Pelvic chlamydial infection predisposes to ectopic pregnancy by upregulating integrin β1 to promote embryo-tubal attachment

Syed F. Ahmad[1†], Jeremy K. Brown[1†], Lisa L. Campbell[1], Magda Koscielniak[1], Catriona Oliver[1], Nick Wheelhouse[2], Gary Entrican[6], Stuart McFee[1], Gillian S. Wills[3], Myra O. McClure[3], Patrick J. Horner[4], Sevasti Gaikoumelou[1], Kai F. Lee[5], Hilary O.D. Critchley[1], W. C. Duncan[1], Andrew W. Horne[1*]

[1]*MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK*

[2]*Moredun Research Institute and Napier University, Edinburgh, Midlothian, UK*

[3]*Jefferiss Research Trust Laboratories, Imperial College London, London, UK*

[4]*Department of Medical Microbiology, North Bristol NHS Trust, Bristol, UK*

[5]*Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong, China*

[6]*Moredun Research Institute and the Roslin Institute at the University of Edinburgh, Midlothian, UK*

[†]*These authors contributed equally.*

*Correspondening author:
Prof. Andrew W Horne
MRC Centre for Reproductive Health
University of Edinburgh
Queen's Medical Research Institute
47 Little France Crescent
Edinburgh EH16 4TJ
UNITED KINGDOM
Tel: +44 131 242 6988
andrew.horne@ed.ac.uk

Abstract

Tubal ectopic pregnancies are a leading cause of global maternal morbidity and mortality. Previous infection with *Chlamydia trachomatis* is a major risk factor for tubal embryo implantation but the biological mechanism behind this association is unclear. Successful intra-uterine embryo implantation is associated with increased expression of endometrial 'receptivity' integrins (cell adhesion molecules). We examined integrin expression in Fallopian tubes of women with previous *C. trachomatis* infection, in mice experimentally infected with *C. trachomatis*, in immortalised human oviductal epithelial cells (OE-E6/E7) and in an *in vitro* model of human embryo attachment (trophoblast spheroid-OE-E6/7 cell co-culture). Previous exposure with *C. trachomatis* increased Fallopian tube/oviduct integrin-subunit beta-1 (ITGB1) in women and mice compared to controls. *C. trachomatis* increased OE-E6/E7 cell ITGB1 expression and promoted trophoblast attachment to OE-E6/E7 cells which was negated by anti-ITGB1-antibody. We demonstrate that infection with *C. trachomatis* increases tubal ITGB1 expression, predisposing to tubal embryo attachment and ectopic pregnancy.

Keywords: Ectopic pregnancy, *Chlamydia trachomatis,* Integrins, embryo implantation; Fallopian tube

Introduction

An ectopic pregnancy is a pregnancy that implants outside the main cavity of the uterus, most commonly in the Fallopian tube. It occurs in 1-2% of all pregnancies worldwide and remains the most common cause of maternal morbidity and mortality in the first trimester of pregnancy (Jurkovic and Wilkinson 2011). *Chlamydia trachomatis* (*C. trachomatis*) is the most prevalent curable bacterial sexually transmitted disease worldwide, with estimated incidence of greater than 100 million cases per year (WHO 2008). Epidemiological studies indicate that previous pelvic *C. trachomatis* infection is a major risk factor for ectopic pregnancy (Bakken et al. 2007). However, the mechanism by which *C. trachomatis* infection leads to tubal implantation is not understood and does not appear to be a direct consequence of tissue destruction by the organism (J. L. V. Shaw et al. 2011). We propose that *C. trachomatis* infection of tubal epithelial cells may alter their phenotype predisposing to ectopic embryo attachment and implantation later in a woman's reproductive life.

In the human uterus, the putative 'window of receptivity' to the embryo (that is required for successful intra-uterine implantation to occur), in the mid-luteal phase of the menstrual cycle, is accompanied by increased endometrial expression of integrin heterodimers, composed of the integrin subunits (ITG) alpha 1 (ITGA1), beta 1 (ITGB1), alpha 4 (ITGA4), alpha v (ITGAV) and beta 3 (ITGB3) (Lessey 1998). Integrins are a family of widely-expressed cell surface

receptors that mediate cell–cell and cell–extracellular matrix adhesion and, as a result, regulate many aspects of cell behavior. Twenty-four different integrin heterodimers are currently recognized in humans, each comprising a pair of non-covalently associated ITGA and ITGB subunits (Barczyk et al. 2010). In addition to providing a physical transmembrane link between the extracellular environment and the cytoskeleton, they are capable of transducing bi-directional signals across the cell membrane (Hynes 2002). Unlike the uterus, all five of the ITG markers of receptivity (ITGB1, ITGB3, ITGA1, ITGA4 and ITGAV) are constitutively expressed throughout the menstrual cycle in the Fallopian tube epithelium (Brown et al. 2012). We therefore hypothesized that previous infection with *C. trachomatis* may predispose to tubal implantation by increasing tubal integrin expression.

To address our hypothesis, we examined integrin transcript and protein expression in the Fallopian tube of women with serological evidence of previous infection with *C. trachomatis*. We then assessed integrin expression in response to *C. trachomatis* infection in the oviducts of mice and in human immortalized oviductal epithelial cells (OE-E6/E7). Finally, due to the lack of a good *in vivo* animal model of tubal ectopic pregnancy (in animals the abdominal cavity is the most frequent extra-uterine implanation site) (Brown and Horne 2011), we used an *in vitro* human trophoblast spheroid (embryo surrogate) – Fallopian tube epithelial cell co-culture model to investigate the effect of *C. trachomatis* exposure and functional blockage of integrin on embryo attachment.

Materials and Methods

Patient samples

Ethical approval for this study was obtained from the Lothian Research Ethics Committee (LREC 04/S1103/20, 05/S1103/14, 07/S1103/29), with informed, written consent obtained from all study participants. Serum samples and full thickness cross-sections of human Fallopian tube ampulla (total n=26) were collected from women undergoing hysterectomy for benign gynaecological conditions. This group of women had a regular 21-35 day menstrual cycle, were non-smokers, not using contraception and had no obvious evidence of FT pathology on microscopic examination (as assessed by an expert histopathologist). Fallopian tubes samples were saved either into RNAlater (Applied Biosystems, Warrington, UK) for RNA extraction or into neutral-buffered formalin (NBF) for paraffin embedding. Previous *C. trachomatis* infection was determined by an indirect enzyme-linked immunosorbent assay to serum Pgp3 antibody (Wills et al. 2009) with a cut-off value for absorbance at 450 nm of ≥0.473 giving ≥96% specificity (with an observed decline in seropositivity occurring following the last episode of chlamydial infection). Of the 26 women, 8 had serological evidence of previous *C. trachomatis* infection and 18 had no serological evidence of previous *C. trachomatis* infection.

Animal studies

The animal studies were approved by the Moredun Research Institute Ethics Committee and were conducted adhering to the institution's guidelines for

125 animal husbandry under licence from the UK Home Office. Eight week old
126 female C57/BL6 mice were infected with *C. trachomatis* (Figure 2a) following a
127 modified protocol published by Darville *et al* (Darville et al. 1997) and described
128 in more detail in Supplementary Information (*Animal Studies*).

129

130 *Isolation of DNA from vaginal swabs and quantitative real-time PCR*
131 DNA was extracted from vaginal swabs using a DNeasy® Blood and Tissue Kit
132 (Qiagen, Cat No. 69504) according to the manufacturer's instructions. Evidence
133 of infection with *C. trachomatis* was determined by TaqMan real-time PCR
134 using the *C. trachomatis* specific primers and probes (see Supplementary
135 Table 1) (Darville et al. 1997). DNA extraction and qRT-PCR methods are
136 described in detail in Supplementary Information (*Isolation of DNA from vaginal*
137 *swabs and quantitative real-time PCR)*.

138

139 *Quantitative reverse transcription PCR for integrin mRNA expression*
140 TaqMan real-time PCR (qRT-PCR) was performed to quantify mRNA
141 expression levels of human and mouse integrins using specific primers (see
142 Supplementary Table 1) following the protocol described in Supplementary
143 Information (*Quantitative reverse transcription PCR for integrin mRNA*
144 *expression*).

