Laparoscopic endometrioma resection increases peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression

Onder Celik, M.D.,[a] Cihat Unlu, M.D.,[b] Baris Otlu, Ph.D.,[c] Nilufer Celik, M.D.,[d] and Eray Caliskan, M.D.[e]

[a] Private Clinic, Obstetrics and Gynecology, Usak; [b] Department of Obstetrics and Gynecology, School of Medicine, Acıbadem University, Istanbul; [c] Department of Medical Microbiology, Inonu University, School of Medicine, Malatya; [d] Department of Biochemistry, Behçet Uz Children's Hospital, İzmir; and [e] Department of Obstetrics and Gynecology, Medical Park Hospital, Kocaeli, Turkey

Objective: To determine whether laparoscopic endometrioma resection alters peri-implantation endometrial HOXA-10, HOXA-11, LIF, ITGB3 and ITGAV mRNA expression.
Design: Case-control study.
Setting: Medical school.
Patient(s): Twenty infertile patients with uni- or bilateral endometrioma, five infertile patients having nonendometriotic benign ovarian cyst, and five fertile control subjects.
Intervention(s): Mid–luteal-phase endometrial sampling was performed at the time of surgery. Second endometrial biopsies were obtained 3 months after laparoscopic endometrioma resection during the mid-luteal phase of the cycle.
Main Outcome Measure(s): Endometrial HOXA-10, HOXA-11, LIF, ITGAV, and ITGB3 mRNA expressions were evaluated with the use of reverse-transcription polymerase chain reaction.
Result(s): Significantly decreased endometrial ITGAV mRNA expression was noted in biopsies obtained from endometrioma and nonendometriotic cyst groups before surgery. Trends toward decreased endometrial HOXA-10, HOXA-11, LIF, and ITGB3 mRNA expressions were noted in the endometrioma and nonendometriotic cyst groups before surgery compared with the fertile subjects. However, the differences failed to show statistical significance. Compared with preoperative values, significantly increased HOXA-10 (12.1-fold change) and HOXA-11 (17.2-fold change) mRNA expressions were noted in endometrial biopsies obtained from subjects who were undergoing endometrioma surgery. Fold change in endometrial ITGAV mRNA after endometrioma surgery was found to be 30.1 and indicated a positive regulation. However, this fold increase was statistically insignificant. Expressions of these endometrial receptivity markers did not change significantly after surgical removal of nonendometriotic benign ovarian cysts.
Conclusion(s): Laparoscopic endometrioma resection increases peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression, suggesting an improvement in endometrial receptivity. (Fertil Steril® 2015;104:356–65. ©2015 by American Society for Reproductive Medicine.)
Key Words: Endometrioma, eutopic endometrium, laparoscopy, homeobox genes

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/celiko-endometrioma-surgery-hoxa10-hoxa11/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received February 26, 2015; revised and accepted April 30, 2015; published online June 13, 2015.
O.C. has nothing to disclose. C.U. has nothing to disclose. B.O. has nothing to disclose. N.C. has nothing to disclose. E.C. has nothing to disclose.
Supported by the Turkish German Gynecologic Research and Education Foundation.
Reprint requests: Onder Celik, M.D., Professor, Metin Oktay Mah, 52/96 Sokak No 3/39, Kat:7, Karabağlar, İzmir, Turkey (E-mail: celiksudenaz@gmail.com).

Fertility and Sterility® Vol. 104, No. 2, August 2015 0015-0282/$36.00
Copyright ©2015 American Society for Reproductive Medicine, Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.fertnstert.2015.04.041

Infertility in endometriosis is not exclusively due to poor oocyte quality or embryo development, but is also the result of mechanical factors, defective folliculogenesis, reduced fertilization, and implantation capacity, as well as the abnormal embryogenesis that might be possible causes of endometrioma-associated infertility

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

(1–4). Studies fail to show any significant difference between implantation rates in women with and without endometriosis after in vitro fertilization (IVF) (5, 6). However, others have reported that implantation rates of women with endometriosis were only one-half of those achieved for other causes of infertility (7, 8). Although the receptivity of eutopic endometrium of patients having ovarian endometrioma might not be altered during the window of implantation (WOI) (9), the underlying mechanisms of endometrioma-associated infertility remain elusive. However, recent human and other animal studies with the use of different technologies have revealed differences in the expression of several genes and inflammatory cytokines in eutopic endometrium of women with endometriosis or endometrioma (2, 10, 11). Genetic research of the endometrium in women with endometriosis showed abnormal expression of putative implantation markers, such as homeobox genes, $\alpha v\beta 3$ integrins, and leukemia inhibitory factor (LIF) (12–16). Differences in gene expression suggest that the eutopic endometrium of women with endometrioma may appear histologically normal but in fact may be genetically abnormal during the WOI.

