

***Chlamydia trachomatis* infection of human endometrial stromal cells induces defective decidualisation and chemokine release.**

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Supplementary figures and tables

Supplementary figure and table legends

Supplementary Figure S1: hESC infected with *Ct* at an estimated multiplicity of infection (MOI) 1, 2 and 3. In infected wells, between 0.05% (at an MOI 1) and 20% (at either an MOI of 2 or 3) of ESC contained chlamydial inclusions. No differences were observed in the numbers of inclusions in cells from two different patients. An MOI 2 was used for all subsequent experiments. Scalebars equal 500µm.

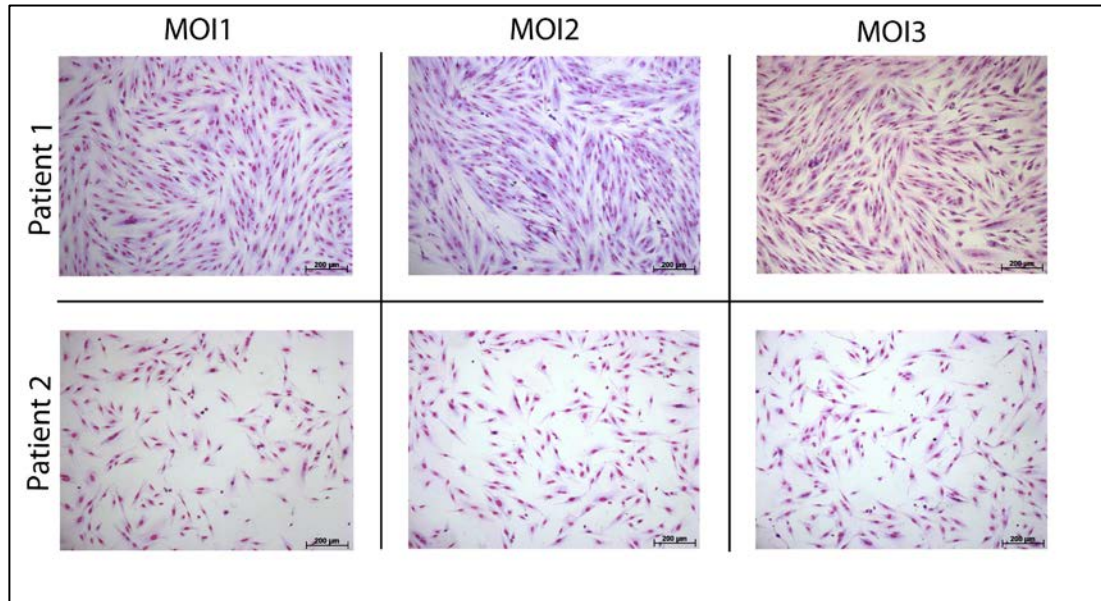
Supplementary Table S2. Proteomic chemokine array results (measured in percentage of positive control of each membrane) and information for the role of each chemokine in decidualisation, early pregnancy/trophoblast invasion, miscarriage and *Ct* infection.

Supplementary Figure S3: Prolactin expression and release remains unaffected by exposure of decidualised ESC to LPS. a. PRL mRNA remains unaffected by exposure of decidualised ESC to either rough LPS (rLPS; derived from *S. minnesota*) or smooth LPS (sLPS; derived from *E. coli*) (n=5, One way ANOVA Friedman's test with Dunn's multiple comparisons test) b. PRL protein levels remain unaffected by exposure to LPS (n=5, One way ANOVA Friedman's test with Dunn's multiple comparisons test).

