University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Menkhorst, EM;Gamage, T;Cuman, C;Kaitu'U-Lino, TJ;Tong, S;Dimitriadis, E

Title:
Galectin-7 acts as an adhesion molecule during implantation and increased expression is associated with miscarriage

Date:
2014-03-01

Citation:
Menkhorst, E. M., Gamage, T., Cuman, C., Kaitu'U-Lino, T. J., Tong, S. & Dimitriadis, E. (2014). Galectin-7 acts as an adhesion molecule during implantation and increased expression is associated with miscarriage. Placenta, 35 (3), pp.195-201. https://doi.org/10.1016/j.placenta.2014.01.004.

Publication Status:
Accepted manuscript

Persistent Link:
https://hdl.handle.net/11343/41905

ACCEPTED MANUSCRIPT

Galectin-7 acts as an adhesion molecule during implantation and increased expression is associated with miscarriage

Ellen M Menkhorst[1,2]*, Thilini Gamage[1,2], Carly Cuman[1,2], Tu'uhevaha Kaitu'u-Lino[3], Stephen Tong[3], Evdokia Dimitriadis[1,,2,4]

1. Embryo Implantation Laboratory, Prince Henry's Institute, Clayton, VIC, Australia.
2. Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia.
3. Obstetrics and Gynecology, University of Melbourne, Melbourne, Australia.
4. Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

*Address for correspondence:

Ellen Menkhorst

Prince Henrys Institute of Medical Research,

PO Box 5152, Clayton Vic 3168 Australia

Phone: +61-3- 9594 4392

Fax: +61-3- 9594 6125

Email: ellen.menkhorst@princehenrys.org

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abstract

Introduction: Galectins are expressed at the fetal-maternal interface and have multiple roles including during blastocyst implantation. The expression of galectin-7 however has not been investigated in the uterus. We aimed to localise galectin-7 to the endometrium of women with normal fertility and with a history of miscarriage and prospectively determine whether serum levels are altered in women who subsequently miscarry. We also investigated the role of galectin-7 on trophoblast–endometrial epithelial cell adhesion.

Methods: Immunohistochemistry localised galectin-7 to endometrium throughout the menstrual cycle in women (normal fertility or with history of miscarriage) and in first trimester implantation sites. Galectin-7 serum levels were determined by ELISA. We used both endometrial epithelial–trophoblast cell lines and primary cells for cell-cell adhesion experiments.

Results: Galectin-7 immunolocalized to endometrial luminal and glandular epithelium in normally fertile women and was upregulated in epithelium and stroma of women with a history of miscarriage. Similarly, gelectin-7 serum levels were elevated at 6 weeks gestation in women who subsequently miscarried compared to gestation matched controls. Exogenous galectin-7 reduced endometrial epithelial–trophoblast adhesion in cell-line and primary cell assays. However, when endometrial epithelial cells were isolated from women with endometrial disorders, galectin-7 increased epithelial–trophoblast adhesion.

Conclusions: Galectin-7 is produced by endometrial epithelium and is abnormally elevated in the endometrium of women with a history of miscarriage. Serum levels may be useful as a predictive biomarker of miscarriage. Our data suggests that galectin-7 facilitates adhesion of the embryo to the endometrium and upregulated galectin-7 may result in abnormal adhesion.

Keywords: trophoblast; endometrium; uterus; luminal epithelium; blastocyst; pregnancy

Introduction

During the initiation of pregnancy, the human blastocyst must implant into the uterus in order to facilitate the formation of a functional placenta. Implantation involves the blastocyst apposing then adhering to the uterine luminal epithelium before trophoblast cells migrate and invade through the endometrial decidua to engraft and remodel maternal spiral arteries. Inadequate, or inappropriate implantation can lead to recurrent miscarriage, placental insufficiency and other obstetric complications (1, 2).

Implantation is initiated during the 'window of implantation', a specific period during the menstrual cycle where the uterus is receptive to the blastocyst (3). To become receptive, the luminal and glandular epithelium undergo significant changes, particularly with respect to their secretory capacity and adhesion molecule expression (3), facilitating the adhesion of two epithelial surfaces; the blastocyst trophectoderm and the uterine luminal epithelium. Adhesion molecules such as mucins, glycoconjugates, trophinin, cadherins and integrins are all regulated during the window of implantation (3).

While implantation failure results in infertility, impaired implantation results in inadequate placentation and associated conditions including early miscarriage (4). More recently, it has been proposed that recurrent miscarriage [defined as 3 or more consecutive miscarriages (5)] is associated with abnormalities in endometrial receptivity that lead to inappropriate implantation and subsequent miscarriage (6). Certainly, both the endometrial epithelium and decidua are altered in women with recurrent miscarriage, including aberrant expression of adhesion molecules by the endometrial epithelium (5, 7) and impaired decidualization (8).

Galectins are animal lectins which bind to surface glycoproteins, with preferential binding to β-galactoside (9). Galectins regulate many cell functions important for implantation such as cell-adhesion and migration, signal transduction, apoptosis, immune cell activation, differentiation and hormone production (9-12). The galectin family has many and varied roles in reproduction (13, 14), including during implantation. For example, galectin-3 is

upregulated in the human endometrial epithelium during the secretory phase (15) and in mice decreased implantation is found following tissue specific knockdown of galectin-3 (16). Further, an association with spontaneous abortion and a splice variant of galectin-9 is found in both mice and women (17), indicating critical roles for at least these lectins in implantation.

Galectin-7 is a 15kDa protein originally identified in the epidermis. It is generally expressed by epithelial cells and localizes to areas of cell-cell contact (9). It is a prototype galectin found as both a monomer and a dimer (18); unlike most mammalian galectins it is known to exist as a monomer in solution (18). Galectin-7 has recently been identified in 1st trimester human endometrial epithelial cells and the syncytiotrophoblast and extravillous trophoblast (Menkhorst et al under review) however to date it has not been investigated in the cycling endometrium.

Galectin-7 is secreted by keratinocytes (19). However, like all galectins its sequence has no typical secretion signal peptide. It is released out of cells through an unusual route which requires intact carbohydrate-binding activity of the secreted protein (12). Galectin-7 has a well characterized role in wound healing; it accelerates re-epithelialisation of corneal wounds more efficiently than most known growth factors (20, 21).

