HOXA-11 mediated dysregulation of matrix remodeling during implantation window in women with endometriosis

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Received: 22 April 2013 / Accepted: 14 August 2013 / Published online: 25 August 2013
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Abstract

Purpose To investigate the Homeobox genes HOXA-10 and HOXA-11 mediated endometrial molecular defects during implantation window in endometriosis-associated infertility cases.

Methods Endometrial biopsies were obtained during implantation window from 31 infertile women with endometriosis (age < 35 years) and 26 age and BMI-matched infertile women without endometriosis were included in the study for comparison purposes. Endometrial expression of HOXA-10 and HOXA-11 genes, MMP-2, -9, $\alpha_v\beta_3$ integrin, leukemia inhibitory factor and surface characteristics including average roughness and topology were assessed.

Results A significantly lower expression of HOXA-10 and HOXA-11 were observed in endometriotic women compared to non-endometriotic controls. Further, a significantly higher endometrial expression of MMP-2 and -9 were observed in women with endometriosis when compared with controls. Interestingly, endometrial surface were observed to be grossly

affected in terms of average roughness and topology in women with endometriosis compared to controls.

Conclusions The findings suggest that aberrant expression of HOXA-10 and -11 genes adversely affects endometrial remodelling and expression of receptivity markers.

Keywords HOXA gene · Endometrial receptivity · Endometrium · Atomic force microscopy · Endometriosis

Abbreviations

HOXA	Homeobox genes A
MMP	Matrix metalloproteinases
SEM	Scanning electron microscope
AFM	Atomic force microscope

Introduction

Endometriosis, a common gynecological disorder, is characterized by benign growth of endometrial tissue outside the uterus. In India, approximately 1 % women undergoing major gynecological surgery, 6–43 % undergoing sterilization, 12–32 % undergoing laparoscopy for pelvic pain, 21–48 % undergoing laparoscopy for infertility are diagnosed with endometriosis. From a global perspective, endometriosis affects almost 10 % of women of reproductive age [1]. Presence of endometriosis may alter the endometrial characteristics, which can result in a dysfunctional endometrium.

The endometrium remodels before attaining a state of receptivity and remains receptive during a limited period, when it is favourable to blastocyst attachment and implantation. This period, known as the implantation window, is related to changes in the endometrial epithelial morphology [2]. Pinopodes, characterized by protrusions from the endometrial epithelial surface, are expressed for a maximum period of 2 days during menstrual cycle corresponding to the presumed window

Capsule Aberrant expression of Homeobox HOXA-10 and HOXA-11 genes causes excessive extracellular matrix degradation during implantation window in women with endometriosis. This results in impairment of endometrial remodeling affecting the receptive status of the endometrium.

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of implantation. These morphological markers are proposed to be the indicators of endometrial receptivity in humans [3]. Inappropriate morphological development leads to unreceptive endometrium that causes defective endometrial/embryonic cross talk. It is generally agreed that one of the main reasons for implantation failure is impairment of the endometrial receptivity [2]. The fact that uterine receptivity might be affected by the presence of endometriosis has drawn considerable attention in recent years.

Homeobox genes HOXA10 and HOXA11 are transcriptional factors necessary for endometrial differentiation, which results in the development of an endometrium receptive to embryo implantation [4]. These genes usually bind to the regulatory regions of their downstream target genes involved in endometrial development and regulate their transcriptional expression [5]. Altered expression of HOX genes are reported to be associated with a number of infertility related disorders with decreased rate of implantation including PCOS, idiopathic infertility, endometriosis and recurrent spontaneous miscarriage [4, 6–9].

The endometrium undergoes cyclic degeneration and regeneration during every normal reproductive cycle mediated by the action of a class of proteolytic enzymes, matrix metalloproteinases (MMPs) [10]. Several studies have reported the diverse pattern of expression of MMPs in the endometrium throughout the menstrual cycle. However, expression of MMP-2 remains consistent throughout the reproductive cycle. MMP-9 also expresses in the endometrium throughout the cycle, though, its expression increases in the glandular cells particularly during midsecretory phase [2].