145

146 *Immunohistochemistry*
147 Immunohistochemistry for ITGB1 in human Fallopian tube samples and Itgb1
148 in mouse oviducts was carried out on NBF fixed paraffin wax embedded (FPE)
149 sections following our previously described protocol (Brown et al. 2012) and

detailed in Supplementary Information (*Immunohistochemistry*). The primary antibodies used to detect ITGB1 (both for human as well as human samples) were rabbit-anti-ITGB1 (Santa Cruz sc-8978, diluted 1:100) or isotype matched control (Rabbit IgG Dako X0903, diluted 1:100).

Histoscore calculation

Sections of immunohistochemical staining for ITGB1 were evaluated using semiquantitative histoscore analysis following previously described method which considers both the intensity and the percentage of cells stained in each of four intensity categories (McCarty et al. 1985). Intensities were classified as 0 (no staining), 1 (weak staining), 2 (strong staining) and 3 (very strong staining). For each stained section, a histoscore was obtained by application of the following algorithm: histoscore=$\sum(i+1) \times Pi$, where i and Pi represent intensity and percentage of cells that stain at each intensity, respectively, and corresponding histoscores were then calculated.

Quantitative dual-fluorescent western blot

Quantitative dual-fluorescent western blot was performed to quantify the ITGB1 and ITGB3 proteins in human Fallopian tube lysates following our previously established protocol (Brown et al. 2012) and detailed in Supplememntary Information (*Quantitative dual-fluorescent western blot*). Primary antibodies used to detect ITGB1 were rabbit-anti-ITGB1 (Santa Cruz sc-8978, dilution 0.5 µg/ml) and for ITGB3 were rabbit anti-ITGB3 (Santa Cruz sc-14009, dilution 0.5 µg/ml).

Oviductal epithelial OE-E6/E7 cell culture and C. trachomatis infection

Immortalised human oviductal epithelial OE-E6/E7 cells (sourced from KF Lee, Hong Kong) were maintained in DMEM/F12 containing 10% fetal bovine serum at 37°C, 5% CO_2. OE-E6/E7 cells were seeded at $5x10^5$ cells per well of a 12-well dish (BD Biosciences) and cultured for 24 hours. Cells were then washed with PBS and incubated overnight with serum-free DMEM/F12. The OE-E6/E7 cells (triplicate wells) were exposed to live *C. trachomatis* (serovar E) at MOI values of 0.1 and 1.0 in serum-free DMEM/F12. Control cells were cultured in medium alone. After 24 hours, medium was removed and the cells were treated with Qiagen RLT buffer and frozen at −80°C before RNA extraction.

Trophoblastic spheroid-oviduct epithelial-cell co-culture model

A previously established co-culture model using human immortalized Swan71 trophoblast cells (kind gift from V. Abrahams, Yale School of Medicine, CT) (Gipson et al. 2008) and human immortalized OE-E6/E7 oviductal epithelial cells, designed to simulate trophoblast attachment, was modified (Kodithuwakku et al. 2012a, Kodithuwakku et al. 2012b). Human immortalized were cultured in Dulbecco's Modified Essential Medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 2mM L-glutamine, penicillin/streptomycin (Invitrogen) and non-essential amino acids (Sigma). Swan71 cells are derived from first trimester trophoblasts and are well characterised (Straszewski-Chavez et al. 2009). Both cell lines were tested for mycoplasma prior to use. Swan71 cells were seeded at 2000 cells per well in a 96 well non-adherent round bottom tissue culture plate to encourage spheroid

development. During this time, confluent 12-well plates of OE-E6/E7 cells were washed and maintained in serum-free conditions. For *C. trachomatis* infection experiments, OE-E6/E7 cells were exposed to *C. trachomatis,* as described in the previous section. Triplicate wells were treated for 1 hour with 0.1 or 0.01 µg/ml mouse anti-ITGB1 (Clone P5D2: R&D Systems) or equivalent concentration of isotype-matched control IgG_1 (Sigma) prior to careful transfer of sixteen Swan71 spheroids onto the OE-E6/E7 monolayers and a further 6 hour incubation. Non-adherent spheroids were removed by gentle washing with PBS before the cells were fixed for 10 minutes in NBF, washed and stored in 70% ethanol. Swan71 spheroids adherence was quantified using light microscopy. Percentage adherence was derived by division of the number of spheroids attached by total number of spheroids.

Statistical analysis

Statistical analysis was performed using GraphPad PRISM, version 6.1. To allow for small sample sizes, non-parametric testing was applied to analysis of human and animal studies. As endometrial integrins are upregulated at the window of receptivity, data were interrogated to detect a significant increase in integrin transcript and protein levels using the one-tailed Mann Whitney test. For *in vitro* work, normality of data was tested using Shapiro-Wilk test and Kruskal-Wallis or one-way ANOVA accordingly applied, with correction for multiple comparisons by Dunn's or Dunnett's tests, respectively. Differences were considered significant if $P<0.05$.

Results

ITGB1 expression is increased in the Fallopian tube of non-pregnant women with evidence of previous C. trachomatis infection

We first investigated mRNA expression levels of ITGB1, ITGB3, ITGA1, ITGA4 and ITGAV in Fallopian tube from women with serological evidence of previous *C. trachomatis* infection (non-pregnant and non-smokers). We found that expression of ITGB1 mRNA was higher ($P<0.05$) in Fallopian tube from women with evidence of previous *C. trachomatis* infection (n=8) compared to those without (n=18) (Figure 1a). ITGB1 protein expression in Fallopian tube from women with previous *C. trachomatis* infection (n=7) correlated with ITGB1 mRNA levels ($R=0.442$, $P=0.026$), but changes in protein expression alone, compared to a control group (n=13), did not reach significance (Figure 1b). Immunohistochemistry demonstrated abundant Fallopian tube epithelium ITGB1 expression in women with previous *C. trachomatis* infection and mild stromal staining (n=8; Figure 1d, 1g). In contrast, in women without previous *C. trachomatis* infection (n=18), only sporadic cell staining was observed (Figure 1c, 1f). Semiquantitative histoscore analysis revealed a significant increase ($P<0.0001$) in ITGB1 expression in Fallopian tube epithelial cells in women with previous *C. trachomatis* infection as compared to women without previous *C. trachomatis* infection (Figure 1h). Although ITGB3 mRNA expression was increased ($P<0.05$) in women with previous *C. trachomatis* infection (Supplementary Figure 1a), ITGB3 protein levels did not show any significant changes (Supplementary Figure 1b) nor did they correlate with mRNA levels.

Tubal expression of ITGA1, ITGA4 and ITGAV were not affected by previous *C. trachomatis* infection (Supplementary Figure 1c, 1d and 1e).

Oviductal Itgb1 expression is increased by C. trachomatis in a mouse model of previous infection

To investigate causality between *C. trachomatis* infection and increased ITGB1, we developed an *in vivo* mouse model of previous *C. trachomatis* infection (Figure 2a). Female C57BL/6 mice were infected intra-vaginally with *C. trachomatis* and confirmed to have cleared the infection by day 30 post-infection by qRT-PCR detection of *C. trachomatis* genomic DNA (n=6) (Figure 2b). Mice infected with *C. trachomatis* displayed increased expression of oviductal Itgb1 mRNA compared to sham-infected controls (n=6) on day 60 post-infection ($P<0.05$) (Figure 2c). Immunohistochemistry to Itgb1 revealed strongly positive epithelial cells in the oviducts isolated from mice exposed to *C. trachomatis* (Figure 2e, 2h), with limited staining in the sham-infected mice (Figure 2d, 2g). Semiquantitative histoscore analysis revealed a significant increase ($P<0.05$) in ITGB1 expression in oviductal epithelial cells in mice exposed to C. trachomatis as compared to controls (Figure 1h). Exposure to *C. trachomatis* did not cause any significant changes in Itgb3 mRNA expression levels in murine oviducts (Supplementary Figure 2).

Exposure to C. trachomatis increases ITGB1 mRNA expression in human immortalised oviductal epithelial cells

ITGB1 mRNA expression in human immortalised oviductal epithelial OE-E6/E7 cells was significantly increased following 24 hours of exposure to 1.0

multiplicity of infection (MOI) *C. trachomatis* compared to control ($P < 0.05$) (Figure 3a). Exposure to 0.1 MOI *C. trachomatis* did not have any significant effect on ITGB1 mRNA expression.