We hypothesized that as an adhesion molecule, epithelial galectin-7 would be important in facilitating blastocyst implantation. We aimed to identify galectin-7 expression in the endometrium during the menstrual cycle of normally fertile women and women who have a history of miscarriage, to see whether tissue and serum levels of galectin-7 are associated with previous miscarriage or predictive of subsequent miscarriage and to investigate the function of galectin-7 during implantation.

Methods

Ethical approvals

This study was approved by the Monash Health Human Research and Ethics Committee (#09317B; #06014C) and the Mercy Hospital for Women Ethics Committee (R03/23). Written and informed consent was obtained from each patient.

Endometrial samples

Endometrial samples (Table 1) from normally fertile women (3-6 samples per stage of the menstrual cycle), women who have a history of miscarriage (3-5 samples per stage of the menstrual cycle except late secretory) and women with unexplained primary infertility (1-3 samples per stage of the menstrual cycle except mid-secretory) were obtained from women undergoing unrelated surgical procedures. The average age of the women was not different between groups (Table 1). The median number of miscarriages that the miscarriage cohort had undergone was 3.0 and the range was 2-12. In 3 cases the women had experienced 2 miscarriages.

First trimester placenta samples

First trimester placental tissue was collected from healthy women undergoing termination of pregnancy for psychosocial reasons (amenorrhea 6-12 weeks). The tissues were examined by an experienced pathologist at Monash Health and showed no pathologies. Implantation sites – sites where the placental villous was adhered to the decidua, were chosen for immunohistochemical analysis (n=4).

Serum samples from pregnant women

The serum used in this study was from a Biobank of serum samples prospectively collected from the mid to late first trimester specifically to investigate miscarriage (Table 2). Serum was collected from pregnant women between weeks 6-10 of gestation as previously described (22). Importantly, fetal cardiac activity was confirmed by ultrasound at the time of

blood collection. From the biobank of samples, 109 controls were chosen from pregnancies that went on to have a healthy live birth and 18 miscarriage samples were chosen. Miscarriage was defined as the loss of the pregnancy at less than 20 weeks gestation (22).

Galectin-7 immunohistochemistry

Formalin-fixed cycling endometrium or placental villous 5µm sections on poly-l-lysine (Sigma) coated glass slides were dewaxed in histosol and rehydrated in ethanol. Antigen retrieval was performed in 0.01M citrate buffer (pH 6) before endogenous peroxidise activity was quenched by incubation in 3%H_2O_2/Methanol for 10min. Sections were blocked in non-immune serum (10% normal horse serum, 2% normal human serum in Tris buffered saline [TBS]) for an hour before application of the primary antibody in non-immune serum (galectin-7, R&D Systems, AF1339, 1µg/ml; Goat IgG, R&D Systems, 1µg/ml) overnight at 4°C. Slides were washed in 0.6% Tween-20 (Sigma) in TBS before incubation in the secondary antibody (Biotinylated horse anti-goat, 1:200) for 30 min at RT. Slides were again washed before addition of streptavidin-biotin complex/HRP (Vector) for 30 min at RT. Sections were stained with the substrate 3'3-diamionbenzidine (K3466, DAKO) and counterstained with haematoxylin. Quality controls were included in each run.

Immunostaining intensity in the endometrium was analysed semiquantitively by two independent and blinded observers as previously described (23). The intensity of galectin-7 staining in the endometrium was assessed and allocated a score between 0 (no staining) and 3 (strong staining) relative to positive and negative controls.

Galectin 7 ELISA

The serum concentration of galectin-7 was assayed using the RayBio® Human Galectin-7 ELISA (ELH-Galectin7-001) according to the manufacturer's instructions. Briefly, neat serum diluted 1:4 with diluent (provided) was incubated on a rotating shaker for 2h before binding

was detected using kit components. The plate was read at 450nm (Envision, Perkin Elmer) and the data analysed using the RayBio ELISA analysis tool (Excel). The minimum detection limit for galectin-7 was 41.5pg/ml.

Isolated primary cells and cell lines

Cell lines

Two cell lines were chosen to model blastocyst attachment: HTR8/ Svneo, derived from first trimester villous trophoblast with characteristics of villous cytotrophoblast and extravillous trophoblast (24); ECC1 derived from an adenocarcinoma with characteristics of the luminal epithelium (24). These cells were grown to sub-confluence in a 12 well plate (n=4 experiments) in RPMI (Gibco) or DMEM/F12 (Gibco) respectively containing 1% antibiotics and antimycotic (penicillin, streptomycin, amphoceterin B; Gibco) and 10% fetal calf serum (Invitrogen) in a 5% CO_2 incubator at 37°C.

Human endometrial epithelial cell isolation

Human endometrial epithelial cells (hEEC) were isolated from endometrial biopsies (n=10) as previously described (25). As limited samples were available at any one time, the tissues were pooled to obtain a confluent monolayer of cells. The pools therefore contained cells obtained from both fertile and infertile women. We have classed these pools into two groups: 1. normally fertile women pooled with women with unexplained primary infertility (n=2 pools) or 2. women with diagnosed endometrial disorders (miscarriage, endometriosis, irregular bleeding) pooled with women with unexplained primary infertility (n=3 pools).

Human villous trophoblast cell isolation

Villous cytotrophoblast were isolated from normal first trimester placental tissue as previously described (26). The cells were cultured on Matrigel$^{(TM)}$ (BD) for 72hr before

treatments. The purity of primary trophoblasts (hT) was confirmed by immunocytochemistry for HLAG (BD Biosciences, CA, USA) as previously described (26).

Cell-cell adhesion assays

Galectin-7 regulation of adhesion was performed using a cell-cell adhesion assay as previously described (25, 27). Briefly, trophoblast (HTR8/Svneo) and epithelial (ECC1) cell lines or hEEC or hT were grown to confluence (except hT which do not proliferate when plated on Matrigel) in DMEM/Hams F12 containing 10% fetal calf serum and antibotics, then treated with galectin-7 (1µg/ml) or vehicle control (BSA, 0.1%) in serum-free media for 18h. The concentration of galectin-7 used was based on the manufacturer's recommendations and determined from preliminary studies. Trophoblast cells were labelled with 4µM calcein-AM (Molecular Probes) and plated on top of the confluent epithelial cell monolayer (HTR8/Svneo: $5x10^4$ cells; hT $8x10^3$ cells). Trophoblast cells were allowed to adhere at 37°C for 90 min before non-adhered cells were removed by several washes with phosphate buffered saline containing calcium and magnesium. Adhered trophoblast was measured by fluorescence using a fluorscein set of excitation 485 and emission 535 (Envision, Perkin Elmer).

