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Decreased endometrial HOXA10 expression associated with use of the copper intrauterine-device

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Abstract

Objective—To characterize human endometrial HOXA10 expression in patients using copper intrauterine devices (IUD).

Design—Case-control study.

Setting—Academic medical center

Patient(s)—Women using copper IUD

Interventions(s)—Immunohistochemical analysis of endometrial HOXA10 expression in biopsies obtained from 24 women using copper Paraguard T380A as well as in biopsies obtained from 10 normal cycling women who were not using IUD or hormonal contraceptives.

Main Outcome Measure(s)—Endometrial HOXA10 expression.

Result(s)—Endometrial HOXA10 expression was markedly decreased in biopsies obtained from women using IUD contraceptive when compared to controls. The mean H score for endometrial stromal cell HOXA10 expression in biopsies obtained from women using Paraguard IUD was 0.21 compared to 2.2 in the control endometrial biopsies ($P < 0.001$). Endometrial glandular expression of HOXA10 was absent in all IUD users.

Conclusion(s)—Decreased endometrial HOXA10 expression was apparent in women who use a copper IUD. Expression of HOXA10 is essential for endometrial receptivity. A novel mechanism of copper IUD action involves suppression of genes required for endometrial receptivity. The dramatic decrease of endometrial HOXA10 in response to IUD use may contribute to contraceptive efficacy.

Keywords

Endometrium; Implantation; Contraception; IUD; HOX

Introduction

Intrauterine devices (IUDs) provide long-lasting, highly effective contraception to approximately 150 million women worldwide (1). Although this form of birth control is one of the most commonly used reversible contraceptives, the exact mechanism of action remains

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incompletely characterized (2,3). Endometrial morphology and biochemical composition are important factors in reproduction; modification of such factors result in the interference of processes required for spermatozoa capacitation or endometrial receptivity.

The copper IUD alters the intrauterine environment by eliciting a local foreign body reaction characterized by a significant influx in polymorphonuclear leukocytes, mast cells, and macrophages. Traditionally, influx of inflammatory cells resulting from copper concentrations in the endometrial tissue has been postulated to create a hostile environment for the embryo, thus contributing to the IUD mode of action. (4–7) Timonen and Kosonen investigated copper release from copper-T IUDs and the corrosive lifespan of copper in utero respectively; they observed sustained low pregnancy rates even when the device was approaching the end of the copper releasing lifespan, raising the question as to whether the mode of action can be explained by the release of copper alone(8,9).

Copper affects the fertilizing capacity of human spermatozoa by interfering with sperm migration, viability, and acrosomal reaction in vitro(10–14). Recovery of viable spermatozoa near the site of fertilization is decreased in IUD users when compared to non-IUD users, yet hindrance of sperm migratory ability only diminishes the chance of fertilization. Chang and Tatum studied the effect of intrauterine copper in rats, demonstrating the transfer of blastocysts from a copper exposed uterus to a normal uterus resulted in normal implantation, whereas normal blastocysts transferred to a copper influenced uterus failed to implant, concluding the altered intrauterine environment was the mechanism underlying the contraceptive effect (15).

Hox genes (HOX in human) encoded evolutionarily conserved transcription factors, which are essential to embryonic development, endometrial development and endometrial receptivity (16–19). During the embryonic period, HOX gene expression is necessary for directing developmental identity along the paramesonephric duct where HOXA9, A10, A11 and A13 are expressed in the developing fallopian tubes, uterus, cervix and upper vagina respectively. (20–25) In the adult human uterus, HOXA10 expression is apparent in endometrial stroma and glands, where it is regulated by the sex steroid hormones 17 β -estradiol and progesterone (22, 26). We have previously demonstrated the differential expression pattern of HOXA10 protein in human endometrial biopsies throughout the menstrual cycle(26,27). HOXA10 expression increases throughout the menstrual cycle, most dramatically during the mid secretory phase, corresponding to the time of implantation.(26,28] Hoxa10 is essential for embryo implantation in mice and humans. Maternal disruption of Hoxa10 results in impaired decidualization and uterine factor infertility due to defective endometrial receptivity. (29–31) In the current study, we investigated the effect of copper containing Paraguard T380A on endometrial HOXA10 gene expression and characterized a novel mechanism of IUD action. As demonstrated by its role in endometrial differentiation and receptivity, decreased endometrial HOXA10 expression, in response to IUD usage would be expected to lead to impaired implantation.

