



Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

HOXA-10 gene expression in ectopic and eutopic endometrium tissues: Does it differ between fertile and infertile women with endometriosis?



Canan Özcan^a, Özkan Özdamar^{b,*}, Merve Ertan Gökbayrak^c, Emek Doğur^a,
Yiğit Çakıroğlu^a, Naci Çine^c

^a Department of Obstetrics and Gynecology, Kocaeli University, Faculty of Medicine, Kocaeli, Turkey

^b Department of Obstetrics and Gynecology, Istanbul Medeniyet University, Faculty of Medicine, Istanbul, Turkey

^c Department of Genetics, Kocaeli University, Faculty of Medicine, Kocaeli, Turkey

ARTICLE INFO

Article history:

Received 15 January 2018

Received in revised form 12 September 2018

Accepted 26 November 2018

Keywords:

Endometriosis

Endometrioma

Infertility

HOXA-10 gene

ABSTRACT

Objective: To compare HOXA-10 gene expression in eutopic endometrium samples, between fertile and infertile endometriosis patients and the fertile control cases, and in endometrium and endometrioma specimens, between severe and moderate endometriosis cases.

Study Design: Prospective clinical study included women without infertility and endometriosis (Group 1); women without infertility but with endometrioma (Group 2); and infertile women with endometrioma (Group 3). In addition, the Group 2 and 3 cohort were assessed based on the findings obtained during laparoscopy, based on the (rAFS) scoring, as women with a rAFS score of 16–40 were evaluated in Group A, whereas those with rAFS score above 40 were considered in Group B. HOXA-10 gene expression was evaluated in both secretory endometrium tissue and endometrioma specimens.

Results: Eutopic endometrium samples from group 2 (reference gene = 0,680 vs. target gene = 0,362) and group 3 (reference gene = 0,641 vs. target gene = 0,183) patients revealed a 1,871-fold and 3,509-fold decrease in HOXA-10 gene expression, respectively, as compared to group 1. Endometrial HOXA-10 gene expression was 1,778-fold down-regulated in group 3 women (reference gene = 1,510 vs. target gene = 0,850), when compared to group 2. Both eutopic endometrium and endometrioma tissue samples from severe endometriosis patients revealed 1,259-fold (reference gene = 1,523 vs. target gene = 1,210) and 1,338-fold (reference gene = 1,274 vs. target gene = 0,952), down-regulation in HOXA-10 gene expressions, respectively, as compared to moderate cases.

Conclusion: Endometrial HOXA-10 gene expression in women with endometriosis is significantly down-regulated than in those without endometriosis. Endometriosis patients with infertility have significantly lower levels of endometrial HOXA-10 gene expression than endometriosis without infertility; thus decreased expression of this gene may, directly or indirectly, be related with the endometriosis-associated infertility. Severe endometriosis cases express, in their both endometrium and endometrioma tissues, significantly lower levels of HOXA-10 gene than moderate endometriosis cases.

© 2018 Elsevier B.V. All rights reserved.

Introduction

Endometriosis is an enigmatic disease affecting approximately 5–10% of reproductive age women [1]. While there is a fair amount of evidence demonstrating that endometriosis has a negative impact on fertility, it is still unknown why some patients with endometriosis are infertile while others are not, in addition to the

fact that causal association between endometriosis and infertility has not been clearly defined [1,2]. Instead, a number of pathogenetic pathways have been postulated to explain the potential insult to the conception process, including pelvic adhesions that may disrupt tubal function; the concomitant presence of pelvic pathologies that is both associated with endometriosis and infertility, such as adenomyosis and endometrial polyps [3–5]; surgery related damage to the ovarian reserve consequent to the excision of ovarian endometriomas, in particular for women operated on for bilateral endometrioma cysts [6]; and chronic inflammatory milieu of the pelvis that may reduce the quality of the oocyte, and interfere with the fertilization process, tubal function and the endometrium [1].

* Corresponding author at: Istanbul Medeniyet University, Faculty of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey.

E-mail addresses: ozkan_ozdamar@yahoo.com,
ozkan.ozdamar@medeniyet.edu.tr (Ö. Özdamar).