Statistics

All statistical analyses were performed using GraphPad Prism (GraphPad, SanDiego, CA, US). T-tests were used to compare galectin-7 staining intensity and galectin-7 serum concentration. One-way ANOVAs were used to compare cell-cell adhesion. Mann-Whitney tests were used to compare maternal age, gravidity and number of miscarriages between normally fertile and history of miscarriage groups. All data except for patient characteristic tables is shown as mean$\pm$SEM and a $p<0.05$ was considered statistically significant.

Results

Galectin 7 expression was upregulated in the non-pregnant endometrium of women with a history of miscarriage

Non-pregnant endometrium

Galectin-7 protein localized to the uterine luminal and glandular epithelium but not to the stroma during the proliferative, early-, mid- and late-secretory phases of the menstrual cycle (Figure 1A-D respectively). There was no difference in staining intensity between the stages.

Non-pregnant endometrium of women with a history of miscarriage

We compared the galectin-7 endometrial staining intensity in non-pregnant, normally fertile women to non-pregnant women who had previously had at least two miscarriages. Endometrial galectin-7 staining intensity was significantly higher in women who had previously had at least two miscarriages compared to normally fertile women (Figure 1E-J): in the luminal epithelium during the proliferative and early secretory phases (Figure 1H; $p<0.05$) and the glandular epithelium during the proliferative, early and mid-secretory phases (Figure 1I; $p<0.05$). Stromal staining was essentially absent in normally fertile women but present in the proliferative and early- and mid-secretory phases (significantly elevated during the early-secretory phase) in women who had previously undergone miscarriage (Figure 1J; $p<0.05$). In comparison, galectin-7 staining in women with unexplained primary infertility clustered with normally fertile women (Figure 1H-J).

Galectin-7 serum concentration was predictive of miscarriage at 6 weeks gestation.

To determine whether galectin-7 serum concentration could predict subsequent miscarriage, the concentration of galectin-7 was measured in serum from non-pregnant women and serum prospectively collected across weeks 6-10 of pregnancy (Figure 2A). Serum concentration of galectin-7 did not change across gestational weeks 6-10 in normal pregnancies (Figure 2A). At week 6, the serum concentration of galectin-7 was significantly

higher in women who went on to miscarry before 20 weeks than those that had a normal pregnancy (Figure 2B; $p<0.05$).

Galectin-7 strongly localized to the region of placental-endometrial attachment

As galectins are implicated in cell-cell interactions we investigated whether galectin-7 was present in vivo in the region of trophoblast-endometrial attachment and invasion. Immunohistochemistry of first trimester placenta showed strong localization of galectin-7 to the syncytiotrophoblast in the placenta and to EVTs in the cell column (Figure 3).

Galectin-7 regulates trophoblast – epithelial adhesion

Given that galectin-7 strongly localized to the region of fetal-maternal attachment, we aimed to model attachment of trophoblast to the endometrium *in vitro* using a cell-cell adhesion assay. Epithelial (ECC1 cell line, hEEC primary cells) and trophoblast (HTR8/Svneo cell line, hT primary cells) cells were pre-treated with soluble galectin-7 before trophoblast cells were trypsinized, plated on top of the epithelial cell monolayer and allowed to adhere. Treatment of ECC1 or HTR8/Svneo cells with galectin-7 had no effect on HTR8/Svneo-ECC1 adhesion (Figure 4A), however combined treatment of HTR8/Svneo and ECC1 cells with galectin-7 significantly suppressed adhesion (Figure 4A; $p<0.05$). We validated this adhesion data using primary cells: endometrial epithelial cells (hEEC) and trophoblast (hT). Because of the difficulty in obtaining enough hEEC cells to form a pure, confluent monolayer, cells were pooled from different women who had various reproductive pathologies (normally fertile, primary unexplained infertility, history of miscarriage, endometriosis or irregular bleeding). When the adhesion assay was performed using hEEC monolayers were derived from pools of women who were normally fertile and/or who had unexplained primary infertility we confirmed the cell line data, whereby galectin-7 suppressed trophoblast-epithelial adhesion (Figure 4B, black bars, n=2). However, when these cell-cell adhesion experiments were

performed using hEEC monolayers derived from pools including at least one women with a diagnosed disorder of the endometrium (miscarriage, endometriosis or irregular bleeding), galectin-7 treatment significantly enhanced hT adhesion to the hEEC monolayer (Figure 4B, grey bars, n=4; $p<0.05$).

Discussion

Here for the first time we identified differential serum expression of galectin-7 at week 6 of gestation in women who go on to miscarry. Furthermore, we showed women with a history of miscarriage have upregulated non-pregnant endometrial epithelial expression of galectin-7. To determine whether galectin-7 was the cause of, or caused by miscarriage, we investigated whether galectin-7 had a functional role in implantation. We showed that galectin-7 mediated trophoblast-epithelial adhesion. We suggest that upregulated endometrial epithelial galectin-7 expression allows inappropriate implantation in women who suffer miscarriage.

The serum concentration of galectin-7 was significantly up-regulated at week 6 of gestation in women with a viable fetus at the time of blood collection that were destined to miscarry compared to normal healthy pregnancies. This very early gestation specific increase supports our hypothesis that excess galectin-7 plays an important role in trophoblast-endometrial adhesion. It is unclear from our data the origin of the elevated serum galectin-7. Galectin-7 localises to the syncytiotrophoblast in the placenta and glandular epithelium in the decidua of early pregnancy , suggesting it may have a placental origin. The serum utilized in this study has previously been used to investigate the expression of many serum proteins in women who go on to miscarry (22, 28). In these studies IL33 is elevated, ST2 and ananamide show no effect and hCG, MIC-1 and PAPP-A are depressed in women who

subsequently go on to miscarry. Future studies should be directed towards whether in combination these molecules can predict failing pregnancies.