Despite extensive research, alterations in endometrial receptivity leading to implantation failure remain controversial in women with endometriosis. The molecular mechanisms that underlie endometrial receptivity in these women are poorly understood. An understanding of endometrial receptivity in women with endometriosis is, therefore, crucial for understanding the fundamental causes of implantation failure which in turn, have significant implications on fertility potential.

From the previous study by Taylor (1999), alterations in HOX signalling is known to occur in endometriosis [11]. According to them, alterations in HOX genes are expected to produce a cascade of other defects in the expression of downstream target genes which are associated with implantation. HOX gene-mediated alterations in the expression of downstream target genes involved in endometrial receptivity and matrix remodeling during implantation window in endometriosis are not reported so far. The present study aimed to show indirect evidence that HOX genes could affect expression profile of endometrial MMP-2, and -9 during implantation window in women with endometriosis. High-resolution scanning electron microscope (SEM) and atomic force microscope (AFM) has emerged as excellent imaging tools for exploring surface properties. These techniques are used for

imaging any alterations in endometrial tissue morphology during implantation window in these women. Further, endometrial receptivity markers including $\alpha_v\beta_3$ integrin and LIF are assessed in these women to investigate the effect of excessive endometrial remodeling on implantation.

Materials & methods

Subject selection

Infertile women with endometriosis ($n=31$) confirmed by diagnostic laparoscopy coming for treatment at Institute of Reproductive Medicine, Kolkata, India and infertile women without the disease ($n=26$; controls) were included in this study. It was ensured that these women had not received any kind of medical or hormonal treatment during the last 3 months. Women with history of removal of chocolate cyst, previous history of any surgery, other possible causes of pain or pelvic pathology including pelvic tuberculosis were excluded. The study was approved by the Institute Ethics Committee and written informed consent was obtained from all couples participating in the study.

Sample collection

Ultrasonography for serial folliculometry was performed everyday D10 onwards in all cases to monitor follicular growth till ovulation occurred. Linear increment of the size of dominant follicle and endometrial thickness was recorded. Following rupture of dominant follicle, i.e. ovulation and subsequent crenated appearance of the follicle, the expected period/window of implantation was calculated between D18 to D23 which corresponds to 5–8 days post-ovulation. This was supported by BBT (persistent elevated temperature of 0.5 °F above the pre-ovulatory period). Endometrial biopsy was performed on the 7th day after confirmation of ovulation under general anaesthesia by uterine curettage (D&C). The endometrial biopsies were sent for routine pathologic analysis where endometrial histological dating was performed according to Noyes criteria. The remaining collected tissue was washed in phosphate buffer saline (PBS) and divided into four parts: the first part was used for stromal and epithelial cells isolation for flow cytometric analysis of different molecular repertoires of the endometrium. The second part was fixed for immunohistochemistry of these markers, studying the roughness of endometrium using atomic force microscopy (AFM) and for examining expression of pinopodes by scanning electron microscopy. The third part was used to isolate mRNA to study MMP-2, MMP-9, HOXA-10 and HOXA-11 gene expression by Real Time PCR. The fourth part was used to study the expression of MMP-2, -9, HOXA-10 and -11 by Western blotting.

Isolation of cells and flow cytometric analysis

Endometrial tissue was first digested in 2 % collagenase-1 (Invitrogen, Grand Island, NY, USA) in DMEM (Himedia, Mumbai, India) for 1.5 to 2 h at 37 °C and then centrifuged to isolate the stromal cells. Undigested glands were then treated with 0.25 % trypsin-0.02 % EDTA (Himedia, Mumbai, India) for 4–8 min, washed with 10 % FBS-DMEM. Single epithelial cells were isolated by centrifugation, as described previously. Isolated cells were washed, RBC lysed using RBC lysis solution and fixed in 2 % paraformaldehyde (20 min at RT). Single cell suspension thus obtained was divided into three parts; two parts were stained with mouse anti-human $\alpha_v\beta_3$ integrin and LIF (R&D Systems, Minneapolis, MN, USA) according to instructions provided by the manufacturer in the manual. The third part remained unstained. Excess antibodies were washed out and the cells again incubated with fluorescein conjugated secondary goat anti-mouse IgG (R&D Systems, Minneapolis, MN, USA). After washing excess antibodies, the stained cells were analyzed using flow cytometer (BD FACS Calibur™, BD Biosciences, San Jose, CA, USA).

