**HOX gene expression is altered in the endometrium of women with endometriosis**

Hugh S. Taylor¹, Catherine Bagot, Andrew Kardana, David Olive and Aydin Arici

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, PO Box 208063, New Haven, CT 06520–8063, USA

¹To whom correspondence should be addressed

**HOXA10 and HOXA11 are homeobox genes that function as transcription factors essential to embryonic development.** We have recently described a role for each of these two genes in regulating endometrial development in the adult during the course of a menstrual cycle. Both HOXA10 and HOXA11 are essential for implantation in the mouse and appear to play a similar role in women. To investigate the role of HOX genes in the endometrium of women with endometriosis, quantitative Northern blot analysis was performed on the endometrium of 40 normal cycling controls and 40 patients with documented endometriosis. Patients with endometriosis failed to show the expected mid-luteal rise in HOX gene expression as demonstrated in the controls. Aberrant HOX gene expression suggests that altered development of the endometrium at the molecular level may contribute to the aetiology of infertility in patients with endometriosis.

**Key words:** endometriosis/endometrium/homeobox genes/ HOX/implantation

**Introduction**

Endometriosis affects at least 10% of reproductive age women and is characterized by the presence of ectopic endometrium. The association between endometriosis and infertility is well established, but the mechanisms responsible are unknown (Olive and Schwartz, 1993). Multiple factors have been implicated including distortion of the pelvic anatomy, abnormalities of hormone secretion (Ayers et al., 1987), alterations in peritoneal fluid (Halme et al., 1987), disorders of fertilization (Mills et al., 1992), and immunoregulatory dysfunction (Witz et al., 1994). Alterations in endometrial development in patients with endometriosis may contribute to endometriosis related infertility. Reports from several in-vitro fertilization (IVF)/embryo transfer programmes indicate patients with endometriosis have decreased implantation rates (Hahn et al., 1986; Simon et al., 1994; Arici et al., 1996). A surgically-induced mouse model of endometriosis also demonstrates failure of implantation as a mechanism of endometriosis associated infertility (Hahn et al., 1986). Although histologically normal, examination of eutopic endometrium from women with endometriosis has revealed other defects. Ultrastructural defects have been reported in the endometrium of women with endometriosis (Fedele et al., 1990). Molecular markers of endometrial receptivity are altered in patients with endometriosis; integrin expression patterns are aberrant in the native endometrium of women with endometriosis (Lessey et al., 1995, 1997; Ota and Tanaka, 1997; Hii and Rogers, 1998). Other alterations of biochemical or molecular markers have been noted including metalloproteinases (Osteen et al., 1996; Sharpe Timms, 1997), soluble urokinase-type plasminogen activator (suPA-R) (Sillem et al., 1997), oestrogen receptor (ER) splice variants (Fujimoto et al., 1997), vascular endothelial growth factor (VEGF) (Shifren et al., 1996), complement (C3) (Bartsik et al., 1987; Isaacs et al., 1990), aromatase (Noble et al., 1996), CA-125 (McBean and Brunstead, 1993), heat shock protein (Ota et al., 1997) and interleukin 6 (IL6) (Tseng et al., 1996).

**HOXA10 and HOXA11 are homeobox genes that mediate embryonic development (Krumlauf, 1992; McGinnis and Krumlauf, 1992) including the development of the reproductive tract (Favier and Dolle, 1997; Taylor et al., 1997).** They are translated into transcription factors that regulate a battery of downstream genes necessary for growth and differentiation. We have recently demonstrated that HOX genes play an analogous role in endometrial development during the adult menstrual cycle (Taylor et al., 1998, 1999). HOX gene expression possibly regulates the growth and development of the human endometrium (Taylor et al., 1997). HOXA10 and HOXA11 gene expression varies in response to sex steroids during the menstrual cycle, with dramatic up-regulation in the mid-secretory phase, the time of implantation.