We speculate that galectin-7 acts as an adhesion molecule during early blastocyst attachment. Galectin-7 localized to the luminal and glandular epithelium during the menstrual cycle. Galectins (except galectin-3) are considered secreted proteins (9). The current paradigm to understand how galectins act as adhesion molecules suggests that low concentrations of soluble galectins promote adhesion of cells to extracellular matrix proteins (eg. fibronectin, laminin, integrins, lysosomal granule membrane protein). However, higher concentrations can impair adhesion, or promote de-adhesion by competitively binding to the extracellular matrix proteins and blocking cell-cell attachment (9). Here we found that the addition of excess soluble galectin-7 impaired trophoblast-endometrial epithelial cell adhesion when it was added to trophoblast or trophoblast plus epithelial cultures, supporting a role for galectin-7 in mediating adhesion at physiological concentrations.

In contrast, pre-treatment with galectin-7 to the primary cultures isolated from women with diagnosed endometrial disorders, including miscarriage, resulted in enhanced cell-cell adhesion. This result is consistent with observations that miscarriage is associated with inappropriate blastocyst adhesion, either of poor quality blastocysts or to an unsuitable region of the uterine endometrium (6). Further research is required to determine the mechanisms by which pre-treatment of trophoblast with galectin-7 enhanced adhesion to epithelial cells cultured from these women. Indeed here we show that women with miscarriage have upregulated endometrial galectin-7 during the menstrual cycle. The expression of galectin-7 in women with irregular bleeding or endometriosis has not been investigated but galectin-3 is upregulated in women with endometriosis (29). Transfection of cells with galectin-7 enhances expression of MMP9 (30), integrin α1, fibrillin, collagen and

vitronectin (31) suggesting upregulated epithelial galectin-7 may enhance the adhesive capability of the epithelium by the extra availability of surface receptors and extracellular matrix molecules. Certainly, enhanced expression of integrins $\alpha_4\beta_1$, $\alpha_1\beta_1$, $\alpha_v\beta_3$ is found in women with recurrent pregnancy loss (5). Overall, the concentration of galectin-7 is likely critical to mediate normal attachment, as is found for other proteins during blastocyst attachment, including LIF (32).

Proteins up- and down-stream of galectin-7 have also been implicated in implantation failure, suggesting that this pathway may be critical during early pregnancy. Galectin-7 is regulated by p53 and is also known as p53-induced gene 1 (19). A polymorphism in p53 codon 72 is significantly higher in women experiencing recurrent miscarriage compared to control women (33), however whether this polymorphism would affect galectin-7 expression is unknown. Galectin-7 induces MMP9 expression in HeLa and lymphoma cells (30) and two recent papers have linked high uterine flushing MMP9 activity with spontaneous pregnancy loss (34, 35). Further research is required to determine whether these observations are linked.

Another important role for galectins is modulating the immune response (12, 13, 36, 37). Immunologic dysregulation has been proposed as a cause of recurrent miscarriage (38). Galectin-7 is a T-cell binding protein (39) and is upregulated in mice with cardiac allografts undergoing acute rejection compared to isograft controls (40). Importantly, galectin-7 levels increased with the level of acute rejection (40) and it was previously shown by proteomics to be upregulated in the serum of renal graft recipients compared to normal volunteers (41). Further studies are required to determine whether serum levels of galectin-7 are associated with abberent T cell activation in pregnancies that go on to miscarry.

Here, we showed that serum galectin-7 elevated at week 6 of gestation and that galectin-7 was aberrantly expressed in the non-pregnant endometrium of women with a history of miscarriage. We further demonstrated that galectin-7 mediates trophoblast-endometrial epithelial adhesion by acting as an adhesion molecule, suggesting that the elevated endometrial and serum galectin-7 associated with miscarriage may be a mechanism for inappropriate blastocyst implantation seen in women with recurrent miscarriage. Overall our data showed a novel role for galectin-7 in implantation and early pregnancy.

Acknowledgements

The authors would like to acknowledge the invaluable contribution of the Doctors and patients for providing tissues and serum and our research nurse Sister Judi Hocking for the consenting of patients and collection of tissues. We would also like to thank Nicole Pascual and Joanne Yap for excellent technical assistance.

PHI data audit 13-39.

This work was supported by NHMRC (Australia) Project/Program Grant (GNT1027100) to ED and EM, and by the Victorian Government's Operational Infrastructure Support Program. EM was supported by NHMRC (Australia) Early Career Fellowship (#611827). TKL was supported by NHMRC (Australia) New Investigator Project Grant (GNT1048707). ST was supported by an NHMRC (Australia) Career Development Fellowship (#1050765). ED was supported by NHMRC (Australia) Senior Research Fellowship (#550905).

References

1. Aplin JD. Developmental cell biology of human villous trophoblast: current research problems. International Journal of Developmental Biology. 2010;54:323-9.
2. Knofler M. Critical growth factors and signalling pathways controlling human trophoblast invasion. International Journal of Developmental Biology. 2010;54:269-80.

