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

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Objective: To characterize human endometrial HOXA10 expression in patients using a copper intrauterine device (IUD).
Design: Case-control study.
Setting: Academic medical center.
Patient(s): Women using copper IUDs.
Intervention(s): Immunohistochemical analysis of endometrial HOXA10 expression in biopsy samples obtained from 24 women using a copper Paraguard T380A as well as in samples obtained from 10 normal cycling women who were not using an IUD or hormonal contraceptives.
Main Outcome Measure(s): Endometrial HOXA10 expression.
Result(s): Endometrial HOXA10 expression was markedly decreased in the biopsy samples obtained from women using the IUD contraceptive when compared with controls. The mean H score for endometrial stromal cell HOXA10 expression in samples obtained from women using the Paraguard IUD was 0.21 compared with 2.2 in the control endometrial biopsy samples. 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. (Fertil Steril® 2009;92:1820–4. ©2009 by American Society for Reproductive Medicine.)

Key Words: Endometrium, implantation, contraception, IUD, HOX

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 incompletely characterized (2, 3). Endometrial morphology and biochemical composition are important factors in reproduction; modification of such factors results in interference with 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 (8) and Kosonen (9) 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 its copper-releasing lifespan, raising the question as to whether the mode of action can be explained by the release of copper alone.

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 with non–IUD users, yet hindrance of sperm migratory ability only diminishes the chance of fertilization. Chang and Tatum (15) studied the effect of intrauterine copper in rats, demonstrating that the transfer of blastocysts from a copper-exposed uterus to a normal uterus resulted in normal implantation, whereas normal blastocysts

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transferred to a copper-influenced uterus failed to implant. They concluded that the altered intrauterine environment was the mechanism underlying the contraceptive effect.

Hox genes (HOX in human) encode evolutionarily conserved transcription factors, which are essential to embryonic development, endometrial development, and endometrial receptivity (16–19). During the embryonic period, the 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 17β-estradiol and progesterone (22, 26).

We have previously demonstrated the differential expression pattern of HOXA10 protein in human endometrial biopsy samples throughout the menstrual cycle (26, 27). Expression of HOXA10 increases throughout the menstrual cycle, most dramatically during the midsecretory 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 our current study, we investigated the effect of the copper-containing Paraguard T380A IUD 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

Study Participants

Twenty-four women using Paraguard T380A were recruited under an approved Human Investigation Committee protocol. Informed consent was obtained from all participants. 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 to 43). The average duration of IUD usage was 17 months (range: 1 to 122 months). Control endometrial biopsy samples were obtained from 10 normally cycling women 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 5-μm 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 0.01 M sodium citrate for 20 minutes and at room temperature for an additional 20 minutes. Slides were rinsed in phosphate-buffered saline (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 nonspecific 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; Santa Cruz Biotechnology, Santa Cruz, CA). After a 5-minute rinse in PBST, slides were incubated for 1 hour at room temperature with a biotinylated secondary antibody, antigoat horse IgG (BA-9500; 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 (Vector Laboratories). After a 5-minute rinse in PBST, tissue sections were incubated for 5 minutes in 3,3'-diaminobenzidine (DAB; 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 three 3-minute washes in ethanol and xylene, respectively, and then mounted with Permount. Normal horse IgG and antigoat 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 score $= \sum \text{Pi}\,(i+1)$, where the Pi represents the percentage of stained cells (0 to 100%) and stain intensity (i) is assigned a value of 1, 2, or 3 (weak, moderate, or strong, respectively) (32, 33). The 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, and $P<.05$was considered statistically significant. Linear regression analysis was performed using Statistical Analysis Software (SAS Institute, Inc., Cary, NC) 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 with non–IUD users (Fig. 1a). Endometrial stromal HOXA10 expression has been previously well