPTSSS ++TQ QL+ L +DL+++ G+NN KNPKL++ML				60
Query 61	TFKFYMPKATELKHLCLEELKPLEEVLNLAQSKFNHLRP-RDLISINIVTVLEKGS				119
Sbjct 61	TFK MPKATELKHLCLE E KNF + ++L+SINIV VL LKGS				115
Query 120	ETTFMCEYADETATIEFLNRWITFCQSIISTLT 153				
Sbjct 116	ET FTCEYODETG TIEFLNKWITFCQSIISTMT 149				

Supplementary Figure 3: The homology of eight inflammatory cytokines in response to

LPS induction was investigated. Utilizing the Human Th Cytokine Panel 13-plex assay from Biolegend, USA, and employing flow cytometry analysis on a BD FACS Calibur instrument from Becton Dickinson, USA, we found interesting parallels between equine and human sequences. Specifically, the percent homology was highest for IFN γ (67%), IL13 (69%), IL5 (73%), IL10 (81%), IL6 (67%), and TNF- α (87%), IL2 (64%) consistent with previous observations by Scheerlinck in 1999. (It's noteworthy that >60% homology suggests potential cross-reactivity with cytokines). While our study encompassed the examination of eight cytokines, particular emphasis was placed on IL6, which emerged as a pivotal cytokine of interest.