3. Salamonsen LA, Nie G, Hannan NJ, Dimitriadis E. Society for Reproductive Biology Founders' Lecture 2009. Preparing fertile soil: the importance of endometrial receptivity. Reproduction, Fertility and Development. 2009;21(7):923-34.
4. Christiansen OB, Steffensen R, Nielsen HS, Varming K. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. Gynecologic and Obstetric Investigation. 2008;66:257-67.
5. Quenby S. Different types of recurrent miscarriage are associated with varying patterns of adhesion molecule expression in endometrium. RBMOnline. 2007;14(2):224-34.
6. Teklenburg G, Salker M, Heijnen CJ, Macklon NS, Brosens JJ. The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. Molecular Human Reproduction. 2010;16(12):886-95.
7. Yurdakan G, Ekem TE, Bahadir B, Gun BD, Kuzey GM, Ozdamar SO. Expression of adhesion molecules in first trimester spontaneous abortions and their role in abortion pathogenesis. Acta Obstetricia et Gynecologica Scandinavica. 2008;87(7):775-82.
8. Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. PLoS One. 2010;5(4):e10287.
9. Elola MT, Wolfenstein-Todel C, Troncoso MF, Vasta GR, Rabinovich GA. Galectins: matricellular glycan-binding proteins linking cell adhesion, migration and survival. Cellular and Molecular Life Science. 2007;64:1679-700.
10. Fitzgerald JS, Germeyer A, Huppertz B, Jeschke U, Knofler M, Moser G, et al. Governing the invasive trophoblast: current aspects on intra- and extracellular regulation. American Journal of Reproductive Immunology. 2010;63(6):492-505.
11. Nakahara S, Raz A. On the role of galectins in signal transduction. Methods in enzymology. 2006;417:273-89.
12. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nature Reviews Immunology. 2009;9:338-52.
13. Blois SM, Barrientos G. Galectin signature in normal pregnancy and preeclampsia. Journal of Reproductive Immunology. 2013 (0).
14. Jeschke U, Hutter S, Heublein S, Vrekoussis T, Andergassen U, Unverdorben L, et al. Expression and function of galectins in the endometrium and at the human feto-maternal interface. Placenta. 2013;34:863-72.
15. von Wolff M, Wang X, Gabius H-J, Strowitzki T. Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. Molecular Human Reproduction. 2004 March 1, 2004;11(3):189-94.
16. Yang H, Lei C, Zhang W. Expression of galectin-3 in mouse endometrium and its effect during embryo implantation. Reproductive BioMedicine Online. 2012 1//;24(1):116-22.
17. Heusschen R, Freitag N, Tirado-González I, Barrientos G, Moschansky P, Muñoz-Fernández R, et al. Profiling Lgals9 Splice Variant Expression at the Fetal-Maternal Interface: Implications in Normal and Pathological Human Pregnancy. Biology of Reproduction. 2013 January 1, 2013;88(1):22, 1-10.
18. Leonidas DD, Vatzaki EH, Vorum H, Celis JE, Madsen P, Ravi Acharya K. Structural basis for recognition of carbohydrates by human galectin-7. Biochemistry. 1998;37:13930-40.

19. Saussez S, Kiss R. Galectin-7. Cellular and Molecular Life Science. 2006;63:686-97.
20. Cao Z, Said N, Amin S, Wu HK, Bruce A, Garate M, et al. Galectins-3 and -7, but not Galectin-1, Play a Role in Re-epithelialization of Wounds. Journal of Biological Chemistry. 2002 November 1, 2002;277(44):42299-305.
21. Cao Z, Said N, Wu HK, Kuwabara I, Liu F-T, Panjwani N. Galectin-7 as a potential mediator of corneal epithelial cell migration. Arch Ophthalmol. 2003;121:82-6.
22. Kaitu'u-Lino TuJ, Tuohey L, Tong S. Maternal serum interleukin-33 and soluble ST2 across early pregnancy, and their association with miscarriage. Journal of Reproductive Immunology. 2012 9//;95(1–2):46-9.
23. Menkhorst E, Salamonsen LA, Robb L, Dimitriadis E. IL11 antagonist inhibits uterine stromal differentiation, causing pregnancy failure in mice. Biology of Reproduction. 2009;80:920-7.
24. Hannan NJ, Paiva P, Dimitriadis E, Salamonsen LA. Models for Study of Human Embryo Implantation: Choice of Cell Lines? Biology of Reproduction. 2009 July 1, 2009;82(2):235-45.
25. Cuman C, Menkhorst EM, Rombauts LJ, Holden S, Webster D, Bilandzic M, et al. Preimplantation human blastocysts release factors that differentially alter human endometrial epithelial cell adhesion and gene expression relative to IVF success. Human Reproduction. 2013;28:1161-71.
26. Menkhorst EM, Lane N, Winship A, Li P, Yap J, Meehan K, et al. Decidual-secreted factors alter invasive trophoblast membrane and secreted proteins implying a role for decidual cell regulation of placentation. PLoS ONE. 2012;7(2):e31418.
27. Marwood M, Visser K, Salamonsen LA, Dimitriadis E. Interleukin-11 and leukemia inhibitory factor regulate the adhesion of endometrial epithelial cells: implications in fertility regulation. Endocrinology. 2009 June 1, 2009;150(6):2915-23.
28. Tong S, Ngian G-L, Onwude JL, Permezel M, Saglam B, Hay S, et al. Diagnostic accuracy of maternal serum macrophage inhibitory cytokine-1 and pregnancy-associated plasma protein-A at 6-10 weeks of gestation to predict miscarriage. Obsetrics and Gynecology. 2012;119(5):1000-8.
29. Noel JC, Chapron C, Borghese B, Fayt I, Anaf V. Galectin-3 is overexpressed in various forms of endometriosis. Applied immunohistochemistry and molecular morphology. 2011;19(3):253-7.
30. Demers M, Magnaldo T, St-Pierre Y. A Novel Function for Galectin-7: Promoting Tumorigenesis by Up-regulating MMP-9 Gene Expression. Cancer Research. 2005 June 15, 2005;65(12):5205-10.
31. Kuwabara I, Kuwabara Y, Yang R-Y, Schuler M, Green DR, Zuraw BL, et al. Galectin-7 (PIG1) Exhibits Pro-apoptotic Function through JNK Activation and Mitochondrial Cytochrome cRelease. Journal of Biological Chemistry. 2002 February 1, 2002;277(5):3487-97.
32. Lalitkumar S, Menezes J, Wransby H, Gemzell-Danielsson K, Lalitkumar PGL. Optimum level of leukemia inhibitory factor is essential for fertility regulation - an in vitro study. Human Reproduction. 2010 June 1, 2010;25(suppl 1):i70.
33. Kay C, Jeyendran RS, Coulam CB. p53 tumor suppressor gene polymorphism is associated with recurrent implantation failure. Reproductive Biomed Online. 2006;13(4):492-6.
34. Nissi R, Talvensaari-Mattila A, Kotila V, Niinimaki M, Jarvela I, Turpeenniemi-Hujanen T. Circulating matrix metalloproteinase MMP-9 and MMP-

2/TIMP-2 complex are associated with spontaneous early pregnancy failure. Reproductive biology and endocrinology. 2013;11:2.