C. trachomatis exposure increases trophoblast spheroid attachment to oviductal epithelial cells by upregulating ITGB1

There are no good animal models of tubal ectopic pregnancy, so to simulate embryo attachment we used an *in vitro* trophoblastic spheroid (embryo surrogate) - Fallopian tube epithelial-cell co-culture model. We demonstrated that 24 hours exposure of oviductal epithelial OE-E6/E7 cells to 1.0 MOI *C. trachomatis* significantly increased trophoblast spheroid attachment ($P<0.0001$) (Figure 3b). However, treatment of the *C. trachomatis* exposed OE-E6/E7 cells with 0.1 µg/ml ITGB1 neutralising antibody (dose selected following optimisation, data not shown) for 1 hour prior to trophoblast spheroid introduction, significantly reduced the numbers of spheroids that attached to the OE-E6/E7 monolayer compared with *C. trachomatis* exposed OE-E6/E7 cells ($P<0.0001$) and isotype control (IgG) exposed OE-E6/E7 cells ($P<0.0001$).

Discussion

It is accepted that *C. trachomatis* infection in women predisposes to tubal ectopic pregnancy; a relationship that continues for many years after the infection has resolved and that cannot be explained by macroscopic tissue damage as a result of inflammation (Barczyk et al. 2010, J. L. Shaw et al. 2011). In this study, we provide mechanistic evidence for changes in cell adhesion molecule expression that may explain this epidemiological association. Using *ex vivo*, animal *in vivo* and *in vitro* functional models, we demonstrate that previous exposure to *C. trachomatis* infection increases oviductal epithelial cell expression of the adhesion molecule ITGB1, predisposing to ectopic embryo attachment.

Fallopian tube from women with serological evidence of previous exposure to *C. trachomatis* expressed higher levels of ITGB1 mRNA, with abundant immunolocalisation of protein to the Fallopian tube epithelium. This upregulation of mRNA and localisation of protein was replicated in our *in vivo* model of previous *C. trachomatis* infection and in immortalised oviductal epithelial cells. Epithelium-specific expression is important in the context of ectopic pregnancy, as it is to these cells the embryo will initially attach *in vivo*. *In utero*, integrins are upregulated at the luminal surface of the endometrium during the window of implantation (Lessey et al. 1992) and interact with corresponding ligands on the blastocyst trophectoderm to enable attachment (Burrows et al. 1993). Through the use of our *in vitro* model of Fallopian tube -

313 embryo attachment, we have for the first time been able to show the effect of
314 over-expression of ITGB1 on embryo attachment. This model allows
315 investigation of causality in ectopic pregnancy which is not possible by
316 examining human biopsies of tubal implantation sites where molecular changes
317 may be an artefact of implantation and/or presence of an embryo as opposed
318 to a predisposition for ectopic implantation. In addition, in the absence of a good
319 animal model of tubal ectopic pregnancy, we have utilised an alternative *in vivo*
320 model, where mice are exposed to *C. trachomatis* and allowed to clear the
321 infection, to study the effects of *C. trachomatis* on the oviduct. The natural
322 history of untreated (or treated) pelvic chlamydial infection in women cannot be
323 observed for ethical and logistical reasons, and randomized controlled trials do
324 not provide this information because the time from the start of the infection is
325 unknown. We propose that further study using this model could significantly
326 contribute to improvements in clinical management of this prevalent infection
327 (Akande et al. 2010, Howie et al. 2011).

328

329 We acknowledge that our results demonstrate that Fallopian tube ITGB1
330 increases in response to *C. trachomatis* infection but do not explain how, the
331 effect endures following elimination of the infection in the face of oviductal
332 epithelial cell turnover and regeneration. This effect is also seen in ocular
333 trachoma where scarring progresses in the absence of detectable *C.*
334 *trachomatis* infection, raising uncertainty about the primary drivers of late-stage
335 trachoma (Burton et al. 2015). Persistence (where the organism adopts a
336 dormant state in the epithelial cells) occurs in a minority of *C. trachomatis*
337 infections and may contribute to some cases of ectopic pregnancy (Bjartling et

al. 2007). In addition, Kessler *et al* have recently demonstrated the existence of Fallopian tube stem cells, present along the Fallopian tube epithelial surface, with the ability to differentiate into an organoid containing both ciliated and secretory epithelial cell types in culture (Kessler et al. 2015). It would be interesting to discover if bacterial alterations to the genome of these cells by *C. trachomatis*, resulting in persistent ITGB1 upregulation, may account for the long-term increased risk of ectopic pregnancy.

We also acknowledge that further work is required to elaborate the full mechanistic pathway of Fallopian tube ITGB1 regulation by *C. trachomatis.* However, we propose that the utilization of host cell ITGB1 that we have observed in oviductal epithelial cells as a result of *C. trachomatis* infection, may be due to a shared bacterial virulence mechanism. *C. trachomatis* is an obligate intracellular, Gram-negative bacterium. *C. trachomatis* switches between an extracellular, metabolically inactive, infectious form, the elementary body (EB), and an intracellular replicative form, the reticulate body. Stallman and Hegemann have recently shown that *C. trachomatis* EBs produce the adhesin and invasin molecule Ctad1 (Stallmann and Hegemann 2016). This specifically binds ITGB1 on epithelial cells and induces clustering of ITGB1 at the epithelial cell membrane to allow EB entry into the host cell. Another Gram-negative bacterium, *Shigella*, upregulates expression of ITGB1 in epithelial cells ITGB1, in this case to stabilize intestinal epithelial cell adhesion to the extracellular matrix and prevent cellular detachment (Kim et al. 2009). *Shigellae* utilize the type III secretion system (T3SS) to introduce the effector protein OspE into the cell, and OspE interacts with the host-cells integrin-linked kinase ILK, which in

turn upregulates ITGB1 (Kim et al. 2009). *C. trachomatis* also makes use of the type III secretion system; a 'membrane-embedded nanomachine' that delivers virulence proteins into a host cell via a hollow needle which then hijack host cell machinery. Chemical inhibition of T3SS dramatically reduces *C. trachomatis* virulence (Muschiol et al. 2006). It is therefore possible that *C. trachomatis* shares a similar bacterial virulence mechanism and that small molecule inhibitors to such bacterial virulence factors might provide an effective preventative therapy for ectopic pregnancy in women previously infected with *C. trachomatis*.

In summary, we have shown that *C. trachomatis* upregulates oviductal epithelial ITGB1 expression which predisposes to ectopic embryo attachment. This provides an explanation for the epidemiological association between *C. trachomatis* and reproductive life-time risk of ectopic pregnancy. The pathways and mechanisms leading to long-term over-expression of ITGB1 require further study but may be a consequence of bacterial effector proteins hijacking cellular pathways to promote virulence leading to more complex disease outcomes.

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Conflicts of Interest

AWH has received consultancy payments from Roche, Ferring and Viramal for work in the field of endometriosis. AWH receives grant funding from Wellbeing of Women, the UK Medical Research Council (MRC), the UK National Institute for Health Research, and Ferring. HODC has clinical research support for laboratory consumables and staff from Bayer AG and provides consultancy advice (but with no personal remuneration) for Bayer AG, PregLem SA, Gedeon Richter, Vifor Pharma UK Ltd, AbbVie Inc, Myovant Sciences GmbH.

Authors Contributions

AWH and HODC designed the study. JKB, SFA, SMcF, MK, CO, GSW, MOM, SG and NW performed the experimental work. AWH, JKB, SFA, and LLC analysed the results and wrote the manuscript. NW, GE, PJH, KFL, HODC and WCD contributed to experimental design and critical feedback on manuscript.

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References

Akande, V., Turner, C., Horner, P., Horne, A., Pacey, A. and Soc, B. F. (2010) Impact of Chlamydia trachomatis in the reproductive setting: British Fertility Society Guidelines for practice. *Human Fertility, 13*(3), pp. 115-125.

Bakken, I. J., Skjeldestad, F. E. and Nordbo, S. A. (2007) Chlamydia trachomatis infections increase the risk for ectopic pregnancy: A population-based, nested case-control study. *Sexually Transmitted Diseases, 34*(3), pp. 166-169.

Barczyk, M., Carracedo, S. and Gullberg, D. (2010) Integrins. *Cell and Tissue Research, 339*(1), pp. 269-280.

Bjartling, C., Osser, S. and Persson, K. (2007) Deoxyribonucleic acid of Chlamydia trachomatis in fresh tissue from the Fallopian tubes of patients with ectopic pregnancy. *Eur J Obstet Gynecol Reprod Biol, 134*(1), pp. 95-100.