MATERIALS AND METHODS

Subjects

Twenty-four women using Paraguard T380A were recruited under an approved Human Investigation Committee protocol. Informed consent was obtained from all subjects. At the time of IUD removal, adherent endometrial tissue was collected from the device at variable times during the menstrual cycle. The average patient age was 29 years (range 19–43). The average duration of IUD usage was 17 months (range 1–122 months). Control endometrial biopsies were obtained from 10 normally cycling who were not using an IUD or hormonal contraceptive method.

Tissue Collection

Endometrium was obtained by collecting tissue incidentally shed at the time of IUD removal. The tissue samples were then fixed in formalin, embedded in paraffin, cut into 5µm sections, and mounted on glass slides.

Immunohistochemistry

Slides were deparaffinized and hydrated through a series of three 10 minute xylene and ethanol washes respectively, followed by permeabilization in cold 95% ethanol. After rinsing with distilled water, antigen retrieval was performed by steaming the slides at 90°C in 0.01M sodium citrate for 20 minutes and at room temperature for an additional 20 minutes. Slides were rinsed in PBS for 3 minutes, followed by an additional 5 minute wash in PBS containing 0.1% Tween 20 (PBST). A 3 minute rinse in 3% hydrogen peroxide was performed to quench endogenous peroxidase activity. To block non-specific binding, tissue sections were incubated at room temperature with 1.5% normal horse serum diluted in PBST for 1 hour. Slides were then incubated overnight at 4°C with HOXA10 polyclonal primary antibody (sc-17159) purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Following a 5 minute rinse in PBST, slides were incubated for 1 hour at room temperature with biotinylated secondary antibody, anti-goat horse IgG (BA-9500), purchased from Vector Laboratories (Burlingame, CA). Slides were rinsed in PBST for 5 minutes. To increase stain intensity and reduce background stain, slides were incubated for 15 minutes in VECTASTAIN® Elite ABC, purchased from Vector Laboratories. After a 5 minute rinse in PBST tissue sections were incubated for 5 minutes in 3,3'-diaminobenzidine (DAB), purchased from Vector Laboratories. To counterstain nuclei, slides were exposed to hematoxylin for 12 seconds and immediately rinsed in distilled water. Finally, slides were rehydrated by a series of 3, 3 minute washes in ethanol and xylene respectively, and then mounted with Permount. Normal horse IgG and anti-goat IgG were used as negative controls.

Data Analysis

Analysis of HOXA10 expression was performed independently by three evaluators blinded to specimen source and quantified using the H-Score. The H-Score was used to calculate glandular and stromal cell staining intensity and was calculated using the following equation: $H\text{-Score} = \sum P_i (i + 1)$, where the P_i represents the percentage of stained cells (0–100%) and stain intensity (i) is assigned a value of 1, 2, or 3 (weak, moderate, or strong respectively)(32,33). H-Score results for endometrial stromal and glandular staining obtained from each evaluator were averaged. Statistical analysis was performed using Mann Whitney Rank Sum Test, where statistical significance was defined as $P < 0.05$. Linear regression analysis was performed using Statistical Analysis Software (SAS) to identify variables confounding endometrial HOXA10 expression.

RESULTS

Copper IUD usage decreases endometrial HOXA10 expression

In women using a copper containing IUD, endometrial stromal HOXA10 expression, independent of menstrual cycle stage, was localized to the nucleus and markedly decreased when compared to non-IUD users (Fig 1a). Endometrial stromal HOXA10 expression has been previously well characterized in non-IUD users and served as a control and basis for comparison. Endometrial biopsies collected from controls throughout the menstrual cycle, demonstrated relatively high levels of endometrial stromal HOXA10 expression (Fig 1b). The mean H score for stromal expression of HOXA10 in women using Paraguard T380A IUD was 0.21 compared to 2.2 in the control endometrial biopsies (Fig 2). The glandular expression of HOXA10 was absent in the endometrium exposed to the IUD. In non-IUD users, endometrial

glandular expression was observed in the late proliferative phase and peaked during the mid-late secretory phase. Endometrial HOXA10 expression was lower in all IUD exposed specimens at any point in the menstrual cycle compared to controls.