Defective implantation is a relatively novel entity proposed to enlighten the underlying mechanisms of endometriosis-related infertility. Numerous studies suggested that endometriosis leads to implantation defects [7,8], although it remains controversial whether defective implantation is due to suboptimal oocyte quality or a compromised endometrial receptivity. Endometrial receptivity is a complex interaction between conceptus and the uterine epithelium, involving changes in the expression of genes related to the attachment of trophoblast, modification of the uterine stromal cells, silencing of genes for immune recognition, activation of genes for nutrient transport into the uterine lumen, and enhanced signaling for pregnancy recognition, alterations in membrane permeability, and increased endometrium revascularization [9]. Changes in endometrial receptivity due to endometriosis has been well investigated in the current literature. Several studies support the idea that endometriosis does not affect endometrial receptivity, and hence, women with and without endometriosis reveal comparable implantation rates [10–13], while others reported that endometriosis decreases the receptivity of endometrium, thus, hinders the development of oocyte and embryo, and jeopardize the chances of infertility [8,14,15]. However, those studies that claim the possible detrimental impact of endometriosis on endometrial receptivity and implantation rates fail to show the underlying mechanisms of endometriosis-associated infertility. In this context, several molecules, genes or inflammatory cytokines that are involved in the implantation process have been investigated [16,17].

One of the best-recognized sequences of signaling events in implantation has been defined with homeobox A (HOXA), a member of the GATA family of transcription factors [18]. HOXA-10 is one gene that is normally up-regulated in the human endometrium during the window of implantation and its levels increase dramatically during the mid-secretory phase of the menstrual cycle [19]. Animal studies demonstrated that HOXA-10-null mice are severely infertile and represent anatomic defects in the reproductive systems [20]. Studies regarding the HOXA-10 gene expression patterns in the endometrium of patients with endometriosis report conflicting results [21–26]. Moreover, it is yet to be clarified whether there is a relationship between the prevalence, stage, location and adhesion formation capacity of endometriosis and HOXA gene expression. Some recent studies reported endometrial HOXA-10 gene expression variations following the surgical treatment of endometrioma lesions, suggesting an improvement in endometrial receptivity [27]. Given the lack of a consensus regarding the role of HOXA-10 gene in the process of implantation in women with endometriosis, relationship between HOXA-10 gene expression aberrations and implantation failure in women with endometriosis still remains to be clarified [28,29]. On the other hand, epigenetic changes in HOXA gene clusters, with downregulation and hypermethylation of GATA2 and hypomethylation and activation of GATA6, have been postulated to be involved in progesterone resistance and altered estrogen responses in endometriosis [30]. Expression profiling studies show that stromal cells of endometriosis lesions have altered patterns of DNA methylation, compared to stromal cells in normal endometrium. Genes found to have differential methylation included examples of signaling components and transcription factors, such as HOXA clusters [31], which constituted the rationale for the comparison of HOXA-10 gene expressions between endometriotic lesions and native endometrium tissue in this study.

In the present study, we aimed to compare HOXA-10 gene expression alterations between eutopic endometrium samples obtained during the secretion period, from fertile and infertile women with endometriosis and from the fertile control cases, who were presumed not to have endometriosis. We also attempted to investigate whether HOXA-10 gene expression within the

endometrium samples and endometrioma specimens, obtained during surgery, is different between severe and moderate endometriosis cases.

Materials and methods

This prospective clinical study was performed in a tertiary referral hospital in the period between October 2014 and December 2015 after the study protocol was approved by the Institutional Review Board (IRB date and number: 30.09.2014 - 256).

The study was conducted with a total of 33 patients who were assigned to either of the three groups, after signed informed consent forms were obtained from each patient. Group 1 consisted 11 women without infertility or pelvic pain symptoms, as controls, who were presumed not to have endometriosis and/or endometrioma, based on the absence of clinical and/or sonographic findings suggestive of endometriosis, whereas Group 2 and 3 comprised 11 fertile and 11 infertile women, respectively, who underwent an endometrioma surgery due to severe dysmenorrhea, dyspareunia and chronic pelvic pain, as well as sonographic evidence of endometrioma.

Patient selection

General inclusion criteria were age ≤ 39 years and regular menstrual periods, lasting from 21 to 35 days. Additional inclusion criteria for the Group 1 were as follows; having at least two pregnancies that resulted in birth within the last 5 years, achievement of their pregnancies within the first 6-month period of the unprotected sexual intercourse and having no diseases that would potentially alter endometrial gene expression, such as leiomyomas, pelvic inflammatory diseases and hydrosalpinges. The assumption that women in this group had no endometriosis was based on the absence of infertility or subfertility, dysmenorrhea and dyspareunia in the history, absence of pain, tenderness, rectovaginal nodules and obliteration of the pouch of Douglas in the pelvic examination and the absence of endometrioma and free fluid in the pouch of Douglas in the ultrasonographic assessment. However, it was not visually confirmed whether any endometriotic lesions existed in this group of women.