35. Yoshii N, Hamatani T, Inagaki N, Hosaka T, Inoue O, Yamada M, et al. Successful implantation after reducing matrix metalloproteinase activity in the uterine cavity. Reproductive Biology and Endocrinology. 2013;11:37.

36. Dam TK, Brewer CF. Lectins as pattern recognition molecules: The effects of epitope density in innate immunity. Glycobiology. 2010;20(3):270-9.

37. Liu F-T, Rabinovich GA. Galectins: regulators of acute and chronic inflammation. Annals of the New York academy of sciences. 2010;1183:158-82.

38. Larsen EC, Christiansen OB, Kolte AM, Macklon NS. New insights into mechanisms behind miscarriage. BMC Medicine. 2013;11:154.

39. Rossi NE, Reiné J, Pineda-Lezamit M, Pulgar M, Meza NW, Swamy M, et al. Differential antibody binding to the surface αβTCR·CD3 complex of CD4+ and CD8+ T lymphocytes is conserved in mammals and associated with differential glycosylation. International Immunology. 2008 October 1, 2008;20(10):1247-58.

40. Luo Z, Ji Y, Zhou H, Huang X, Fang J, Guo H, et al. Galectin-7 in cardiac allografts in mice: increased expression compared with isografts and localization in infiltrating lymphocytes and vascular endothelial cells. Transplantation Proceedings. 2013;45:630-4.

41. Gao Y, Wu K, Xu Y, Zhou H, He W, Zhang W, et al. Characterization of acute renal allograft rejection by human serum proteomic analysis. J Huazhong Univ Sci Technol [Med Sci]. 2009 2009/10/01;29(5):585-91.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure legends

Figure 1. Galectin-7 immunolocalized to the endometrial luminal and glandular epithelium.

A-G. Galectin-7 immunolocalization in the endometrium during a normally fertile menstrual cycle (A-D) and in women who have a history of miscarriage (E-G). Negative controls shown directly below positive.

A & E. Proliferative phase

B & F. Early secretory phase

C & G. Mid-secretory phase

D. Late secretory phase

H. Immunoscoring of galectin-7 in the luminal epithelium of normally fertile women (black), women with a history of miscarriage (red) and women with unexplained primary infertility (blue).

I. Immunoscoring of galectin-7 in the glandular epithelium of normally fertile women (black), women with a history of miscarriage (red) and women with unexplained primary infertility (blue).

J. Immunoscoring of galectin-7 in the stroma of normally fertile women (black), women with a history of miscarriage (red) and women with unexplained primary infertility (blue).

A-G. Scale bar represents 100µm. H-J. Bar represents mean. *, $p<0.05$ normally fertile vs miscarriage.

Figure 2. Serum concentration of galectin-7 in non-pregnant (np) women, women with healthy pregnancies (black) and women who go on to miscarry (red).

A. Serum concentration at in non-pregnant women and women at weeks 6-10 of gestation.
B. Serum concentration at week 6 of gestation only.

Bar represents mean. *, $p<0.05$.

Figure 3. Galectin-7 immunolocalized to the region of placental villous-decidual attachment during the first trimester.

Galectin-7 immunolocalised to the syncytiotrophoblast (st), the cell column (cc) and to extravillous trophoblast (evt) in first trimester placental tissue.

Negative IgG control is shown below.

Bar represents 100µm. d, decidua. Representative of n=4 tissues.

Figure 4. Effect of exogenous soluble galectin-7 on epithelial-trophoblast adhesion in an in vitro cell-cell adhesion assay. Cells were pre-treated with 1µg/ml galectin-7 or vehicle (BSA) control for 18 hours and then trophoblast cells were labelled with a fluorescent dye and allowed to adhere to a confluent epithelial cell layer for 90 minutes. Unattached cells were washed off and adhesion measured by fluorescence.

A. Adhesion of HTR8/Svneo (trophoblast cell line) to ECC1 (endometrial epithelial cell line) (n=4). Treatment of both HTR8/Svneo and EEC1 cells with galectin-7 impaired adhesion.
B. Adhesion of primary trophoblast (hT) to human endometrial epithelial cells (hEEC) isolated from tissues collected from women with primary infertility pooled with women with normal fertility (black bars; n=2) or isolated from tissues collected from women with primary infertility pooled with women with a diagnosed endometrial disorder including miscarriage (grey bars; n=4). Treatment of hT with galectin-7 impaired adhesion to hEEC isolated from women with primary infertility (black) but enhanced hT adhesion to hEEC isolated from women with diagnosed endometrial disorders (grey).

Data shows mean±sem. *, $p<0.05$ compared to diluent control.

ACCEPTED MANUSCRIPT

Table 1. Characteristics of non-pregnant participants.

	Normally fertile (n=17)	**Miscarriage (n=11)**	**Unexplained infertility (n=6)**
Maternal age (years)	35.0 (28-40)	32.0 (25-44)	31.5 (24-38)
Gravidity [a]	2.0 (2-3)	5.0 (2-12)	0
Parity[b] **median**	2.0 (2-4)	1.0 (0-2) *	0
0 **[%(n)]**	0	33.3 (3)	100 (6)
1	0	44.4 (4)	0
2	63.6 (7)	22.2 (2)	0
≥3	36.3 (4)	0	0
Number of previous miscarriages[c]	0	3.0 (2-12)	0

Data provided as the median, with range given in brackets.

[a] Data unavailable for 10 fertile patients; 2 miscarriage patients

[b] Data unavailable for 7 fertile patients; 2 miscarriage patients

[c] Data unavailable for 2 miscarriage patients – defined as recurrent miscarriage only.