Immunohistochemistry

3–5 μm thick sections obtained from formaldehyde fixed, paraffin-embedded tissue were dehydrated in graded ethanol. After antigen retrieval, the slides were blocked using 3 % BSA in PBS and incubated with mouse anti-human $\alpha_v\beta_3$ integrin (R&D Systems, Minneapolis, MN, USA) and LIF monoclonal antibody (Santa Cruz biotechnology, INC., Santa Cruz, California, USA). Excess primary antibody was washed with PBS and the sections again incubated with anti-mouse biotinylated secondary antibody (Santa Cruz biotechnology, INC., Santa Cruz, California, USA) according to the manufacturer's protocol, before incubation with avidin biotinylated horseradish peroxidase (Santa Cruz biotechnology, INC., Santa Cruz, California, USA). Labeled cells were visualized with diaminobenzidine (DAB) and sections counterstained with hematoxylin. Next, the slides were dehydrated using series of alcohol gradient and mounted using distrene, tricresyl phosphate (DPX) and xylene. The slides were then examined under bright field microscope (Carl Zeiss, Jena, Germany).

Atomic force microscopy

Formaldehyde-fixed tissues were washed in PBS and dehydrated in a series of alcohol gradient (50 %, 70 %, 90 %, 95 %, 100 %), each for 10 min, dipped in HMDS (1,1,1,3,3,3-hexamethyl disilazane; SRL, Bombay, India) and air dried. Dried tissues were then mounted and examined under AFM. A commercial AFM (CP II, Veeco Instruments

Inc., USA) was used in the tapping mode to measure the endometrial epithelial surface roughness. Mathematical tools help in extracting quantitative information on mean surface roughness from AFM images. Average roughness (R_a) is given by:

$$R_a = \frac{1}{n_x n_y} \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} |Z(i, j) - Z_{ave}|$$

where, $Z(i, j)$ denotes the topography data for the surface after specimen tilt-correction, Z_{ave} is the average surface height, i and j correspond to pixels in x and y direction. The maximum number of pixels in the two directions are given by n_x and n_y ($n_x = n_y = 256$).

A V-shaped, silicon nitride cantilever (MMP-11123, Veeco Instruments Inc., USA) with a tip curvature <10 nm, length 115–135 μm and a spring constant in the range of 0.20–80 N/m was used. All images were acquired with a resolution of 256×256 data points per scan at a scan rate of 0.5 Hz per line and a scan size of 10×10 μm . All images were processed and analyzed using Image Processing and Data Analysis software (version 2.1.15; copyright TMMicroscopes USA).

Scanning electron microscopy

Dried tissues were mounted and coated with gold and the endometrial surface thoroughly examined under SEM (Jeol JSM-5800 Scanning Microscope, Tokyo, Japan).

Real-time PCR

Gene expression of MMP-2, -9, HOXA-11 and -10 were analyzed by real time PCR (RT-PCR), which was performed with ABI Prism 7000 Sequence Detection System (Applied Biosystems Inc., Carlsbad, California, USA) using SYBR® green master mix (Applied Biosystems Inc., Carlsbad, California, USA). Total RNA was isolated from tissue by RNA isolation kit (Trizol Reagent, Invitrogen, Carlsbad, California, USA) and 10 μl of total RNA isolated was subjected to reverse transcription for cDNA synthesis with high-capacity cDNA reverse transcription kits (Applied Biosystems, Carlsbad, California, USA), according to the manufacturer's instructions. After synthesis, 5 μl of cDNA was mixed with SYBR® green and then used for RT-PCR. At the end of each reaction, cycle threshold (C_t) was manually set at the level that reflected the best kinetic PCR parameters, and melting curves acquired and analyzed. Relative quantification was used to measure gene expression by relating the PCR signal. GAPDH had been used as an internal control.