Group 2 consisted of patients similar to those in Group 1, in terms of reproductive potential, with the exception of endometrioma status. Women in Group 2 had ultrasonographic evidence of endometrioma and were destined to surgical intervention due to complaints such as severe dysmenorrhea, dyspareunia or chronic pelvic pain. On the other hand, women who conceived by any of the assisted reproductive techniques (ART), such as ovulation induction, controlled ovarian stimulation, intrauterine insemination (IUI) or *in vitro* fertilization (IVF), and who is still using contraceptive methods by means of any hormonal methods, were not included in the Groups 2 or 3.

Group 3 comprised of those who were under treatment regimens of IUI or IVF, due to failure to conceive in spite of unprotected sexual intercourse for at least 24 month-period, in addition to the ultrasonographic evidence of endometrioma. Women in the Group 3 had been destined to surgical intervention due to ovarian endometrioma and complaints such as severe dysmenorrhea, dyspareunia or chronic pelvic pain. Similar to Groups 1 and 2, women with diseases that would potentially alter the endometrial gene expression, such as leiomyoma, pelvic inflammatory disease and hydrosalpinx, were excluded. Moreover, women with a history of conception, either natural or assisted, within the last 2 year-period, and those who were under hormonal suppression therapy, as well as infertility reasons of male factor (Total progressive motile sperm count $<10 \times 10^6$), bilateral

complete tubal obstruction, uterine anomaly or decreased ovarian reserve, which was described as follicle stimulating hormone (FSH) >12 mIU/ml or anti-Müllerian hormone (AMH) <0.5 ng/ml and antral follicle count (AFC) <5, were excluded.

The included patients were assessed in terms of HOXA-10 gene expression within the endometrium tissue. Also, an additional group analysis was also performed by classifying the cases in groups 2 and 3, according to the findings obtained during surgery, based on the revised American Fertility Society (rAFS) scoring. Patients with a rAFS score of 16–40 were considered moderate endometriosis and enrolled in group A (n = 10), whereas those with a rAFS score above 40, who were considered as severe endometriosis cases, were enrolled in group B (n = 12). In these women, HOXA-10 gene expression was evaluated in both native secretory endometrium tissue and endometrioma specimens.

Sample collection

Serum FSH and AMH levels were measured between the 2nd and 4th days of the menstrual cycle in all cases, whereas CA125 levels were measured on the hospital admission day for endometrioma surgery. Endometrial sampling was performed after the 18th day and before 24th day of the menstrual cycle; within the endometrial secretory phase following the ovulation having been proven by either ultrasonographic demonstration of corpus luteum or measurements of serum progesterone levels. In Group 1, endometrium was sampled from all four aspects of the uterine cavity with a canula after the cervix was visualized at the dorsal lithotomy position and was infiltrated prilocain hydrochloride (Priloc Flacon, Vem, Istanbul, Turkey), 4 ml, at both 5 and 7 o'clock positions. In Groups 2 and 3, endometrial sampling was performed under general anesthesia, just before the endometrioma operation began, without prilocain hydrochloride administration and just the same way the Group 1 cases. At least 1 cc endometrial sample from each case was transported inside the liquid nitrogen and conserved at the deep freezer at -80°C , in all patient groups.

Uni- or bilateral endometrioma cysts were removed by laparotomic or laparoscopic approach in group 2 and 3 patients.

Additional intraabdominal pathologies, such as adhesions, peritoneal endometriotic implants or deep rectovaginal endometriotic nodules were removed, excised or cauterized, if necessary, and all were noted. In the both groups, severity of endometriosis was assessed by the rAFS scoring system and groups A and B were created, accordingly. There were no prior intraabdominal surgery cases or patient with pelvic inflammatory disease diagnosis, in groups 2 and 3. Endometrioma specimens obtained from either groups, by laparotomic or laparoscopic surgeries, were transported inside the liquid nitrogen and preserved at the deep freezer at -80°C .