Brown, J. K. and Horne, A. W. (2011) Laboratory models for studying ectopic pregnancy. *Curr Opin Obstet Gynecol, 23*(4), pp. 221-6.

Brown, J. K., Shaw, J. L. V., Critchley, H. O. D. and Horne, A. W. (2012) Human Fallopian tube epithelium constitutively expresses integrin endometrial receptivity markers: no evidence for a tubal implantation window. *Molecular Human Reproduction, 18*(3), pp. 111-120.

Burrows, T. D., King, A. and Loke, Y. W. (1993) Expression of integrins by human trophoblast and differential adhesion to laminin or fibronectin. *Human Reproduction, 8*(3), pp. 475-84.

Burton, M. J., Rajak, S. N., Hu, V. H., Ramadhani, A., Habtamu, E., Massae, P., Tadesse, Z., Callahan, K., Emerson, P. M., Khaw, P. T., Jeffries, D., Mabey, D. C., Bailey, R. L., Weiss, H. A. and Holland, M. J. (2015) Pathogenesis of progressive scarring trachoma in Ethiopia and Tanzania and its implications for disease control: two cohort studies. *PLoS Negl Trop Dis, 9*(5), pp. e0003763.

Darville, T., Andrews, C. W., Laffoon, K. K., Shymasani, W., Kishen, L. R. and Rank, R. G. (1997) Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response. *Infection and Immunity, 65*(8), pp. 3065-3073.

Gipson, I. K., Blalock, T., Tisdale, A., Spurr-Michaud, S., Allcorn, S., Stavreus-Evers, A. and Gemzell, K. (2008) MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: In vitro evidence that MUC16 is a barrier to trophoblast adherence. *Biology of Reproduction, 78*(1), pp. 134-142.

Howie, S. E. M., Horner, P. J., Horne, A. W. and Entrican, G. (2011) Immunity and vaccines against sexually transmitted Chlamydia trachomatis infection. *Current Opinion in Infectious Diseases, 24*(1), pp. 56-61.

Hynes, R. O. (2002) Integrins: Bidirectional, allosteric signaling machines. *Cell, 110*(6), pp. 673-687.

Jurkovic, D. and Wilkinson, H. (2011) Diagnosis and management of ectopic pregnancy. *Bmj-British Medical Journal, 342*.

Kessler, M., Hoffmann, K., Brinkmann, V., Thieck, O., Jackisch, S., Toelle, B., Berger, H., Mollenkopf, H. J., Mangler, M., Sehouli, J., Fotopoulou, C. and Meyer, T. F. (2015) The Notch and Wnt pathways regulate stemness and

differentiation in human fallopian tube organoids. *Nat Commun, 6*, pp. 8989.

Kim, M., Ogawa, M., Fujita, Y., Yoshikawa, Y., Nagai, T., Koyama, T., Nagai, S., Lange, A., Fassler, R. and Sasakawa, C. (2009) Bacteria hijack integrin-linked kinase to stabilize focal adhesions and block cell detachment. *Nature, 459*(7246), pp. 578-U109.

Kodithuwakku, S. P., Pang, R. T., Ng, E. H., Cheung, A. N., Horne, A. W., Ho, P. C., Yeung, W. S. and Lee, K. F. (2012a) Wnt activation downregulates olfactomedin-1 in Fallopian tubal epithelial cells: a microenvironment predisposed to tubal ectopic pregnancy. *Laboratory Investigation, 92*(2), pp. 256-264.

Kodithuwakku, S. P., Pang, R. T., Ng, E. H., Cheung, A. N., Horne, A. W., Ho, P. C., Yeung, W. S. and Lee, K. F. (2012b) Wnt activation downregulates olfactomedin-1 in Fallopian tubal epithelial cells: a microenvironment predisposed to tubal ectopic pregnancy. *Laboratory Investigation, 92*(2), pp. 256-64.

Lessey, B. A. (1998) Endometrial integrins and the establishment of uterine receptivity. *Human Reproduction, 13*, pp. 247-258.

Lessey, B. A., Damjanovich, L., Coutifaris, C., Castelbaum, A., Albelda, S. M. and Buck, C. A. (1992) Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *J Clin Invest, 90*(1), pp. 188-95.

McCarty K. S., Jr, Miller L. S., Cox E. B., Konrath J. and McCarty K. S., Sr (1985) Estrogen receptor analyses. Correlation of biochemical and

immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med.* 109(8), pp. 716–721

Muschiol, S., Bailey, L., Gylfe, A., Sundin, C., Hultenby, K., Bergstrom, S., Elofsson, M., Wolf-Watz, H., Normark, S. and Henriques-Normark, B. (2006) A small-molecule inhibitor of type III secretion inhibits different stages of the infectious cycle of Chlamydia trachomatis. *Proc Natl Acad Sci U S A, 103*(39), pp. 14566-71.

Shaw, J. L., Wills, G. S., Lee, K. F., Horner, P. J., McClure, M. O., Abrahams, V. M., Wheelhouse, N., Jabbour, H. N., Critchley, H. O., Entrican, G. and Horne, A. W. (2011) Chlamydia trachomatis infection increases fallopian tube PROKR2 via TLR2 and NFkappaB activation resulting in a microenvironment predisposed to ectopic pregnancy. *American Journal of Pathology, 178*(1), pp. 253-60.

Shaw, J. L. V., Wills, G. S., Lee, K. F., Horner, P. J., McClure, M. O., Abrahams, V. M., Wheelhouse, N., Jabbour, H. N., Critchley, H. O. D., Entrican, G. and Horne, A. W. (2011) Chlamydia trachomatis Infection Increases Fallopian Tube PROKR2 via TLR2 and NF kappa B Activation Resulting in a Microenvironment Predisposed to Ectopic Pregnancy. *American Journal of Pathology, 178*(1), pp. 253-260.

Stallmann, S. and Hegemann, J. H. (2016) The Chlamydia trachomatis Ctad1 invasin exploits the human integrin beta1 receptor for host cell entry. *Cell Microbiol, 18*(5), pp. 761-75.

Straszewski-Chavez, S. L., Abrahams, V. M., Alvero, A. B., Aldo, P. B., Ma, Y., Guller, S., Romero, R. and Mor, G. (2009) The isolation and

characterization of a novel telomerase immortalized first trimester trophoblast cell line, Swan 71. *Placenta, 30*(11), pp. 939-48.

Wills, G. S., Horner, P. J., Reynolds, R., Johnson, A. M., Muir, D. A., Brown, D. W., Winston, A., Broadbent, A. J., Parker, D. and McClure, M. O. (2009) Pgp3 antibody enzyme-linked immunosorbent assay, a sensitive and specific assay for seroepidemiological analysis of Chlamydia trachomatis infection. *Clinical and Vaccine Immunology, 16*(6), pp. 835-43.

Figure Legends

Figure 1: The effect of previous *C. trachomatis* infection on Fallopian tube ITGB1 expression in women. (a) Box-and-whisker plots of relative levels of ITGB1 mRNA expression (measured by qRT-PCR) in Fallopian tube biopsies from non-pregnant, non-smoking women who tested negative (*Ct*–ve; n=18) or positive (*Ct*+ve; n=8) for previous *C. trachomatis* infection. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (*P<0.05, one-tailed Mann Whitney test). **(b)** Box-and-whisker plots of levels of ITGB1 protein (measured by western blot analysis) from the same women (where there was sufficient sample). The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (P=0.2, one-tailed Mann Whitney test). **(c)** and **(d)** Representative images of immunohistochemical localization of ITGB1 in Fallopian tube tissue from *Ct*–ve and *Ct*+ve women, respectively. Bar = 50μm. **(f)** and **(g)** Higher magnification of c and d respectively. Bar = 20μm. **(e)** Negative IgG control. Bar = 50μm. **(h)** Box and whicker plots of ITGB1 histoscore in Fallopian tube biopsies from women with and without previous *C. trachomatis* infection. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (**** P<0.0001, one tailed Mann Whitney test).