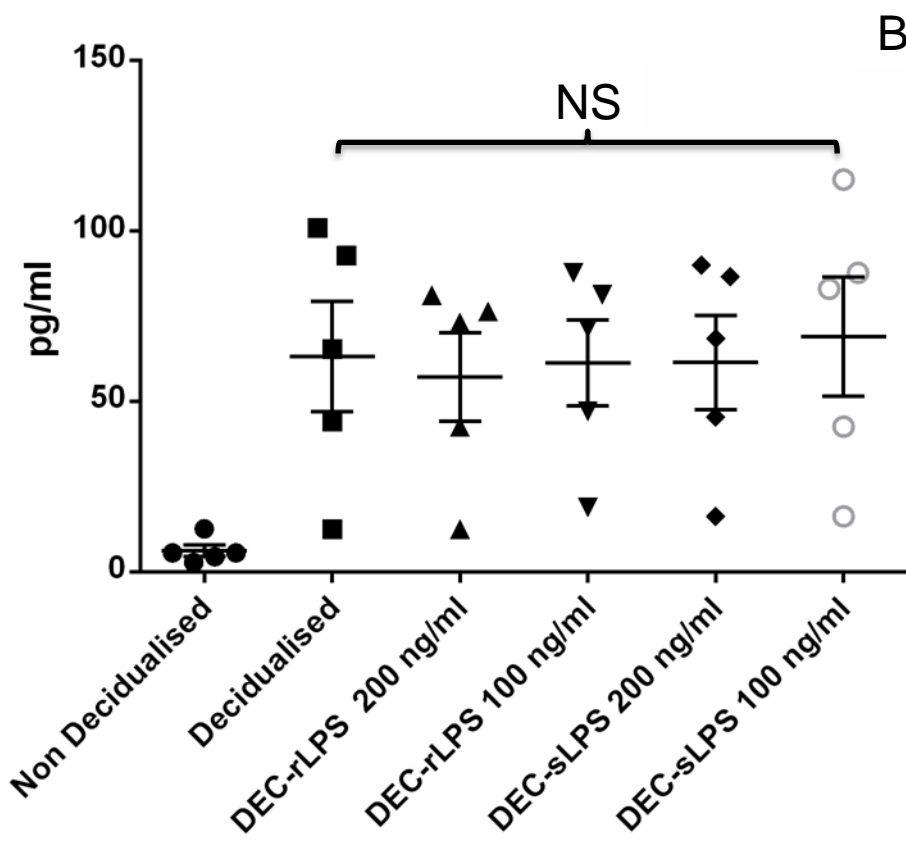
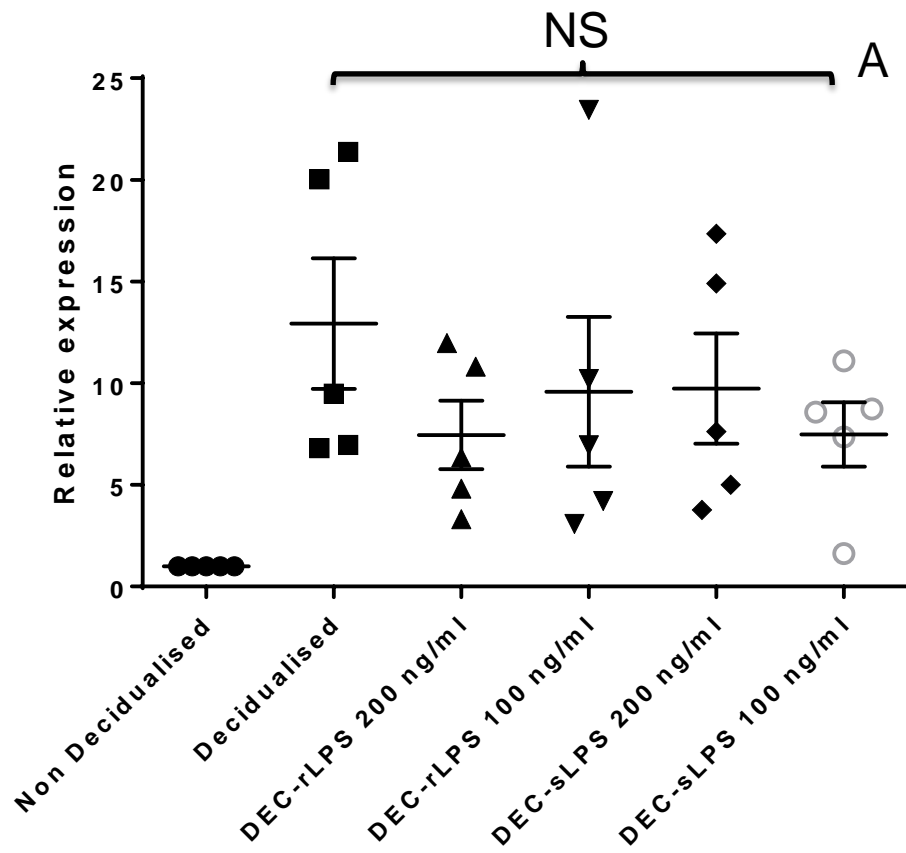
Supplementary Table S4: Decidualisation and infection protocol for human endometrial stromal cells (ESC). Treatments of progesterone (P4) and cyclic-AMP (cAMP) were administered in the cell media on days 1, 3 and 5. On day 6 the cells were infected with *C. trachomatis* or vehicle control and 48 hours later samples were collected.