Analysis of the samples for detection of HOXA-10 expression

RNeasy Mini Kit extraction procedure (Qiagen, Valencia, CA, USA) was used with column-based, high-sensitivity discrimination kits to obtain ribonucleic acid (RNA) from the healthy endometrium and endometrioma tissue samples stored in the deep-freezer at -80°C . The tissues placed in Magna Lyser Green Beads (Roche, Mannheim, Germany) were homogenized in a lysis RLT buffer containing β -mercaptoethanol using Thermo Savant FastPrep FP120 homogenizer. By centrifuging the cell lysate, the RNA is separated from other cellular macromolecules. The RNA products were purified with DNAase I, by using Buffer RLT and Buffer RPE solution in the kit. The quality and accuracy of the RNA products were checked prior to the gene expression study in terms of quality with Agilent 2100 Bioanalyzer (Agilent, Palo Alto, USA) and quantitatively with the Nanodrop ND 1000 Spectrophotometer.

Validation of the relative gene expression with quantitative reverse transcription polymerase chain reaction (Q-RT PCR) (LightCycler): Total RNA (1 μg) was used for the synthesis of c-deoxyribonucleic acid (cDNA) (First Strand cDNA synthesis kit; Roche, Mannheim, Germany). Specific gene primers were derived from IDT. The gene primer sequences for HOXA-10 are as follows:

HOXA-10 F: TACACGAAGCACCAGACTGGA

HOXA-10 R: ATCCTGCGTTCTGAAACCAGA

Using sequence dilutions of the beta-globulin gene, standard curves were obtained (DNA control kit, Roche, Mannheim, Germany). Fast-Start Master SYBR green mix was used with 2 μg

Table 1
Demographic and clinical characteristics of patients in Groups 1, 2 and 3.

	Group 1 (n = 11)	Group 2 (n = 11)	Group 3 (n = 11)	p value
Age (years) ^a	36 ± 3.68 (27-39)	33.18 ± 5.23 (23-39)	30.54 ± 5.87 (23-39)	0.11
BMI (kg/m ²) ^a	23.88 ± 2.88 (19-28)	24.46 ± 3.71 (18.3-31.1)	25.89 ± 4.19 (19.3-31.2)	0.89
Duration of marriage (months) ^a	95.45 ± 33.42 (35-145)	118 ± 50.33 (18-170)	44.45 ± 22.31 (12-67)	0.13
Gravidity (n) ^b	3.36 ± 1.56 (2-6)	2.54 ± 0.82 (2-4)	–	0.21
Parity (n) ^b	2.63 ± 1.02 (2-5)	2.09 ± 0.3 (2-3)	–	0.11
Endometrioma diameter (mm) ^b	–	57.72 ± 25.42 (30-110)	50.09 ± 14.07 (30-66)	0.44
AFS score ^b	–	48 ± 26.61 (20-92)	45.18 ± 23.9 (20-76)	0.60
FSH (mIU/ml) ^a	7.21 ± 1.38 (4.8-9.1)	8.07 ± 2.66 (2.54-12)	8.06 ± 2.55 (4.86-12)	0.34
AMH (ng/ml) ^a	1.84 ± 1.06 (0.7-4.21)	1.38 ± 0.65 (0.50-2.41)	1.54 ± 1.09 (0.52-4.03)	0.41
CA 125 (U/ml) ^a	20.15 ± 7.06 (7.6-30)	67.73 ± 43.03 (7.2-175.9)	55.29 ± 48.6 (5.4-172.6)	0.001 ^c

Data are presented as mean ± standard deviation (minimum-maximum).

BMI: Body mass index, AFS: American Fertility Society, FSH: Follicle stimulating hormone, AMH: Anti-müllerian hormone.

^a Data were analyzed using Kruskal Wallis test.

^b Data were analyzed using Mann Whitney U test.

^c Statistically significant.

cDNA and 10 pmol/l primer. The amplification schedule was programmed to be 10 s. at 95 °C; 45 cycles, 5 s. at 55 °C and 72 °C for 10 s. annealing. In the melting curve analysis at the time of the amplification, the result was generally 0 s. at 95 °C, 10 s. at 65 °C and 0 s. at 95 °C per cycle. In each assay, the negative control proceeded with no cDNA-containing water. Each assay contained duplicated reactions for dilution and recurrence. At each gene concentration, the kinetic approach was obtained using the basis software (LightCycler).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 16.0 software (SPSS, Chicago, IL, USA). Binary and triple group comparisons were made by Mann Whitney-U and Kruskal-Wallis test, respectively. Statistical analysis of RNA data was performed by The Relative Quantification Relative Expression Software Tool (REST) software (Germany). Values of $p < 0.05$ were considered statistically significant.