Figure 2: The effect of previous *C. trachomatis* infection on Itgb1 expression in the murine oviduct. (a) Schematic representation of *C. trachomatis* infection *in vivo* mouse model **(b)** *C. trachomatis* genome copy number (as a marker of infection) in C57/BL6 mice infected with 10^7 IFU of *C. trachomatis* Serovar E (filled circles) or vehicle alone (dashed line, indistinguishable from x-axis). **(C)** Box-and-whisker plots of relative levels of Itgb1 mRNA expression (measured by qRT-PCR) on day 60 post-infection in oviducts of control (*Ct*–ve; n=6) and infected (*Ct*+ve; n=6) mice. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (*P<0.05, one-tailed Mann Whitney test). **(d)** and **(e)** Representative images of immunohistochemical localization of Itgb1 in oviducts of *Ct*–ve and *Ct*+ve mice respectively. Bar = 50μm. **(g)** and **(h)** Higher magnification of c and d respectively. Bar = 20μm. **(f)** Negative IgG control. Bar =50μm. **(i)** Box and whicker plots of Itgb1 histoscore in oviducts of C. trachomatis infected mice as compare to controls. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (* P<0.05, one tailed Mann Whitney test).

Figure 3: Effect of *C. trachomatis* infection on immortalised human Fallopian tube epithelial OE-E6/E7 cells and an *in vitro* model of human embryo attachment. (a) Box and whisker plots of relative levels of ITGB1 mRNA expression (measured by qRT-PCR) in Fallopian tube epithelial OE-E6/E7 cells following exposure to *C. trachomatis* for 24 hours (MOI = multiplicity of infection). The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. Data are the mean of six biological replicates. (*P<0.05, Kruskal-Wallis test with Dunn's multiple comparisons post-test). **(b)** Trophoblast spheroid-oviductal epithelial cell attachment following 24 hours exposure to *C. trachomatis* +/- 1 hour pre-treatment with 0.1µg/ml anti-ITGB1 antibody. The box-and-whisker plots illustrate percentage adherence (number of spheroids attached/total number of spheroids) of SW-71 trophoblast spheroids to oviductal epithelial OE-E6/E7 cells. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. Data are the mean of four biological replicates. (**** P<0.0001, one-way Anova and Dunnett's multiple comparisons post-test).

Highlights

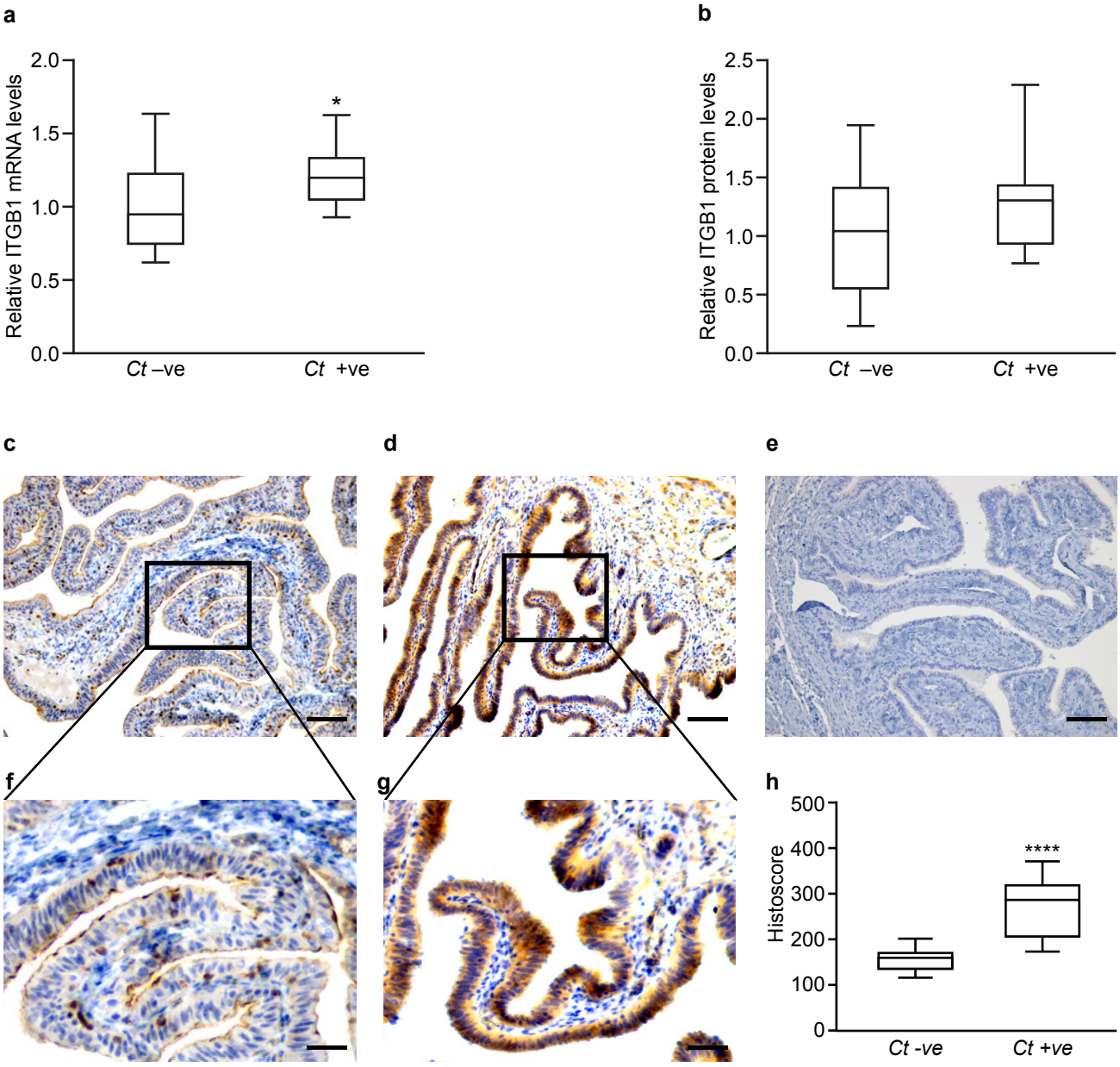
- Integrin subunit beta 1 is increased in Fallopian tubes of women and mice with evidence of past exposure to *C. trachomatis*.

- *C. trachomatis* increases integrin subunit beta 1 in oviductal epithelial cells and promotes trophoblast attachment.

- Functional blockage of integrin subunit beta 1 abrogates the attachment of trophoblast to oviductal epithelial cells.

Research in Context

We present exciting data, derived from a combination of ex-vivo, in-vivo and in-vitro models, to explain the mechanism behind the epidemiological association of past pelvic chlamydial infection and increased risk of tubal ectopic pregnancy. Our data demonstrate that past infection with *C. trachomatis* increases integrin subunit beta 1 expression in Fallopian tubes in women and in oviducts in mice. We also show that *C. trachomatis* promotes attachment in an embryo-surrogate co-culture tubal attachment model and that this effect is negated by functional blockage of the integrin subunit beta 1.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Relative ITGB1 mRNA levels
2.0
1.5
1.0
0.5
0.0
*
Ct –ve
Ct +ve
b
Relative ITGB1 protein levels
2.5
2.0
1.5
1.0
0.5
0.0
Ct –ve
Ct +ve
c
d
e
f
g
h
Histoscore
500
400
300
200
100
0
****
Ct -ve
Ct +ve

Figure 1.