Western blotting

The endometrial tissue was homogenized in tissue lysis buffer. The tissue lysate was then centrifugated at 15,000 g for 15 min and the protein concentration of the homogenate determined by GeNei™ protein estimation kits (Bangalore Genei, India). 30 µg of homogenate protein was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the separated proteins electroblotted onto a Hybond nitrocellulose membrane (GE Healthcare) at 30 V for 13 h. After blocking the non-specific binding sites with non-fat dry milk in TBST buffer for 1 h at room temperature, the blots were incubated overnight at 4 °C with mouse monoclonal antibody against MMP-2, -9 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) and rabbit polyclonal antibody against HOXA-10 and -11 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). The blots were then washed three times with TBST buffer, incubated for 1 h at room temperature with horseradish peroxidase-linked goat anti-rabbit immunoglobulin G (IgG) and goat anti-mouse immunoglobulin G (IgG) (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). After further washing, the immunoreactive proteins were revealed using 3,3'-Diaminobenzidine (DAB) as substrate.

Statistical analysis

Data were compared using independent two sample *t* test and chi-square test, as applicable. Ky Plot version 2.0 beta 13 software and Graphpad Prism Software were used for this purpose. Statistical significance was defined as $p \leq 0.05$ and the results were expressed in Mean $\pm$ SD. Correlation coefficients were calculated by Pearson linear correlation using Ky Plot version 2.0 beta 13 software.

Result

The endometriotic women were comparable to their non-endometriotic counterparts in terms of age, BMI and endometrial thickness. Level of serum estrogen was found to be significantly higher and progesterone significantly less in women with endometriosis as compared with controls (Table 1).

Table 1 Clinical characteristics during the implantation window period of women with endometriosis and controls

Parameters	Endometriosis	Control	<i>P</i> value
Age	29.5 $\pm$ 0.61	29.32 $\pm$ 0.83	$P > 0.05$
BMI	28.18 $\pm$ 0.6956	26.51 $\pm$ 0.6095	$P > 0.05$
Endometrial thickness (cms)	9.247 $\pm$ 0.2490	8.250 $\pm$ 0.4059	$P > 0.05$
Serum estrogen level (pg/ml)	258.5 $\pm$ 13.83	193.6 $\pm$ 14.66	$P \leq 0.05$
Serum progesterone level (ng/ml)	12.39 $\pm$ 1.283	26.43 $\pm$ 2.011	$P \leq 0.05$

Results are expressed in Mean $\pm$ SD

HOXA-10 and 11 gene expression

Expressions of HOXA-10 and -11 mRNA in endometrium of women with or without endometriosis were assessed by RT-PCR. A significantly lower expression of HOXA-10 and HOXA-11 were observed in women with endometriosis in comparison to non-endometriotic controls. Western blot analysis evidenced a similar trend, showing decreased expression of both the proteins in endometrium of women with endometriosis when compared with controls (Fig. 1c–e).

MMP-2 and MMP-9 expression

mRNA expression of MMP-2 and -9 in endometrium of women with or without endometriosis were assessed by RT-PCR. A significantly higher expression of both MMP-2 and -9 was observed in women with endometriosis in comparison to non-endometriotic controls. Further, as evidenced by Western blotting, MMP-2 and -9 were observed to be significantly higher in endometriotic women as compared to controls (Fig. 1a, b, e).