Results

In our study, median infertility period in Group 3 patients was 40 months (min-max 24–72). In this group, two women was administered IUI, whereas four women underwent a total of six ICSI cycles. Demographic and clinical characteristics of the patients in the groups 1, 2 and 3 are presented in Table 1. Demographic and clinical characteristics of the patients in the groups A and B are presented in Table 2. Mean and delta-cycle threshold (CT) values and their comparisons between target and reference genes are presented in Table 3. In the endometrium tissue samples, mean CT value was 2,244 in Group 1 as compared to 2,390 in Group 2 and 2,489 in Group 3. In the comparison of the mean CT between Group 2 and Group 3, the values were 2,466 and 2,489, respectively. Statistical analysis of RNA data with REST software revealed a 1,871-fold and 3,509-fold down-regulation in HOXA-10 gene expression in the eutopic endometrium samples of group 2 (reference gene = 0,680 vs. target gene = 0,362) and group 3 (reference gene = 0,641 vs. target gene = 0,183) cases, respectively, as compared to group 1 cases. Moreover, endometrial HOXA-10 gene expression was demonstrated to decrease 1,778-fold in the samples of group 3 women (reference gene = 1,510 vs. target gene = 0,850), when compared to those of group 2 (Table 3).

In the comparison of native endometrium tissues between Group A and Group B, mean CT value was 2,488 in Group A, while it was 24,6 in Group B. Endometrioma tissue sample comparison

revealed a mean CT value of 2,422 in Group A and 2,429 in Group B. When compared to Group A, endometrium and endometrioma tissue samples of the Group B patients revealed 1,259-fold (reference gene = 1,523 vs. target gene = 1,210) and 1,338-fold (reference gene = 1,274 vs. target gene = 0,952), decrease in HOXA-10 gene expressions, respectively (Table 3).

Discussion

In the present study, we demonstrated that HOXA-10 gene expression in the eutopic endometria of women with endometriosis is significantly down-regulated than those without endometriosis. Also, our results suggest that endometriosis patients with infertility have significantly lower levels of HOXA-10 gene expression than those without infertility; thus decreased expression of this gene may, directly or indirectly, be related with the endometriosis-associated infertility. Moreover, more severe endometriosis cases appear to express significantly lower levels of HOXA-10 gene than moderate cases do.

In patients with endometriosis, the HOXA-10 gene in the eutopic endometrium was reported to be down-regulated in the mid-secretory phase when compared with the control group, and it was extrapolated that embryo implantation decreased due to alterations of gene expression [21–23,32]. In accordance with these, our results suggest that HOXA-10 gene expression in the eutopic endometrium samples is 1.8-fold and 3.5-fold down-regulated in fertile and infertile endometriosis patients, respectively, as compared to the women without infertility and endometriosis. Our results are compatible with the study by Matsuzaki et al. [24], which reported significantly lower levels of HOXA-10 mRNA and protein expression levels in infertile patients with different types of endometriosis as compared with the healthy fertile controls. However, it is not known whether similar HOXA-10 expression alterations emerge in endometriosis patients without infertility and, to the best of our knowledge, no study comparing fertile and infertile endometriosis patients with healthy fertile controls is available in the current English literature. Our results indicate that the decreased expression of this gene may directly be related with the endometriosis-associated implantation failure or may surrogate different, or not yet known, underlying mechanisms that ultimately deteriorate fertility potential of the woman. This result may be an important indicator of the dramatic effect of endometriosis on implantation. Although some studies [33] reported that the cause of infertility was not an inappropriate endometrial environment and that uterine receptivity was not affected in IVF cycles with donor oocytes in women with endometriosis, our results indicate that HOXA-10 expression in mid-secretory endometrium is not up-regulated in this population and that the lower the gene expression, the greater the likelihood of infertility.