Expression of each of these Hox genes is necessary for implantation in the mouse. Mouse with a targeted mutation of either of these genes have uterine factor infertility, producing normal embryos, but with a uterus which lacks the ability of wild-type embryos to implant (Hsieh-Li et al., 1995; Satokata et al., 1995; Favier and Dolle, 1997). We have recently shown that HOXA10 and HOXA11 likely play a similar role in human implantation (Taylor et al., 1997, 1998, 1999). HOXA10 and HOXA11 are expressed in the adult human endometrial stroma and glands and are differentially expressed in the developing endometrium during the menstrual cycle. HOX genes may affect endometrial development in a way analogous to their role in embryonic development, leading to endometrial growth, differentiation and receptivity. In women HOXA10 and HOXA11 are up-regulated in the mid-secretory endometrium, at the time of implantation. The extraordinarily high conservation of HOX gene function and the spatial and temporal
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Figure 1. HOXA10 expression in the eutopic endometrium of patients with endometriosis as demonstrated by Northern blot analysis. Expression was seen in the first and second half of the proliferative phase (P1 and P2 respectively) of the menstrual cycle after hybridization to a probe specific to the HOXA10 or G3PD genes. Representative samples showed a lack of significant up-regulation between the proliferative phase (P1 and P2) and the mid- and late secretory phase (S2 and S3).

Figure 2. HOXA10 expression in the endometrium of women with and without endometriosis. Endometrial HOX expression from 40 normal cycling women and 40 women with endometriosis are presented. Average expression of HOXA10 normalized to GAPDH was demonstrated throughout the menstrual cycle. Normal controls showed an increase in HOXA10 expression in the mid-luteal phase. Endometriosis patients showed altered HOXA10 expression. There was no statistically significant change in HOXA10 expression throughout the menstrual cycle. *Indicates a significant difference between controls and endometriosis patients (P < 0.01). P = proliferative phase; S1 = early secretory phase; S2 = mid-secretory phase; S3 = late secretory phase of the menstrual cycle.

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expression pattern of HOXA10 and HOXA11 in the endometrium suggest that these HOX genes play an essential role in human implantation. In this study we determined the levels of expression of HOXA10 and HOXA11 in the eutopic endometrium of patients with endometriosis. Alterations of the HOXA10 and HOXA11 genes, whose expression is necessary for implantation, may provide evidence of molecular alterations in the endometrium of these patients, and would suggest a defect in the development and receptivity of the endometrium in patients with endometriosis.

Materials and methods

Tissue collection
Endometrium was collected from 40 normal cycling women or from an equal number of women with histologically proven endometriosis, by endometrial biopsy under an approved institutional Human Investigations Committee protocol. Half of the tissue was immediately frozen in the liquid nitrogen and stored at –72°C. The other half of the tissue sample was fixed in formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Menstrual cycle dating was determined by menstrual history and confirmed by histological examination using the criteria of Noyes et al. (1950). Patients receiving hormonal therapy or currently undergoing evaluation of infertility were excluded.

Northern blot analysis
Tissues were individually homogenized in 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl, and 0.1 M 2-mercaptoethanol. Total RNA was size-fractioned on a 1% agarose-cyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl, and 0.1 M bioanhydride gel and sequentially hybridized with a 32P-labelled riboprobe as described below. Hybridization was performed overnight at 60°C in 50% formamide, 1× sodium chloride/sodium citrate (SSC), 5× Denhardt’s reagent, 0.2% tRNA, and 32P-labelled riboprobe at 37°C. The filter was washed twice at 86°C for 30 min in 0.1× SSC and 0.1% SDS. Kodak (Rochester, NY, USA) X-Omat AR film was exposed overnight at –70°C.

Probe preparation
Plasmids used for probe preparation were a generous gift from E.Boncinnelli. pGEM plasmids containing sequence from the 3’ untranslated region of either human HOXA10 or HOXA11 were linearized with EcoRI or HindIII (New England Biolabs, Beverly, MA, USA), ethanol precipitated and used as a template for generation of riboprobes. Radiolabelled RNA probes were generated by in-vitro transcription using the Promega Riboprobe Kit (Promega, Madison, WI, USA). Antisense probes were generated using the appropriate RNA polymerase (T7 or SP6) and labelled with α-[32P]-UTP (Amersham, Arlington Heights, IL, USA).