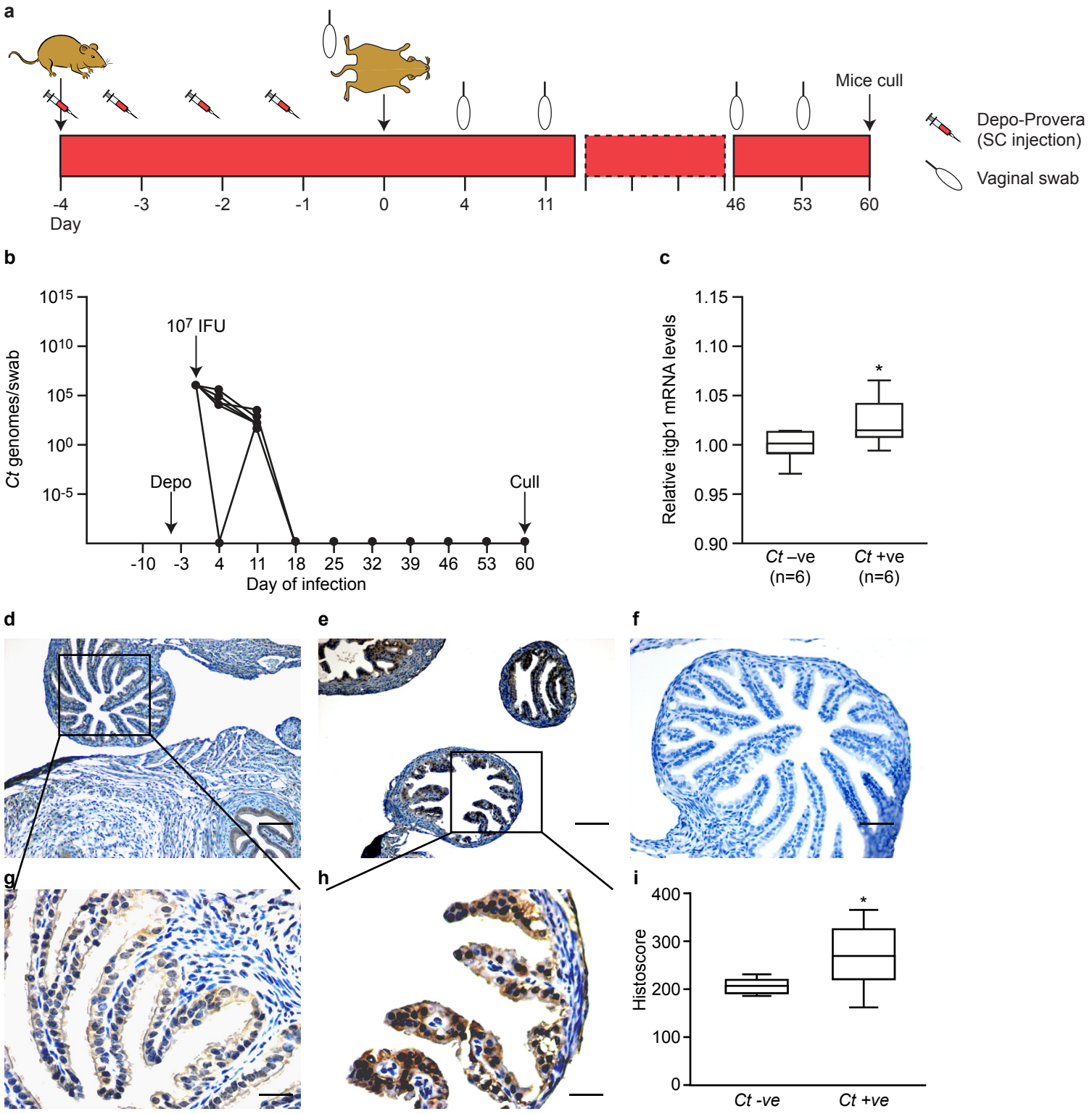

a
Mice cull
Depo-Provera
(SC injection)
Vaginal swab
-4
-3
-2
-1
0
4
11
46
53
60
Day
b
10^{15}
10^{10}
10^{5}
10^{0}
10^{-5}
10^{7} IFU
Depo
Cull
Ct genomes/swab
-10
-3
4
11
18
25
32
39
46
53
60
Day of infection
c
Relative itgb1 mRNA levels
1.15
1.10
1.05
1.00
0.95
0.90
*
Ct –ve
(n=6)
Ct +ve
(n=6)
d
e
f
g
h
i
Histoscore
400
300
200
100
0
*
Ct -ve
Ct +ve

Figure 2.

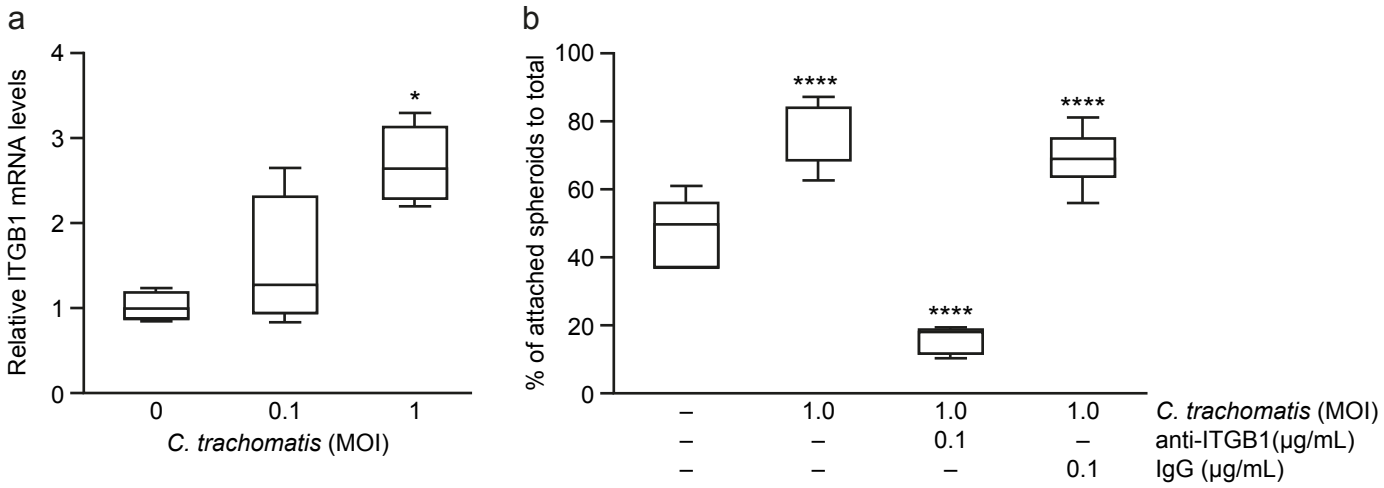

a
Relative ITGB1 mRNA levels
4
3
2
1
0
*
0
0.1
1
C. trachomatis (MOI)
b
% of attached spheroids to total
100
80
60
40
20
0
****
****
****
– 1.0 1.0 1.0 C. trachomatis (MOI)
– – 0.1 – anti-ITGB1(μg/mL)
– – – 0.1 IgG (μg/mL)

Figure 3.

Supplemental Information

Animal studies

Mice received 2.5 mg of medroxyprogesterone acetate (Depo-Provera) subcutaneously for 4 days to increase susceptibility to infection. Thereafter, 10^7 inclusion-forming units (IFU) of *C. trachomatis* human serovar E suspended in 30ul of vehicle (SPG: 250 mM sucrose, 10 mM sodium phosphate, 5 mM L-glutamic acid, pH 7.2), were introduced to the vaginal vault (n=6). Control animals (n=6) received vehicle alone. Mice were sacrificed 60 days from infection. With n=6 per group, we predicted that we would be able to detect a standardised difference of ≥1.85 with power of 90% at 5% significance level. Animals were randomly allocated between treatment and control groups, no animals were excluded from analysis and there was no investigator blinding.

Isolation of DNA from vaginal swabs and quantitative real-time PCR

Vaginal swabs were rehydrated by vigorous vortexing in 200µl of PBS prior to the addition of 200µl AL buffer. The samples were vortexed again before the addition of 20µl proteinase K solution (supplied with kit) and incubation for 10 minutes at 70°C. 200µl of 100% ethanol was added to each of the samples before the mixture was added directly onto a DNeasy® column. For the remaining stages of the protocol, the DNA was prepared on the column as described by the manufacturer and finally eluted in a total volume of 200 µl elution buffer. Quantitative real-time PCR was carried out using the primers and probe specific for Ch23S (Supplementary table 1) (Darville et al. 1997). The PCR consisted of 12.5 µl 2X TaqMan® Universal master mix II

(Invitrogen, Warrington, United Kingdom), 900 nM of each primer, 250 nM fluorescent probe and 1µl gDNA, made up to a final volume of 25 µl with sterile deionised water. Amplification and detection were performed using an ABI Prism 7500 sequence detection system (Invitrogen), following the manufacturer's standard protocols. Thermal cycling conditions were: 50°C for 2 min, 95°C for 10 min, then 45 cycles of 95°C for 15 sec and 60°C for 1 min. Genome copy numbers were quantified against a standard curve prepared from *C. abortus* genomic DNA as previously described (Livingstone et al. 2009). Each sample, standard and non-template control was examined in triplicate.