Surface characteristics of endometrium

The endometrial surface characteristics including average roughness and topology were assessed by AFM and SEM (Fig. 2). Endometrium roughness as well as mean height was found to significantly increased in women with endometriosis (13.66 $\pm$ 0.8709 nm; 32.26 $\pm$ 2.525) as compared to controls (5.726 $\pm$ 0.1906 nm; 12.50 $\pm$ 0.5628 nm). SEM observation showed expression of pinopodes on endometrial epithelial surface. Pinopodes formation was also evaluated semi-quantitatively depending on their stage of development on the surface of the endometrium, and scored as (i) well-developed (ii) poorly developed and (iii) absent. Their occurrence was scored as (i) abundant (ii) moderate (iii) few. Few poorly developed pinopodes were expressed in women with endometriosis whereas endometrium of non-endometriotic controls showed abundant, well formed pinopodes (Fig. 3).

Expression of endometrial receptivity markers

Expression of endometrial receptivity markers, $\alpha_v\beta_3$ integrin and LIF were studied by immunohistochemistry and flow

Fig. 1 Graphical representation of mRNA expression of **a** MMP-2 **b** MMP-9 **c** HOXA-11 **d** HOXA-10 in the endometrium of women with endometriosis and controls. **e** Endometrial expression of MMP-2, MMP-9, HOXA-11, HOXA-10 and β -actin in women with endometriosis and controls. E: endometriosis and C: control

cytometry. Immunostaining of these markers were less in women with endometriosis in contrast to strong immunostaining of controls (Fig. 4). In addition, mean expression of these molecular markers, as detected by flow cytometry, was significantly less in both, stromal and epithelial cells of women with endometriosis as compared to controls (Fig. 4).

Discussion

Embryo-endometrial interaction is necessary for successful implantation to occur. Ultrastructural defects in the endometrium reported in endometriosis [11] may disrupt this synchrony between the embryo and endometrium, adversely affecting endometrial receptivity and embryo implantation. The structural and functional changes of the endometrial tissue during peri-implantation period are regulated by the transcriptional factors HOXA-10 and -11, which in turn regulate the expression of cell adhesion molecules and MMPs, respectively [12, 13]. We observed a significant decrease in HOXA-10 and -11 expressions in women with endometriosis as compared with controls. Our findings are in good agreement with the report of Taylor (1999) where a significant decrease in the expression of both the genes is reported [11]. It is worthwhile to mention here that we used Western blotting as an additional method to validate our mRNA expression findings. However, whether this defect in the expression of HOXA genes in endometriosis is inherent to the eutopic endometrium or a consequent of other factors associated with the disease still remains unknown.

It is well accepted that HOXA-11 gene regulates the downstream expression of MMPs, key enzymes responsible for

extracellular matrix (ECM) degradation [13]. Animal studies have shown that upon knock-down of HOXA-11, there is an increase in the activity of MMPs evidencing an overall increase in uterine ECM degradation by [13, 14]. This motivated us to determine whether HOXA-11 is associated with the expression of MMPs in women with endometriosis. A negative correlation was observed between the endometrial expression of HOXA-11 and that of MMP-2 and -9 (r value -0.63 and -0.68 , respectively). However, no significant correlations were observed between HOXA-10 and MMP-2 and -9. The findings clearly suggest that excessive endometrial matrix degradation is a consequence of reduced HOXA-11 expression. This is further evidenced by flattened endometrial epithelial surface morphology in endometriotic women resulting decrease in average endometrial surface roughness compared to controls (Fig. 2).

To investigate the effect of excessive endometrial remodeling on implantation in these women, expression of established endometrial receptivity markers including $\alpha_v\beta_3$ integrin, pinopodes and LIF were assessed. All markers were found to be significantly less, thereby suggesting reduced receptivity of the endometrium towards blastocyst implantation. Further, HOXA-10 gene is known to directly regulate β_3 -integrin subunit [12]. A significant positive correlation was observed between the endometrial expression of HOXA-10 and that of stromal and epithelial $\alpha_v\beta_3$ integrin (r value -0.67 and -0.7 , respectively) and no significant correlations were observed between HOXA-11 and stromal and epithelial $\alpha_v\beta_3$ integrin. This is possibly due to the fact that diminished endometrial HOXA-10 protein in endometriotic women is unable to bind directly to the transcription start site of the β_3 -integrin gene