Age and menstrual cycle stage predict endometrial HOXA10 expression level

Clinical variables were correlated with endometrial HOXA10 expression using multiple linear regression analysis. Patient age and last menstrual period were significant predictors of endometrial HOXA10 expression, $p = 0.008$ and $p = 0.012$ respectively. Duration of IUD usage and prolonged vaginal bleeding associated with use had no effect on endometrial HOXA10 expression in IUD users. (Table 1)

DISCUSSION

Copper containing IUDs are the most widely used reversible contraceptive worldwide(1–3). Copper-T IUD's demonstrate a persistently low failure rate, even at the end of the corrosive lifespan of copper in utero; thus suggesting that the mode of action cannot be explained by copper alone(8,9). Alterations in endometrial morphology and biochemical composition disrupt processes required for endometrial receptivity and implantation(34). Blastocyst implantation fails in copper exposed endometrium, while transfer of copper exposed blastocysts to a normal endometrium results in successful implantation; demonstrating the direct endometrial effect of the IUD (15). Previous studies from our laboratory demonstrate the essential contribution of endometrial HOXA10 expression to implantation and thus suggested a possible mechanism underlying the IUD's contraceptive effect. As copper release is not essential for maximal contraceptive efficacy, the effect of the IUD on HOXA10 expression is likely independent of the copper itself.

The use of non-hormonal IUD contraceptives results in a local foreign body effect characterized by a significant influx of inflammatory cells including polymorphonuclear leukocytes, mast cells, and macrophages(4–6). IL-1 β , a pleiotropic macrophage- derived cytokine, is involved in the inflammatory immune response(41). We have previously characterized the effects of inflammatory cytokines on HOX gene expression where we reported the effect of IL-1 β on HOXA10 expression in decidual cells. Exposure of decidual cells to IL-1 β resulted in an approximate 90% decrease in HOXA10 expression. (42) We propose the contraceptives local foreign body effect increases inflammatory cytokines, such as IL-1 β , resulting in a significant decrease in endometrial HOXA10 expression.

Adult endometrial cyclic HOXA10 expression is required to achieve endometrial receptivity (26–28). Maternal Hoxa10 expression is necessary for decidualization and embryo implantation. Mice with targeted Hoxa10 disruption demonstrate uterine factor infertility. Hoxa10 knockout mice produce a normal number of embryos, yet neither these embryos nor wild-type embryos implant in Hoxa10 (–/–) uteri. (29–31) Similarly, altered HOXA10 gene expression is associated with decreased implantation in humans. Endometriosis, a poorly understood gynecological disorder, is characterized by the presence of endometrial tissue outside of the uterus and often results in reproductive infertility. Endometrial expression of HOXA10 and HOXA11 are downregulated in women with endometriosis.(35,36) In addition, decreased endometrial HOXA10 expression is apparent in women with hydrosalpinges, polycystic ovary syndrome and submucosal uterine leiomyomas; all associated with reduced implantation rates (37–40). Diminished endometrial HOXA10 expression is a common mechanism by which multiple gynecological disease states impact endometrial receptivity.

The variables confounding uterine HOXA10 expression in IUD users include patient age and last menstrual period. The inverse relationship between age and uterine HOXA10 expression correlates with the well know association between age and fertility. The cyclic variation of

endometrial HOXA10 expression is well characterized; therefore it is not surprising that menstrual cycle stage influences HOXA10 expression, even in IUD users(26). The variables including duration of usage and prolonged vaginal bleeding had no influence on HOXA10 expression or contraceptive efficacy; this correlates with both immediate and prolonged efficacy of IUDs. Duration of use doesn't improve or hinder IUD efficacy. Similarly, prolonged vaginal bleeding in the presence of a copper IUD does not impair contraceptive efficacy. Decreased endometrial HOXA10 expression in IUD users suggests a novel mechanism of copper IUD mode of action involving the suppression of genes required for endometrial receptivity, primarily endometrial HOXA10 expression.