Quantitative reverse transcription PCR for integrin mRNA expression

RNA was extracted and treated with DNase using a RNeasy fibrous tissue mini kit (Qiagen). cDNA was synthesised from 200ng of RNA using SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). TaqMan real-time PCR (qRT-PCR) was used to quantify levels of integrin, housekeeping (UBC and GAPDH, predetermined by geNorm assay, Primerdesign Ltd) and loading control (18s) transcripts. Specific primers (Supplementary Table 1) were designed using the Universal Probe Library Assay Design Center (www.roche-applied-science.com) and used in conjunction with Universal Probe Library (UPL) FAM labelled probes (Roche Applied Science, Burgess Hill, UK) or purchased as validated primer-probe sets (Perfect-Probe: Primer Design, UK). Reactions were performed in triplicate, using 18s to control for loading variation (FAM labelled probe), under standard conditions in an ABI Prism 7900 (Invitrogen). 18s–integrin ΔCt values obtained were normalised against mean values of 18s-UBC and 18s-GAPDH ΔCt, prior to normalisation against a cDNA positive control (Fallopian tube from a post-menopausal patient). For mouse samples, integrin gene expression

was normalized to GAPDH expression, using the 2-ΔΔCt method, and then normalised against pooled Fallopian tube cDNA from the control group.

Immunohistochemistry

5μm sections of paraffin embedded tissue (Human Falloipan tube and mouse oviducts) were mounted onto microscope slides, dewaxed and rehydrated, before antigen retrieval in 10mM Tris 1mM EDTA pH 9 with 5 min of pressure-cooking. Slides were washed, incubated with 3% hydrogen peroxide for 30 min, then blocked in normal horse serum diluted 1:12 in TBS-T20 for 30 min. Slides were incubated with primary antibody overnight at 4°C (anti-ITGB1 Santa Cruz sc-8978, diluted 1:100) or isotype matched control (Rabbit IgG Dako X0903, diluted 1:100). They were washed in TBS-T20 before incubation with species specific impress kit for 30 min at room temperature (Vector Laboratories, Peterborough, UK). After washing and incubation with 3, 3'-diaminobenzidine for 5 min, slides were counterstained with hematoxylin, dehydrated and visualized by light microscopy, using an Olympus Provis microscope equipped with a Kodak DCS330 camera (Olympus Optical Co., London, UK, and Kodak Ltd., Herts, UK).

Quantitative dual-fluorescent western blot

50mg wet tissue per sample of Fallopian tube was stabilized in RNALater and was homogenized in 1ml pH 8.0 lysis buffer (50mM Tris-HCl; 150mM NaCl; 1mM EDTA; 1% Triton-X100, 1% Na-deoxycholate; EDTA-free complete mini protease inhibitors (Roche Diagnostics, Welwyn Garden City, UK); and Halt Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Loughborough, UK)) using a TissueLyser bead mill

(Qiagen). Protein quantification was performed by Bradford Assay, adapted for the Cobas Fara centrifugal analyzer (Roche Diagnostics), and samples adjusted to 2mg/ml total protein in lysis buffer, before further 1:1 dilution in 2x NuPAGE LDS sample buffer (Invitrogen) containing 100mM DTT (Sigma). Gel electrophoresis (1DGE) was performed in 15-well NuPAGE 4-12% Bis-Tris gels (Invitrogen) using 20 µg of total protein/lane alongside SeeBlue® Plus2 pre-stained molecular weight standards (Invitrogen). A positive control (Fallopian tube from a post-menopausal patient) was included in every gel to allow intra-blot comparisons to be made. Gels were equilibrated for 15 minutes in transfer buffer (50 mM Tris, 40 mM Glycine, 0.05% SDS), before blotting at 20V (limited to 80 mA/gel) onto polyvinylidene fluoride membrane (Immobilon P: Millipore, Livingston, UK) in the presence of transfer buffer + 10% methanol using a Transblot SD (Bio-Rad Laboratories, Hemel Hempstead, UK). Blots were then blocked for 30 minutes in TBS-T20 (TRIS-buffered saline containing 0.5% Tween20, pH 7.4) + 2% Marvel (Premier Foods, St Albans, UK) and incubated for 2 hours with combinations of 0.5 µg/ml rabbit anti-ITGB1 (Santa Cruz sc-8978) or rabbit anti-ITGB3 (Santa Cruz sc-14009) together with 0.5 µg/ml mouse anti-GAPDH and anti-β actin and/or negative control antibodies (Abcam) diluted in TBS-T20 + 2% Marvel. Blots were then washed in TBS-T20 (6 x 3 minutes) and incubated for 1 hour with ImmPRESS anti-rabbit Ig peroxidase Polymer Detection Kit (Vector Laboratories, Peterborough, UK) (diluted 1/250) and 0.5 µg/ml of goat anti-Mouse DyLight-488 in TBS-T20 + 2% Marvel. Blots were then washed (6 x 3 minutes) in TBS-T20 and DyLight-488 and Cy5 labelling imaged using a FLA-5100 (FUJIFILM Europe GmbH, Düsseldorf, Germany). Protein band intensities were measured using ImageJ software (Schneider et al. 2012). After normalizing against the mean value of GAPDH and β-actin, values for integrin-specific labelling were expressed relative to the positive

control. After imaging, blots were stained with Imperial Protein Stain (Fisher Scientific UK, Loughborough, UK) to confirm uniform blotting efficiency.

Supplementary Figure Legends

Supplementary Figure 1: The effect of *C. trachomatis* infection on Fallopian tube expression of integrin endometrial receptivity markers in women. Box-and-whisker plots illustrating relative levels of integrin mRNA expression (measured by qRT-PCR) and protein levels (measured by western blot analysis) in Fallopian tube biopsies from non-pregnant, non-smoking women who tested negative (Ct-ve; n=18) or positive (Ct+ve; n=8) for previous *C. trachomatis infection*. (a) mRNA expression levels of ITGB3. (b) protein levels (where sample sufficient) of ITGB3. (c) (d) and (e) mRNA expression levels of ITGA1, ITGA4 and ITGAV, respectively. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. *P<0.05 (one-tailed Mann Whitney test).

Supplementary Figure 2: The effect of previous *C. trachomatis* infection on Itgb3 mRNA levels in the murine oviduct. C57/BL6 mice were infected with 10^7 IFU of *C. trachomatis* Serovar E or vehicle alone, and levels of *C. trachomatis* genomic DNA monitored until not detectable (day 30). Oviducts were then collected for integrin expression analysis. Box-and-whisker plots of show relative Itgb3 mRNA levels on day 60 post-infection in oviducts of control (Ct –ve; n=6) and infected (Ct +ve; n=6) mice. The boxes represent mean values ±1 standard deviation and the whiskers denote the full range of the data. (P=0.1526, one-tailed Mann Whitney test).