Fig. 2 **a, b, d, e** Typical atomic force microscopic images of endometrial tissue: **a** 2D image of endometrial tissue of control **b** 3D image of endometrial tissue of control **d** 2D image of endometrial tissue of women with endometriosis **e** 3D image of

endometrial tissue of women with endometriosis. **c** Typical height profile of endometrial tissue of control **f** Typical height profile of endometrial tissue of women with endometriosis

[12, 15]. Further, it is evidenced that HOXA-10 plays an essential role in pinopodes development in endometrial cells [16]. SEM images clearly indicate poorly developed pinopodes (Fig. 3) in the same cohort of patients.

LIF is another important receptivity marker which plays a regulatory role in implantation through its influence on luminal epithelium and stromal decidualization. There are controversial reports on LIF expression during mid-secretory phase in women with endometriosis [17, 18]. Moreover, there appear to be no

study which correlates HOX-10 gene expression with LIF in human. There is only one study where normal expression of LIF in HOXA-10 deficient mice is reported [19]. We also found no significant correlation between HOX-10 and stromal and epithelial LIF ($r = -0.19$ and 0.03 , respectively) in the present study.

Summarizing, alterations in HOX-11 gene results in excessive endometrial matrix remodeling which, in turn, affects endometrial receptivity and implantation. The unreceptive state of the endometrium is further evidenced by the aberrant expression

Fig. 3 a–f Typical scanning electron microscopic images of pinopodes: a well developed b developing c absent d abundant e moderate f few. g–h graphical representation of pinopodes formation in women with endometriosis and controls

of HOX-10 mediated $\alpha_v\beta_3$ integrin and pinopodes. Another key regulatory factor, LIF was also found to be dysregulated. Additional molecular repertoires such as cytokines, angiogenic

and vasculogenic factors that may provide in-depth insight into the molecular and ultrastructural defects of the endometrium need to be investigated.

Fig. 4 a–d Immunohistological images of different biochemical markers expression: a $\alpha_v\beta_3$ integrin in controls b $\alpha_v\beta_3$ integrin in women with endometriosis c LIF in controls d LIF in endometriosis. Graphical representation of e $\alpha_v\beta_3$ integrin expression in the stromal and epithelial

cells of endometrial tissues in endometriosis and control f LIF expression in the stromal and epithelial cells of endometrial tissues in endometriosis and control

Acknowledgments The authors are grateful to all the volunteers who participated in this study, as well as to Dr. Ratna Chattopadhyay and Dr. Pallavi Pasricha from IRM, Kolkata for helping in collection of samples. The first author would like to acknowledge Vishmadeb Pramanik for helping with Real time PCR experiments.