Current data available is scarce regarding the impact of endometrioma resection on the expression of HOX genes and the associated reproductive outcomes. A case-control study by Celik et al. [27] compared numerous endometrial gene expressions, including HOXA-10 and HOXA-11, between two endometrial sampling, one performed at the time of surgery and the other obtained 3 months after endometrioma resection. The authors reported that endometrioma resection increased peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression, suggesting an improvement in endometrial receptivity, and concluded that endometrioma was associated with endometrial receptivity defect that affects fertility regardless of other causes of infertility, such as defective folliculogenesis, poor oocyte quality, adhesions. However, they did not assess whether reproductive outcomes improved in line with the increase in the HOXA gene expressions.

Table 2
Demographic and clinical characteristics of patients in Groups A and B.

	Group A (n = 10)	Group B (n = 12)	p value
Age (years)	30.4 ± 7.08 (23-39)	33.08 ± 3.87 (25-39)	0.45
BMI (kg/m ²)	25.53 ± 4 (19.3-31.1)	24.88 ± 4.02 (18.3-31.2)	0.82
CA 125(U/ml)	42.05 ± 23.41 (7.2-73)	77.7 ± 53.18 (5.4-175.9)	0.06
Endometrioma diameter (mm)	55.1 ± 17.12 (30-80)	52.91 ± 23.54 (30-110)	0.62
AFS score	21.4 ± 2.06 (20-25)	67.58 ± 10.31 (60-92)	<0.001 ^a

Data were analyzed using Mann Whitney U test. Data are presented as mean ± standard deviation (minimum-maximum).

BMI: Body mass index, AFS: American Fertility Society.

^a Statistically significant.

Table 3Mean and Δ CT values within and between the patient groups.

GROUP COMPARISONS	MEAN CT VALUE		STANDARD DEVIATION		CV %95		Δ CT VALUE		CVs OF RELATIVE GENE EXPRESSION	
	HOXA Gene	Reference Gene	Up Regulated	Down Regulated	HOXA Gene	Reference Gene	Up Regulated	Down Regulated		
Group 1 vs. Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	0,363	0,680	–	1,871
	22,44	23,90	1,61	0,36	7,15	1,51				
Group 1 vs. Group 3	Group 1	Group 3	Group 1	Group 3	Group 1	Group 3	0,183	0,641	–	3,509
	2,244	2,489	1,61	1,14	7,15	4,56				
Group 2 vs. Group 3	Group 2	Group 3	Group 2	Group 3	Group 2	Group 3	0,850	1,510	–	1,778
	24,66	24,89	1,95	1,14	7,92	4,56				
Group A vs. Group B ^a	Group A	Group B ^a	Group A	Group B ^a	Group A	Group B ^a	1,210	1,523	–	1,259
	24,88	24,61	2,41	0,77	9,69	3,11				
Group A vs. Group B ^b	Group A	Group B ^b	Group A	Group B ^b	Group A	Group B ^b	0,952	1,274	–	1,338
	24,22	24,29	0,49	1,50	2,02	6,19				

CV, coefficient of variation; CT, cycle threshold.

^a Data represents the comparison of native endometrium tissues between groups A and B.^b Data represents the comparison of endometrioma tissues between groups A and B.

Another insufficiently researched issue is whether there is a relationship between the severity of the disease and gene expression. Experimental endometriosis studies revealed that the HOXA-10 gene expression decreased in eutopic endometrium specimens of endometriosis subjects, and also there was a further decrease in the gene expression as the severity of the disease, demonstrated by the serial laparoscopy and sampling procedures, increased [19]. It is not yet known whether the inferior ART outcomes are associated with the decreased the expression of the HOX genes, although the probability of achieving pregnancy with IVF in the human is assumed to decrease as the stage of endometriosis increase, [34,35]. In their study investigating the association between the type of endometriosis lesions and HOXA-10 gene expression, Matsuzaki et al. [24] reported a more severe deterioration of HOXA-10 mRNA and protein expression in women with superficial peritoneal endometriosis as compared to those with deep infiltrating endometriosis and ovarian endometriosis. In our study, we investigated whether any association exists between HOXA-10 gene expression in both eutopic and ectopic endometrium tissues and the severity of intraperitoneal disease, which was assessed by the rAFS classification based on the findings in the surgery. Our results indicate that HOXA-10 expression in patients with severe disease was significantly lower than those with moderate cases, in both endometrium and endometrioma tissues. However, our results do not reveal any causal relationship between the severity of disease and the level of the gene expression; hence, it remains awaiting to be investigated whether the decrease in HOXA-10 gene expression results in more severe disease or levels of the gene expression drops as the disease advances.