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References

1. Xia X, Xie C, Wang Y, Cai S, Zhu C, Yang X. The forces imposed by the novel T-shape Cu/LDPE nanocomposite intrauterine devices on the simulated uterine cavity. *Contraception* 2007;76(4):326–30. [PubMed: 17900446]
2. Bahamondes L, Faundes A, Sobreira-Lima B, Lui-Filho JF, Pecci P, Matera S. TCu 380A IUD: a reversible permanent contraceptive method in women over 35 years of age. *Contraception* 2005;72(5):337–341. [PubMed: 16246658]
3. Grimes DA, Lopez LM, Manion C, Schulz KF. Cochrane systematic reviews of IUD trials: lessons learned. *Contraception* 2007;75(6 Supplement 1):S55–S59. [PubMed: 17531618]
4. Sandvei R, Wollen AL, Flood PR, Anker C. Mast cells in the tubal wall in women using an intrauterine contraceptive device. *Br J Obstet Gynaecol* 1986;93(7):758–64. [PubMed: 3730347]
5. Cuadros A, Hirsch JG. Copper on intrauterine devices stimulates leukocyte exudation. *Science* 1972;175(18):175–6. [PubMed: 4621480]
6. Salaverry G, Méndez Mdel C, Zipper J, Medel M. Copper determination and localization in different morphologic components of human endometrium during the menstrual cycle in copper intrauterine contraceptive device wearers. *Am J Obstet Gynecol* 1973;115(2):163–8. [PubMed: 4570954]
7. Sheppard BL. Endometrial morphological changes in IUD users: A review. *Contraception* 1987;36(1):1–10. [PubMed: 3117492]
8. Timonen H. Copper release from Copper-T intrauterine devices. *Contraception* 1976;14(1):25–38. [PubMed: 975809]
9. Kosonen A. Corrosion of copper in utero. *Fertil Steril* 1978;30(1):59–65. [PubMed: 680184]
10. Tredway DR, Umezaki CU, Mishell DR Jr, Settlege DS. Effect of intrauterine devices on sperm transport in the human being: preliminary report. *Am J Obstet Gynecol* 1975;123(7):734–5. [PubMed: 1200066]
11. Aref I, Kandil O, El-tagi A, Morad MR. Effects of non-medicated and copper IUDs on sperm migration. *Contracept Deliv Syst* 1983;4(3):203–6. [PubMed: 12265363]
12. Ullmann G, Hammerstein J. Inhibition of sperm motility in vitro by copper wire. *Contraception* 1972;6(1):71–6. [PubMed: 4659586]
13. Roblero L, Guadarrama A, Lopez T, Zegers-Hochschild F. Effect of copper ion on the motility, viability, acrosome reaction and fertilizing capacity of human spermatozoa in vitro. *Reprod Fertil Dev* 1996;8(5):871–4. [PubMed: 8876046]
14. Kadanali S, Varoglu E, Komec D, Uslu H. Evaluation of active and passive transport mechanisms in genital tracts of IUD-bearing women with radionuclide hysterosalpingoscintigraphy. *Contraception* 2001;63(1):41–5. [PubMed: 11257248]
15. Chang CC, Tatum HJ, Kincl FA. The effect of intrauterine copper and other metals on implantation in rats and hamsters. *Fertil Steril* 1970;21(3):274–8. [PubMed: 5435750]
16. Daftary GS, Taylor HS. Endocrine regulation of HOX genes. *Endocr Rev* 2006;27(4):331–55. [PubMed: 16632680]