* $p<0.05$ compared to normally fertile

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 2: Characteristics of pregnant participants

	Controls (n=109)	Miscarriage (n=18)
Maternal age (years)	30.0 (21-44)	30 (19-43)
Gravidity[%(n)]		
Primiparous	34.8 (38)	27.7 (5)
Multiparous	65.2 (71)	72.3 (13)
Parity [%(n)] [a]		
0	54 (59)	62.5 (10)
1	34 (37)	25 (4)
≥2	12 (13)	12.5 (2)
Gestation at sampling (wks + days)	8+2 (6+0 – 10+6)	7+4 (6+2 – 9+2) *
Gestation at 1st symptoms (wks + days) [b]	n/a	11+0 (7+0 – 14+5)
Gestation when the miscarriage was confirmed by ultrasound (wks + days)	n/a	11+0 (7+0 – 18+0)
Gestation at delivery (wks + days)	39+1 (32+0 – 42+0)	n/a
Birth weight (g)	3475 (1785-4680)	n/a

Data provided as the median, with range given in brackets.
* $p<0.05$ compared to control
[a] Data unavailable for two miscarriage patients
[b] Data unavailable for four miscarriage patients

ACCEPTED MANUSCRIPT

Figure 1

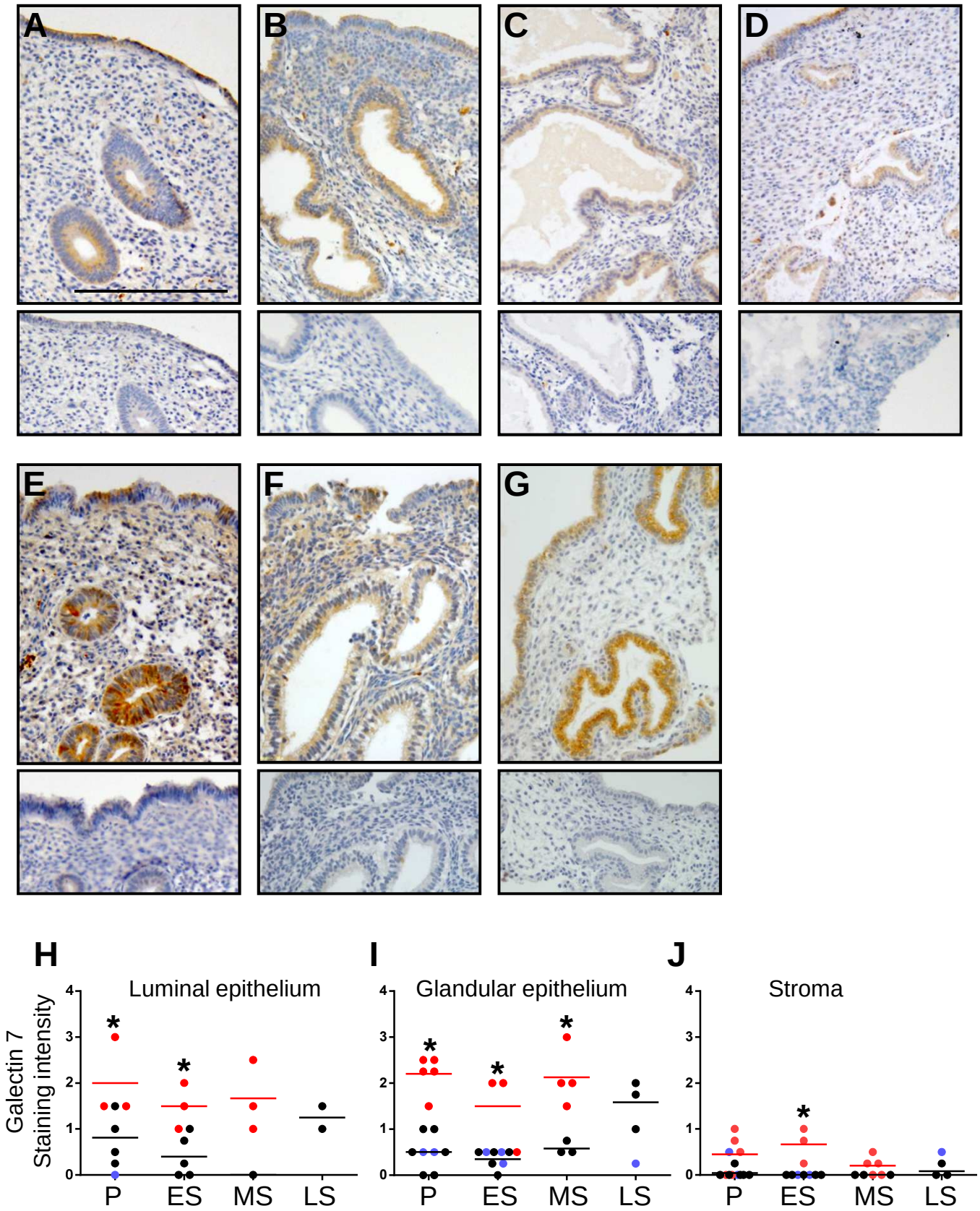

A
B
C
D
E
F
G
H
Luminal epithelium
Galectin 7
Staining intensity
0
1
2
3
4
P
ES
MS
LS
I
Glandular epithelium
J
Stroma

Figure 2.

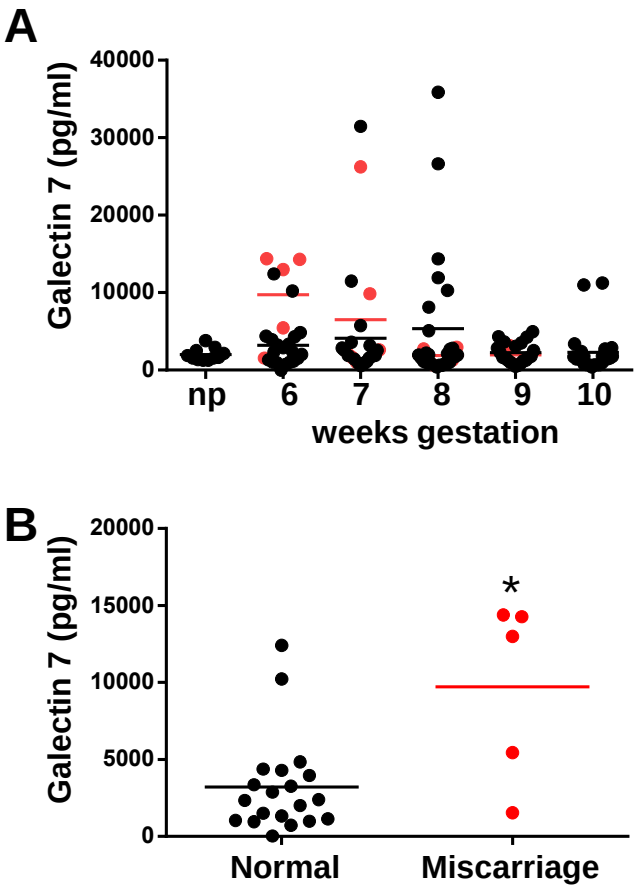

A
Galectin 7 (pg/ml)
40000
30000
20000
10000
0
np
6
7
8
9
10
weeks gestation
B
Galectin 7 (pg/ml)
20000
15000
10000
5000
0
*
Normal
Miscarriage