Supplementary Table S5: qPCR sample using SYBR green

Supplementary Figure S1



Supplementary Figure S2



Supplementary Table S3

Gene	Decidualised Ct infected % of positive control	Decidualised uninfected % of positive control	Δ % of positive control	Role in decidualisation/early pregnancy/miscarriage/association with Ct in humans
6Ckine/ CCL21/ Exodus-2	14.90	19.43	-4.53	mRNA detected in the endometrium throughout the menstrual cycle and increased in early pregnancy (GCID:GC09M034709). No information on miscarriage and association with Ct infection.
CCL28/ MEC	6.42	7.78	-1.36	Detected by immunohistochemistry in decidualised hESC and first trimester decidua, was shown to induce apoptosis in hESC and its receptors were increased in hESC from miscarriage compared to controls ¹ . No information on association with Ct infection.
CXCL16 / SRPSOX	24.59	53.31	-28.72	Human trophoblasts recruited T lymphocytes and monocytes into decidua by secretion of CXCL16 in early pregnancy ² . CXCL16 also induces invasion and proliferation of first-trimester human trophoblast cells ³ . No information on decidualisation, miscarriage or association with Ct infection.
Chemerin /TIG-2/ RARRES 2	91.66	99.69	-8.03	Chemerin serum concentration was significantly higher in pregnant women in the 3rd trimester than in non-pregnant and pregnant women in the 1st trimester, whilst no differences were observed during the menstrual cycle ⁴ . No information on miscarriage or association with Ct infection.
ENA-78/ CXCL5	10.67	14.48	-3.82	Elevated ENA-78 levels were associated with increased risk of miscarriage ⁵ . mRNA levels were increased in trachoma patients ⁶ . No information on decidualisation.
Eotaxin-3/ CCL2	16.52	23.75	-7.23	IL-33 enhances proliferation and invasiveness of ESC by up-regulation of CCL2 and its receptor CCR2 ⁷ .

				No association between maternal circulation levels during the first trimester of CCL2 and miscarriage ⁸ . No information on association with <i>Ct</i> infection.
Fractalkine/ CX3CL1 / Neurotactin	28.68	30.31	-1.63	Is expressed in maternal decidua and promotes human trophoblast invasion ⁹ . No association between maternal circulation levels during the first trimester of CCL2 and miscarriage ⁸ . No information on association with <i>Ct</i> infection.
GRO α / CXCL1	94.17	66.23	27.94	Expressed in early pregnancy decidua (GCID:GC04P073869). Upregulated in women with antiphospholipid antibodies inducing a placental inflammatory response via the TLR-4/MyD88 pathway, which in turn compromises trophoblast survival ¹⁰ . Associated with asymptomatic <i>Ct</i> infection in men ¹¹ .
HCC-1/ CCL14/ HCC-3	10.22	15.23	-5.01	Promotes human trophoblast invasion ⁹ . No information on miscarriage or association with <i>Ct</i> infection.
I-309/ CCL1/ TCA3	10.82	18.54	-7.72	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.
IL-8/ CXCL8	98.02	100.15	-2.13	Promotes trophoblast invasion ¹² . Upregulated in women with antiphospholipid antibodies inducing a placental inflammatory response via the TLR-4/MyD88 pathway, which in turn compromises trophoblast survival ¹⁰ . No association of serum levels with miscarriage (Whitcomb et al., 2007). Induced in response to <i>Ct</i> infection ¹³ .
IL-16/ LCF	20.71	36.59	-15.88	Expressed in decidua during the implantation window (GCID:GC15P081159). Induced in response to <i>Ct</i> infection in cell lines ¹⁴ . No information on miscarriage.
IP-10/ CXCL10	36.13	44.75	-8.62	Increased in decidua during the implantation window ¹⁵ . Also reported to be increased in women with <i>Ct</i> associated infertility ¹⁶ . No information on miscarriage.
I-TAC/ CXCL11	8.70	13.58	-4.88	Increased levels in euthyroid women with autoimmune thyroiditis with recurrent miscarriage and compared to diabetics with successful pregnancy ¹⁷ .