Statistical analysis
The autoradiographic bands were quantified using a laser densitometer (Molecular Dynamics Inc, Sunnyvale, CA, USA). Each HOXA10 or HOXA11 band was normalized to the value obtained from the
same lane hybridized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analysed using analysis of variance. \( P < 0.05 \) was considered to be statistically significant.

**Results**

Endometrium from 40 control patients was analysed for **HOXA10** and **HOXA11** expression by Northern blot analysis. In all patients, the expected up-regulation of **HOXA10** and **HOXA11** expression in the mid-luteal phase was observed, as we have previously demonstrated (Taylor et al., 1998, 1999).

Endometria from 40 patients with endometriosis were analysed in the same fashion. The eutopic endometria obtained from patients with endometriosis failed to show the equivalent up-regulation of either the **HOXA10** or **HOXA11** genes at the time of implantation. Representative samples are shown in Figure 1. Densitometry was performed on the Northern blot analysis of all samples normalized to GAPDH, and summarized in Figure 2. Levels of **HOXA10** expression were similar in the proliferative and early secretory phases and not statistically different. In both the mid- and late segments of the secretory phase, a statistically significant difference was noted in endometrial **HOXA10** expression between patients with or without endometriosis \( (P < 0.01) \). A similar difference was noted with **HOXA11** (Figure 3). This failure to increase **HOX** gene mRNA levels did not depend on the stage of the disease and occurred despite in-phase endometrial history.

**Discussion**

The pathogenesis of endometriosis associated infertility is unclear. These data suggest a defect in regulation of **HOX** gene expression in the endometrium of patients with endometriosis. Expression of each of these genes is necessary for implantation. Failure of the normal increase in **HOXA10** and **HOXA11** mRNA levels to occur at the beginning of the window of implantation, may be one mechanism responsible for endometriosis related infertility. Whether this defect is inherent to the eutopic endometrium or the result of other factors associated with endometriosis remains to be demonstrated. These data suggest that a defect in endometrial development exists in patients with endometriosis, and that failure of implantation may contribute to their infertility.

Alterations in other molecules expressed in the endometria have been reported in endometriosis (Isaacs et al., 1990; Lessey et al., 1994; Noble et al., 1996; Shifren et al., 1996; Fujimoto et al., 1997; Sharpe Timms, 1997; Sillem et al., 1997). In the absence of histological alteration, molecular defects in the endometrium may be responsible for failure of implantation. In mice with a targeted disruption of either the **Hoxa10** or **Hoxa11** gene, implantation cannot occur despite a histologically normal endometrium (Hsieh-Li et al., 1995; Satokata et al., 1995). Similarly a defect in **HOX** expression in patients with endometriosis may lead to a decrease in implantation without an appreciable pathology noted on histological examination. Very few molecules are known to affect implantation specifically when a targeted mutation is produced in mice; it is interesting to note that these defects are often undetectable on histological examination. Molecular markers may be a more valuable way to assess the receptivity of the endometrium.

**HOX** genes function as transcription factors and are early regulators of tissue identity in embryonic development (Krumlauf, 1992; McGinnis and Krumlauf, 1992). It is likely they function in an analogous role in cyclic endometrial development (Taylor et al., 1998, 1999). Other molecular markers of implantation or of endometrial development are likely downstream target genes of **HOX** genes – either direct or indirect. It will be interesting to determine if any of the structural molecules or growth factors that are involved in implantation are regulated by **HOXA10** or **HOXA11**. Alterations in **HOX** genes can be expected to produce a cascade of other defects in the expression of downstream target genes. **HOX** genes may be important early initiators of signal transduction that lead to the proper molecular development of the endometrium and to endometrial receptivity. It is likely many of the molecular, ultrastructural and clinical alterations seen in patients with endometriosis are mediated through alterations in **HOX** gene expression.

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**References**


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