Figure 3

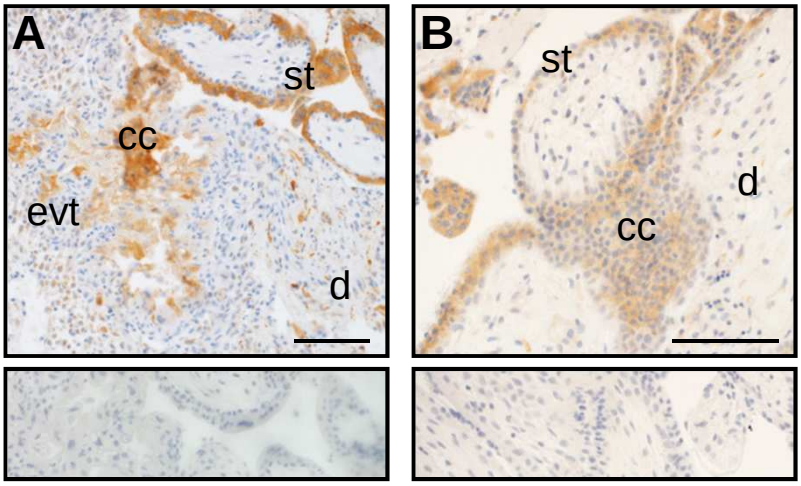
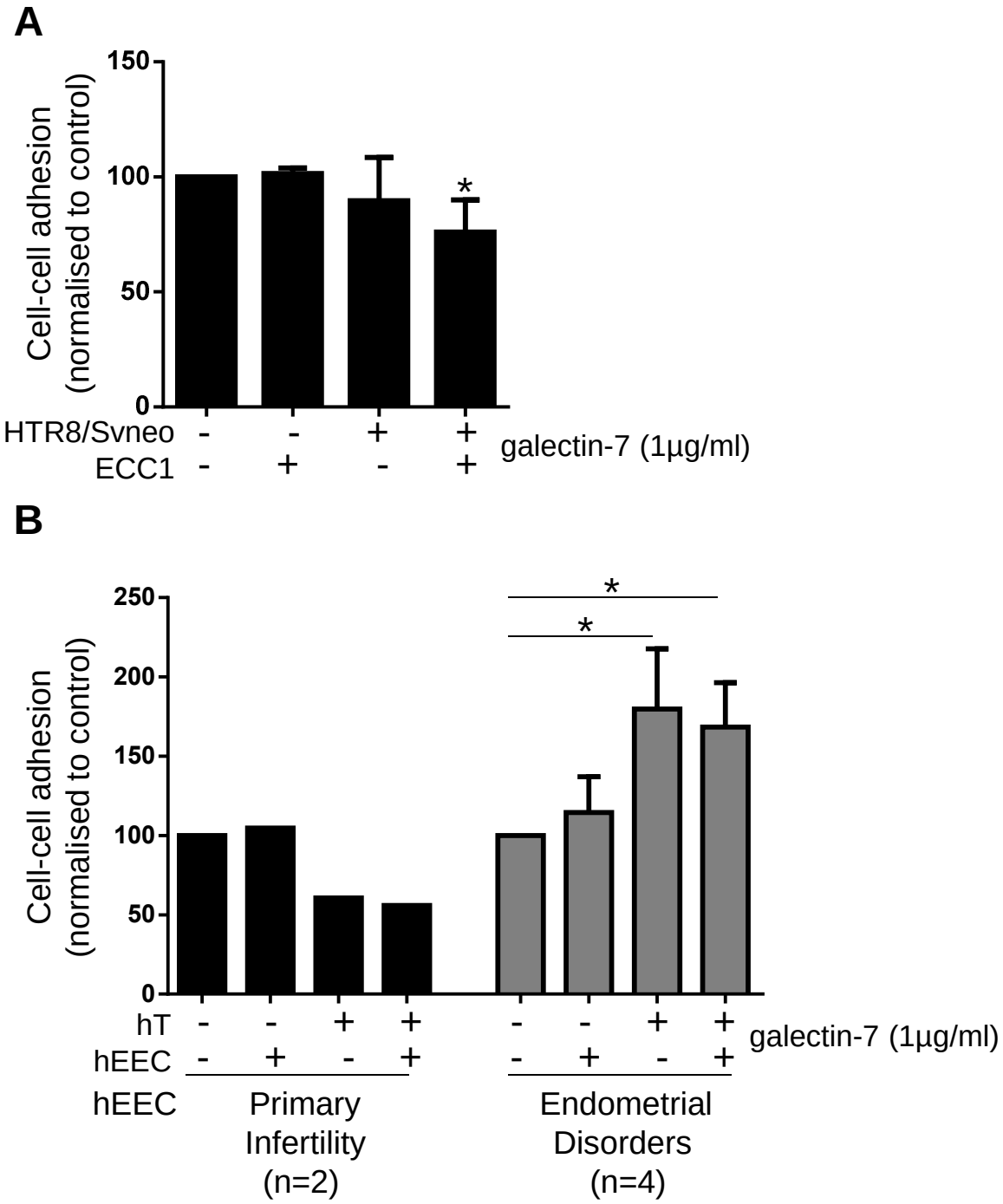


Figure 4.


A
Cell-cell adhesion
(normalised to control)
150
100
50
0
*
HTR8/Svneo - - + +
ECC1 - + - +
galectin-7 (1µg/ml)
B
Cell-cell adhesion
(normalised to control)
250
200
150
100
50
0
*
*
hT - - + + - - + +
hEEC - + - + - + - +
galectin-7 (1µg/ml)
hEEC
Primary
Infertility
(n=2)
Endometrial
Disorders
(n=4)