References

1. Dutta M, Joshi M, Srivastava S, Lodh I, Chakravarty B, Chaudhury K. A metabonomics approach as a means for identification of potential biomarkers for early diagnosis of endometriosis. *Mol Biosyst*. 2012;8:3281–7. doi:[10.1039/c2mb25353d](https://doi.org/10.1039/c2mb25353d).
2. Jana SK, Banerjee P, Thangaraju S, Chakravarty B, Chaudhury K (2012) Alteration in endometrial remodeling: a cause for implantation failure in endometriosis? In: Chaudhury K (ed) *Endometriosis - basic concepts and current research trends*, 1st edn. InTech, Croatia, pp. 325–342.
3. Nikas G, Makrigiannakis A. Endometrial pinopodes and uterine receptivity. *Ann N Y Acad Sci*. 2003;997:120–3. doi:[10.1196/annals.1290.042](https://doi.org/10.1196/annals.1290.042).
4. Taylor HS. The role of HOX genes in human implantation. *Hum Reprod Update*. 2000;6:75–9. doi:[10.1093/humupd/6.1.75](https://doi.org/10.1093/humupd/6.1.75).
5. Xu B, Geerts D, Qian K, Zhang H, Zhu G. Myeloid ecotropic viral integration site 1 (MEIS) 1 involvement in embryonic implantation. *Hum Reprod*. 2008;23:1394–406. doi:[10.1093/humrep/den082](https://doi.org/10.1093/humrep/den082).
6. Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2003;88:238–43. doi:[10.1210/jc.2002-021072](https://doi.org/10.1210/jc.2002-021072).
7. Szczepanska M, Wirstlein P, Luczak M, Jagodzinski P, Skrzypczak J. Expression of HOXA-10 and HOXA-11 in the endometria of women with idiopathic infertility. *Folia Histochem Cytobiol*. 2011;49:111–8. doi:[10.5603/FHC.2011.0016](https://doi.org/10.5603/FHC.2011.0016).
8. Kulp JL, Cakmak H, Taylor HS. HOX Genes and Endometriosis. In: Giudice LC, Evers JLH, Healy DL, editors. *Endometriosis: science and practice*. Oxford: Blackwell Publishing Ltd; 2012. p. 184–9.
9. Sarno J, Schatz F, Huang SJ, Lockwood C, Taylor HS. Thrombin and interleukin-1 β decrease HOX gene expression in human first trimester decidual cells: implications for pregnancy loss. *Mol Hum Reprod*. 2009;15:451–7. doi:[10.1093/molehr/gap030](https://doi.org/10.1093/molehr/gap030).
10. Curry Jr TE, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev*. 2003;24:428–65. doi:[10.1210/er.2002-0005](https://doi.org/10.1210/er.2002-0005).
11. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. *Hum Reprod*. 1999;14:1328–31. doi:[10.1093/humrep/14.5.1328](https://doi.org/10.1093/humrep/14.5.1328).
12. Daftary GS, Troy PJ, Bagot CN, Young SL, Taylor HS. Direct regulation of beta3-integrin subunit gene expression by HOXA10 in endometrial cells. *Mol Endocrinol*. 2002;16:571–9. doi:[10.1210/me.16.3.571](https://doi.org/10.1210/me.16.3.571).
13. Connell KA, Guess MK, Chen H, Andikyan V, Bercik R, Taylor HS. HOXA11 is critical for development and maintenance of uterosacral ligaments and deficient in pelvic prolapse. *J Clin Invest*. 2008;118:1050–5. doi:[10.1172/JCI34193](https://doi.org/10.1172/JCI34193).
14. Ma Y, Guess M, Datar A, Hennessey A, Cardenas I, Johnson J, et al. Knockdown of Hoxa11 in vivo in the uterosacral ligament and uterus of mice results in altered collagen and matrix metalloproteinase activity. *Biol Reprod*. 2012;86:100. doi:[10.1095/biolreprod.111.093245](https://doi.org/10.1095/biolreprod.111.093245).
15. Ekker SC, Jackson DG, von Kessler DP, Sun BI, Young KE, Beachy PA. The degree of variation in DNA sequence recognition among four Drosophila homeotic proteins. *EMBO J*. 1994;13:3551–60.
16. Bagot CN, Kliman HJ, Taylor HS. Maternal Hoxa10 is required for pinopod formation in the development of mouse uterine receptivity to embryo implantation. *Dev Dyn*. 2001;222:538–44. doi:[10.1002/dvdy.1209](https://doi.org/10.1002/dvdy.1209).
17. Mikolajczyk M, Wirstlein P, Skrzypczak J. Leukaemia inhibitory factor and interleukin 11 levels in uterine flushings of infertile patients with endometriosis. *Hum Reprod*. 2006;21:3054–8. doi:[10.1093/humrep/del225](https://doi.org/10.1093/humrep/del225).
18. Dimitriadis E, Stoikos C, Stafford-Bell M, Clark I, Paiva P, Kovacs G. Interleukin-11, IL-11 receptor α and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. *J Reprod Immunol*. 2006;69:53–64. doi:[10.1016/j.jri.2005.07.004](https://doi.org/10.1016/j.jri.2005.07.004).
19. Benson GV, Lim H, Paria BC, Satokata I, Dey SK, Maas RL. Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. *Development*. 1996;122:2687–96.