The specific roles of the HOX genes in the endometrial receptivity during the implantation window are not fully understood, as there are limitations to the studies made for this purpose. In-vivo studies can be performed in cycles without pregnancy, thus, cyclic expressions of endometrial factors are not associated with subsequent pregnancy. In addition, gene suppression assays in animals or *in vitro* cell cultures provide indirect evidence of human function of these markers. In the present study, there is a number of limitations, one of which is the lack of an endometrial dating through pathologic examination that predicts the implantation window period, although it was ensured that all cases were in secretory phase after ovulation. Furthermore, with the assumption that the control cases that constitute Group 1 do not have endometriosis, we did not have the chance to demonstrate it laparoscopically. While obtaining an endometrial sample from elective tubal ligation volunteers would have been an appropriate option, formation of a control group from these women was waived due to the potential inequality that would have

occurred between the groups, given the more advanced ages of the tubal ligation candidates. On the other hand, lack of evaluation of the expression of the protein product encoded by the HOXA gene in endometrial samples may also be considered as a limitation, because even if the expression of a given gene is altered, the end-product of that gene may not change at the same level. Another limitation is the lack of the surveillance of the gene expression changes and reproductive outcomes after endometriosis surgery, as mentioned above. Another limitation of our study is that it does not evaluate the variations in the expression of HOXA-10, as well as in the fertility outcomes, after endometriosis surgery. This topic might be one of the main investigation topics of future studies.

In conclusion, HOXA-10 gene expression in women with endometriosis is significantly lower than in those without. Moreover, endometriosis patients with infertility have significantly lower levels of HOXA-10 gene expression than those without infertility; thus decreased expression of this gene may, directly or indirectly, be related with the endometriosis-associated infertility. On the other hand, severe endometriosis cases express significantly lower levels of HOXA-10 gene than moderate cases.

Conflict of interests

None.

Acknowledgement

None.

References

- [1] de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. *Lancet* 2010;376:730–8.
- [2] Somigliana E, Viganò P, Benaglia L, Busnelli A, Berlanda N, Vercellini P. Management of endometriosis in the infertile patient. *Semin Reprod Med* 2017;35(31):e7.
- [3] Vercellini P, Consonni D, Barbara G, Buggio L, Frattaruolo MP, Somigliana E. Adenomyosis and reproductive performance after surgery for rectovaginal and colorectal endometriosis: a systematic review and meta-analysis. *Reprod Biomed Online* 2014;28(6):704–13.
- [4] Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine adenomyosis and in vitro fertilization outcome: a systematic review and meta-analysis. *Hum Reprod* 2014;29(5):964–77.
- [5] Di Spiezio Sardo A, Di Carlo C, Minozzi S, Spinelli M, Pistotti V, Alviggi C, et al. Efficacy of hysteroscopy in improving reproductive outcomes of infertile couples: a systematic review and meta-analysis. *Hum Reprod Update* 2016;22(4):479–96.
- [6] Somigliana E, Berlanda N, Benaglia L, Viganò P, Vercellini P, Fedele L. Surgical excision of endometriomas and ovarian reserve: a systematic review on serum antimüllerian hormone level modifications. *Fertil Steril* 2012;98(6):1531–8.
- [7] Simon C, Gutierrez A, Vidal A, de los Santos MJ, Tarín JJ, Remohí J, et al. Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. *Hum Reprod* 1994;9:725–9.