				<p>Secreted by progesterone primed primary endocervical epithelial cells infected with <i>Ct</i> ¹⁸.</p> <p>No information on decidualisation or early pregnancy.</p>
Lympho tactin/ XCL1/ Lptn/ ATAC/ SCM-1 α	46.07	23.09	22.98	<p>Upregulated during implantation window ¹⁹.</p> <p>No information on miscarriage or association with <i>Ct</i> infection.</p>
MCP-1/ CCL2/ MCAF	101.83	99.08	2.75	<p>Localised in glandular epithelium and endothelial cells, not secreted by blastocyst during early implantation window (GCID: GC17P034255).</p> <p>Upregulated in women with antiphospholipid antibodies inducing a placental inflammatory response via the TLR-4/MyD88 pathway, which in turn compromises trophoblast survival ¹⁰. Elevated mRNA due to <i>Ct</i> infection ²⁰.</p>
MCP-3/ CCL7	38.34	61.95	-23.61	<p>Detected in plasma of women during 1st trimester of pregnancy but no difference between miscarriage cases and controls ⁸. Does not induce trophoblast migration ⁹. Not detected in sera of infertile women with past <i>Ct</i> infection ²¹.</p>
MDC/ CCL22/ STCP-1/ ABCD-1	31.95	46.88	-14.93	<p>Detected on maternal stromal cells and increased in placentae from miscarriage cases ²².</p> <p>No information on association with <i>Ct</i> infection.</p>
MDK/M K/ Midkine	76.92	97.66	-20.75	<p>No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.</p>
MIG/ CXCL9	10.27	17.56	-7.29	<p>Increased levels in euthyroid women with autoimmune thyroiditis with recurrent miscarriage compared to diabetics with successful pregnancies¹⁷.</p> <p>Associated with asymptomatic <i>Ct</i> infection in men ¹¹.</p> <p>No information on decidualisation or early pregnancy.</p>
MIP- 1 α / β / CCL3/ CCL4	10.44	18.14	-7.70	<p>Shown to be secreted by endometrial stromal cells in response to hCG ²³. Promotes trophoblast migration ⁹. No association of serum levels with miscarriage ⁵. Levels were not affected by <i>Ct</i> infection ²⁴.</p>

MIP-1 δ / CCL15/ Leukotactin 1/ MIP-5/ HCC-2	10.74	16.56	-5.82	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.
MIP-3 α / CCL20/ LARC/ Exodus-1	14.12	14.36	-0.24	Was shown to be increased due to <i>Ct</i> infection ²⁵ . No information on decidualisation, early pregnancy or miscarriage.
MIP-3 β / CCL19/ ELC/ Exodus-3	36.15	37.03	-0.88	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.
NAP-2/ CXCL7/ CTAP III	78.93	18.09	60.84	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.
PARC/ CCL18/ MIP-4/ AMAC-1	15.85	25.42	-9.57	Increased in trachoma patients in response to IL-17 ⁶ . No information on decidualisation, early pregnancy or miscarriage.
PF4/ CXCL4	19.27	31.85	-12.57	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.
RANTES / CCL5/ SISd	34.42	25.36	9.07	Localised in endometrial stromal and endothelial cells during the implantation window and is not produced by the trophoblast (GCID:GC17M035871). No association of serum levels with miscarriage ⁵ .
SDF-1/ CXCL12 / PBSF	15.12	94.86	-79.75	Associated with trophoblast invasion and implantation in primary stromal cells ^{26,27} . There is also a study showing inhibition of CXCL12 due to cytomegalovirus infection that resulted in impaired migration and invasion of human extravillous cytotrophoblasts ²⁸ . Levels not altered in semen of men with asymptomatic <i>Ct</i> infection ¹¹ . No information on association with miscarriage.
TARC/ CCL17	17.75	30.25	-12.50	Induces trophoblast migration ²⁹ . No information on decidualisation, miscarriage or association with <i>Ct</i> infection.
VCC-1/ CXCL17 / DMC	6.77	10.00	-3.23	No information on decidualisation, early pregnancy, miscarriage or association with <i>Ct</i> infection.

Supplementary Table S4

	Day 1	Day 3	Day 5	Day 6	Day 8
1° ESC/ SHT290	P4/cAMP treatment	P4/cAMP treatment	P4/cAMP treatment	<i>Ct</i> /mock infection	Sample collection

Supplementary Table S5

Stock	Final concentration	Volume per 10µl
2x Brilliant III SYBR Mix	1X	5µl
Primer pair	10µM	0.5µl
Diluted Reference Dye		0.15 µl
Nuclease Free H ₂ O		3.35 µl
cDNA	2.5ng	1µl

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