17. 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(9):2687–96. [PubMed: 8787743]
18. Krumlauf R. Hox genes in vertebrate development. *Cell* 1994;78(2):191–201. [PubMed: 7913880]
19. Vitiello D, Pinard R, Taylor HS. Gene expression profiling reveals putative HOXA10 downstream targets in the periimplantation mouse uterus. *Reproductive Sciences* 2008;15(5):529–534. [PubMed: 18579861]
20. Eun Kwon H, Taylor HS. The role of HOX genes in human implantation. *Ann N Y Acad Sci* 2004;1034:1–18. [PubMed: 15731295]
21. Vitiello D, Kodaman P, Taylor HS. HOX Genes in Implantation. *Semin Reprod Med* 2007;(06):431–36. [PubMed: 17960527]
22. Ma L, Benson GV, Lim H, Dey SK, Maas RL. Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in müllerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 1998;197(2):141–54. [PubMed: 9630742]
23. Favier B, Dolle P. Developmental functions of mammalian Hox genes. *Mol Hum Reprod* 1997;3(2):115–31. [PubMed: 9239717]
24. Taylor HS, Vanden Heuvel GB, Igarashi P. A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. *Biol Reprod* 1997;57(6):1338–45. [PubMed: 9408238]
25. McGinnis W, Krumlauf R. Homeobox genes and axial patterning. *Cell* 1992;68 (2):283–302. [PubMed: 1346368]
26. Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest* 1998;101(7):1379–84. [PubMed: 9525980]
27. Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. *J Clin Endocrinol Metab* 1999;84(3):1129–35. [PubMed: 10084606]
28. Sarno JL, Kliman HJ, Taylor HS. HOXA10, Pbx2, and Meis1 protein expression in the human endometrium: formation of multimeric complexes on HOXA10 target genes. *J Clin Endocrinol Metab* 2005;90(1):522–8. [PubMed: 15494461]
29. Du H, Taylor HS. Molecular regulation of müllerian development by Hox genes. *Ann N Y Acad Sci* 2004;1034:152–65. [PubMed: 15731308]
30. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa 10 deficient mice. *Nature* 1995;374(6521):460–463. [PubMed: 7700356]
31. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. *Gene Ther* 2000;7(16):1378–84. [PubMed: 10981664]
32. Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, et al. Aberrant integrin expression in the endometrium of women with endometriosis. *J Clin Endocrinol Metab* 1994;79(2):643–9. [PubMed: 7519194]
33. Sharpe-Timms KL, Rieke EA, Piva M, Horowitz GM. Differential expression and localization of de-novo synthesized endometrial haptoglobin in endometrium and endometrial lesions. *Hum Reprod* 2000;15(10):2180–5. [PubMed: 11006195]
34. Johannisson E. Mechanism of action of intrauterine devices: biochemical changes. *Contraception* 1987;36(1):11–22. [PubMed: 3311620]
35. Kim JJ, Taylor HS, Lu Z, Ladhani O, Hastings JM, Jackson KS, et al. Altered expression of HOXA10 in endometriosis: potential role in decidualization. *Mol Hum Reprod* 2007;13(5):323–32. [PubMed: 17350963]
36. 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 (5):1328–31. [PubMed: 10325287]
37. Daftary GS, Taylor HS. Hydrosalpinx fluid diminishes endometrial cell HOXA10 expression. *Fertil Steril* 2002;78(3):577–80. [PubMed: 12215336]
38. Daftary GS, Kayisli U, Seli E, Bukulmez O, Arici A, Taylor HS. Salpingectomy increases peri-implantation endometrial HOXA10 expression in women with hydrosalpinx. *Fertil Steril* 2007;87(2):367–72. [PubMed: 17173899]

39. 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(1): 238–43. [PubMed: 12519859]
40. Rackow BW, Taylor HS. Submucosal uterine leiomyomas have a global effect on molecular determinants of endometrial receptivity. *Fertil Steril*. 2008 Epub ahead of press.
41. Lebovic DI, Bentzien F, Chao VA, Garrett EN, Meng YG, Taylor RN. Induction of an angiogenic phenotype in endometriotic stromal cell cultures by interleukin-1beta. *Mol Hum Reprod* 2000;6(3): 269–75. [PubMed: 10694276]
42. Sarno JL, Schatz F, Lockwood CJ, Huang ST, Taylor HS. Thrombin and interleukin-1beta regulate HOXA10 expression in human term decidual cells: implications for preterm labor. *J Clin Endocrinol Metab* 2006;91(6):2366–72. [PubMed: 16551735]

FIGURE 1.

Endometrial HOXA10 expression using immunohistochemical analysis. Endometrial HOXA10 expression was predominantly localized in the stroma and significantly decreased in patients using Paraguard T380A (A) when compared to non-IUD users (B). Representative photomicrographs (40x) illustrate immunohistochemistry results performed with a HOXA10 polyclonal antibody.

FIGURE 2.

The effect of copper IUD usage on endometrial cell HOXA10 expression. Mean H scores for endometrial stromal HOXA10 expression for Paraguard T380A user's is significantly decreased in comparison to non-IUD user's (* $p < 0.001$).

TABLE 1

Selected Demographics of Patient Population Using Copper IUD and Fertile Controls

Variable	IUD Group	Control Group
Age (years)	28.0 (19–39)	39.4 (31–48)
Duration of Use (months)	25.63 (2–122)	-