- [8] Prapas Y, Goudakou M, Matalliotakis I, Kalogeraki A, Matalliotaki C, Panagiotidis Y, et al. History of endometriosis may adversely affect the outcome in menopausal recipients of sibling oocytes. *Reprod Biomed Online* 2012;25:543–8.
- [9] Bazer FW, Spencer TE, Johnson GA. Interferons and uterine receptivity. *Semin Reprod Med* 2009;27(1):90–102.
- [10] Inoue M, Kobayashi Y, Honda I, Awaji H, Fujii A. The impact of endometriosis on the reproductive outcome of infertile patients. *Am J Obstet Gynecol* 1992;167:278–82.
- [11] Bukulmez O, Yarali H, Gurgan T. The presence and extent of endometriosis do not affect clinical pregnancy and implantation rates in patients undergoing intracytoplasmic sperm injection. *Eur J Obstet Gynecol Reprod Biol* 2001;96:102–7.
- [12] Miravet-Valenciano J, Ruiz-Alonso M, Gomez E, Garcia-Velasco JA. Endometrial receptivity in eutopic endometrium in patients with endometriosis: it is not affected, and let me show you why. *Fertil Steril* 2017;108:28–31.
- [13] Kuivasaari P, Hippelainen M, Anttila M, Heinonen S. Effect of endometriosis on IVF/ICSI outcome: stage III/IV endometriosis worsens cumulative pregnancy and live-born rates. *Hum Reprod* 2005;20:3130–5.
- [14] Díaz I, Navarro J, Blasco L, Simon C, Pellicer A, Remohí J. Impact of stage III–IV endometriosis on recipients of sibling oocytes: matched case-control study. *Fertil Steril* 2000;74:31–4.
- [15] Lessey BA, Kim JJ. Endometrial receptivity in eutopic endometrium of women with endometriosis: it is affected, and let me show you why. *Fertil Steril* 2017;108(1):19–27.
- [16] Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* 2003;144:2870–81.
- [17] Dimitriadis E, Stoikos C, Stafford-Bell M, Clark I, Paiva P, Kovacs G, et al. Interleukin-11, IL-11 receptor alpha and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. *J Reprod Immunol* 2006;69:53–64.
- [18] Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. *Science* 2002;296:2185–8 Review.
- [19] 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;13:323–32.
- [20] Benson GV, Nguyen TH, Maas RL. The expression pattern of the murine Hoxa-10 gene and the sequence recognition of its homeodomain reveal specific properties of Abdominal B-like genes. *Mol Cell Biol* 1995;15:1591–601.
- [21] 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.
- [22] Gui Y, Zhag J, Yuan L. Regulation of HOXA 10 and its expression in normal and abnormal endometrium. *Mol Hum Reprod* 1999;5:866–73.
- [23] Wei Q, St Clair JB, Fu T. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. *Fertil Steril* 2009;91:1686–91.
- [24] Matsuzaki S, Canis M, Darcha C, Pouly JL, Mage G. HOXA-10 expression in the mid-secretory endometrium of infertile patients with either endometriosis, uterine fibromas or unexplained infertility. *Hum Reprod* 2009;24(12):3180–7.
- [25] Kao LC, Tulac S, Lobo S. Global gene profiling in human endometrium during the window of implantation. *Endocrinology* 2002;143:2119–38.
- [26] Burney RO, Talbi S, Hamilton AE. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* 2007;148:3814–26.
- [27] Celik O, Unlu C, Otlu B, Celik N, Caliskan E. Laparoscopic endometrioma resection increases peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression. *Fertil Steril* 2015;104:356–65.
- [28] Giudice LC, Telles TL, Lobo S, Kao L. The molecular basis for implantation failure in endometriosis: on the road to discovery. *Ann N Y Acad Sci* 2002;955:252–64.
- [29] Garrido N, Navarro J, García-Velasco J. The endometrium versus embryonic quality in endometriosis-related infertility. *Hum Reprod* 2002;8:95–103.
- [30] Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, et al. Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. *PLoS Genet* 2014;10(3):e1004158.
- [31] Yotova I, Hsu E, Do C, Gaba A, Sczabolcs M, Dekan S, et al. Epigenetic alterations affecting transcription factors and signaling pathways in stromal cells of endometriosis. *PLoS One* 2017;12(1):e0170859.
- [32] Lu H, Yang X, Zhang Y, Lu R, Wang X. Epigenetic disorder may cause down regulation of HOXA 10 in the eutopic endometrium of fertile women with endometriosis. *Reprod Sci* 2013;20:78–84.
- [33] Díaz I, Navarro J, Blasco L. Impact of stage III–IV endometriosis on recipients of sibling oocytes: matched case-control study. *Fertil Steril* 2000;74:31–4.
- [34] Harb HM, Gallos ID, Chu J, Harb M, Coomarasamy A. The effect of endometriosis on in vitro fertilisation outcome: a systematic review and meta-analysis. *BJOG* 2013;120: 1308–132.
- [35] Hamdan M, Omar SZ, Dunselman G, Cheong Y. Influence of endometriosis on assisted reproductive technology outcomes: a systematic review and meta-analysis. *Obstet Gynecol* 2015;125:79–88.