Supplementary Table 1. qRT-PCR primer and probe sequences

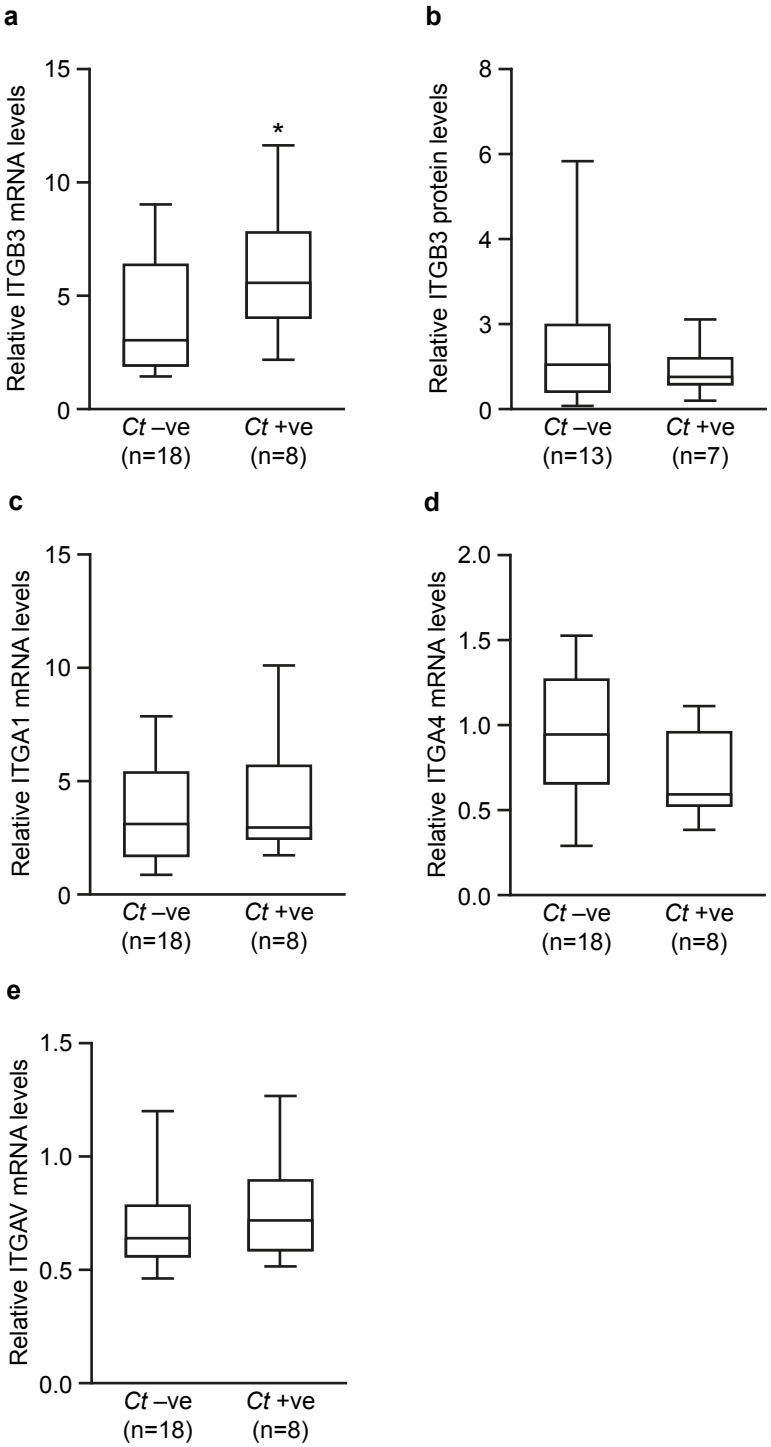
Ch23S forward	5′-CTGAAACCAGTAGCTTATAAGCGGT-3′
Ch23S reverse	5′-ACCTCGCCGTTTAACTTAACTCC-3′
Ch23S probe (FAM)	CTCATCATGCAAAAGGCACGCCG-TAMRA
Human ITGA1 forward	5′-AATTGGCTCTAGTCACCATTGTT-′3
Human ITGA1 reverse	5′-CAAATGAAGCTGCTGACTGGT-′3
Human ITGA1 UPL probe (FAM)	14
Human ITGA4 forward	5′-GGAATATCCAGTTTTTACACAAAGG-′3
Human ITGA4 reverse	5′-AGAGAGCCAGTCCAGTAAGATGA-′3
Human ITGA4 UPL probe (FAM)	57
Human ITGAV forward	5′-GCCGTGGATTTCTTCGTG-′3
Human ITGAV reverse	5′-GAGGACCTGCCCTCCTTC-′3
Human ITGAV UPL probe (FAM)	64
Human ITGB1 forward	5′-CGATGCCATCATGCAAGT-′3
Human ITGB1 reverse	5′-ACACCAGCAGCCGTGTAAC-′3
Human ITGB1 UPL probe (FAM)	65
Human ITGB3 forward	5′-GGGCAGTGTCATGTTGGTAG-′3
Human ITGB3 reverse	5′-CAGCCCCAAAGAGGGATAAT-′3
Human ITGB3 UPL probe (FAM)	13
Mouse ITGB1 forward	5′- CTGCTTCTAAAATTGAGATCAGGA-′3
Mouse ITGB1 reverse	5′- TCCATAAGGTAGTAGAGATCAATAGGG-′3
Mouse ITGB1 UPL probe (FAM)	41
Mouse ITGB3 forward	5′- GTGGGAGGGCAGTCCTCTA-′3
Mouse ITGB3 reverse	5′- CAGGATATCAGGACCCTTGG-′3
Mouse ITGB3 UPL probe (FAM)	31
Human 18S (VIC)	Applied Biosystems (4308329)
Human GAPDH (FAM)	Primer Design (HK-PP-hu-600)
Human UBC (FAM)	Primer Design (HK-PP-hu-600)
Mouse GAPDH (VIC)	Applied Biosystems (4352339E)

Supplementary References

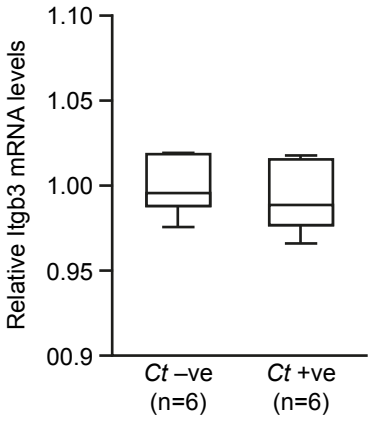
Darville, T., Andrews, C. W., Laffoon, K. K., Shymasani, W., Kishen, L. R. and Rank, R. G. (1997) Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response. *Infection and Immunity, 65*(8), pp. 3065-3073.

Livingstone, M., Wheelhouse, N., Maley, S. W. and Longbottom, D. (2009) Molecular detection of Chlamydophila abortus in post-abortion sheep at oestrus and subsequent lambing. *Veterinary Microbiology, 135*(1-2), pp. 134-141.

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), pp. 671-675.



Supplementary figure 1.



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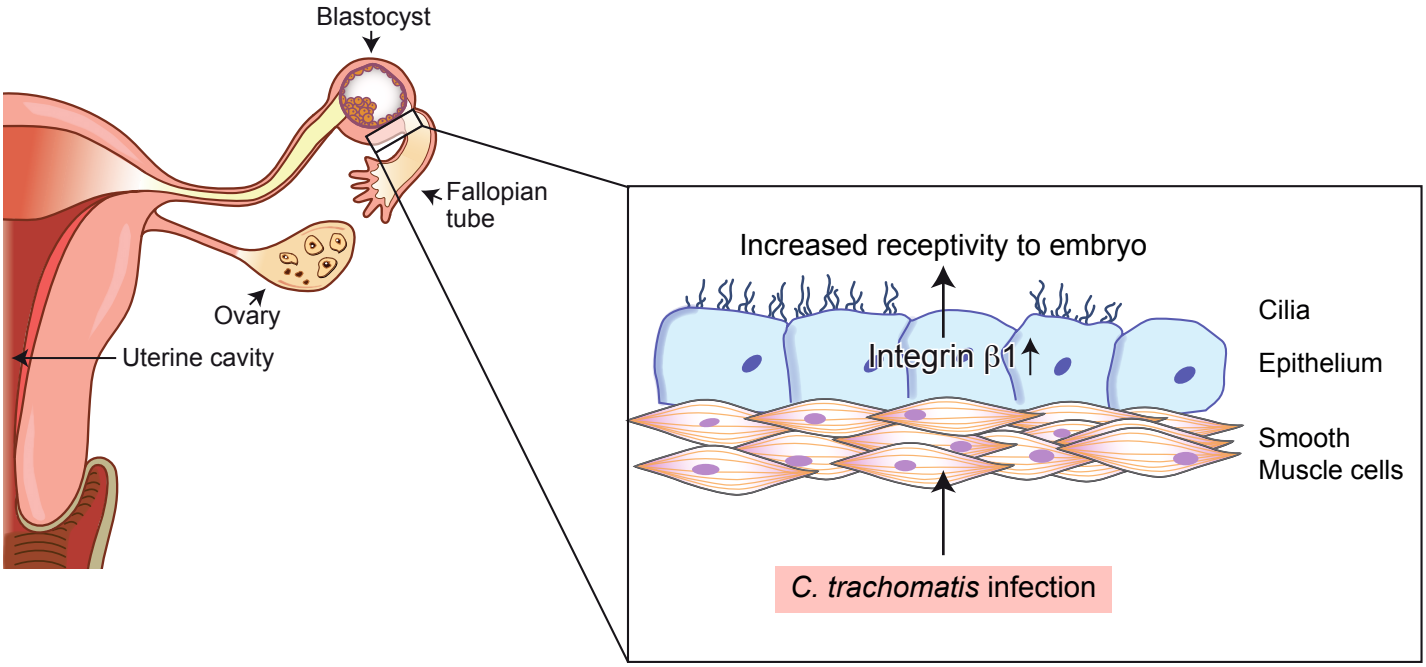
Blastocyst
Fallopian tube
Ovary
Uterine cavity
Increased receptivity to embryo
Integrin β1
Cilia
Epithelium
Smooth Muscle cells
C. trachomatis infection