Different authors have studied the endometrial expression of homeobox genes, integrins, and LIF (13,16–18) in endometriosis patients, with controversial results. HOXA-10 gene is an abdominal B class homeobox gene and it is expressed in the urogenital system (19, 20). HOXA-10–null female mice are severely subfertile and exhibit anatomic defects in their reproductive tracts (21). When mouse endometrium is transfected with a Hoxa10 antisense oligonucleotide at the time of implantation, the number of implanted embryos in to the endometrium are significantly reduced (22). HOXA-10 is initially expressed in the luminal and glandular epithelium of the endometrium, and later its expression shifts to the stroma (19, 20). It is thought that homeobox genes play a physiologic role in endometrial growth, differentiation, implantation, and decidualization through regulating stromal cell responsiveness to progesterone (23). In addition, a number of molecular biomarkers are regulated by homeobox genes, including pinopodes and $\beta 3$ integrin (24–26). Expression of these genes are significantly higher in the secretory phase leading up to embryo implantation (27–29). HOXA-10 transcript levels are down-regulated in the endometrium of patients having endometriosis (12). In contrast, data regarding the importance of HOXA-11 gene in implantation are controversial (30, 31).

Integrins are heterodimeric integral membrane proteins composed of an α chain and β chain. ITGAV gene encodes the αv protein which is a member of the integrin superfamily. This protein has been shown to heterodimerize with β chain, and the heterodimer of αv and $\beta 3$ (ITGB3) has been reported to be a versatile receptor for vitronectin (32, 33). The ITGB3 integrin plays a key role in angiogenesis and facilitates embryo attachment to the surface of the endometrium. ITGAV-knockout nonhuman model is lethal in utero with a presence of large vascular anomalies (32–36).

LIF, a member of the interleukin-6–type cytokine family, is one of the key markers of endometrial receptivity (37). LIF has multiple biologic activities, including proliferation, differentiation, and cell survival (37, 38). LIF has also been linked with infertility in the mouse (39). Expression pattern of LIF mRNA in endometriosis is variable (40, 41).

To date, it remains to be clarified whether surgical treatment of endometrioma improves reproductive outcome (11, 42). There are no controlled studies investigating the effect of surgical removal of endometrioma on the expression levels of homeobox genes, integrins, and LIF in the eutopic endometrium. We therefore attempted to investigate whether surgical removal of endometrioma alters the HOXA-10, HOXA-11, ITGB3, ITGAV, and LIF mRNA expressions in the eutopic endometrium of infertile women with endometrioma. We evaluated expression of these five genes in the eutopic endometrium before and after laparoscopic removal of the ovarian endometrioma during the midsecretory phase.

MATERIALS AND METHODS

Subjects

This case-control study included 30 subjects: 20 with ovarian endometrioma, five with nonendometriotic benign ovarian cyst, and five fertile control subjects with macroscopically normal pelvic cavities who were undergoing tubal sterilization or reversal of tubal sterilization and had no evidence of endometriosis. We evaluated the HOXA-10 HOXA-11, LIF, ITGB3, and ITGAV mRNA expressions in the endometrial tissues obtained before surgery and respective endometrial tissues after the laparoscopic ovarian cystectomy during midluteal phase in infertile women with endometrioma and infertile control subjects with benign nonendometriotic ovarian cyst. In addition, endometrial tissues were obtained from healthy fertile women before surgery during midluteal phase. The study protocol was approved by the Institutional Ethical Committee for Research on Human Subjects.

The presence of endometrioma and nonendometriotic benign ovarian cyst was diagnosed with a transvaginal ultrasonography. The endometrioma was suspected when a diffuse regular-margined cyst with a low level internal echo, indicating hemorrhagic cyst, was present for at least two cycles, to exclude the nonendometriotic hemorrhagic cyst. The benign nonendometriotic ovarian cysts were diagnosed in the absence of characteristic ultrasonographic appearance for endometrioma or malignancy. The endometrioma and nonendometriotic benign ovarian cyst diagnosis was confirmed with the pathologic evaluation of the surgically removed specimen. Histologic classification of the specimens was as follows: endometriotic cyst in 20 cases, serous cyst in two cases, dermoid cyst in two cases, and mucinous cyst in one case.

All of the infertile women underwent routine laboratory and radiologic examinations to diagnose the underlying factors of infertility. The infertile women had normal early follicular FSH, LH, E_2, TSH, and PRL levels, normal midluteal P levels indicating the presence of ovulation, normal two semen analyses evaluated ≥ 3 weeks apart and after 3–7 days of abstinence, and normal hysterosalpingography with bilateral tubal patency and absence of intrauterine mass–forming pathology, and the only detectable cause of

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

infertility was endometrioma or otherwise unexplained. The fertile women enrolled as the control groups had at least two children, and had no history of infertility or habitual abortion. All of the women recruited to the study had regular menstrual cycles.

The surgical technique involved the aspiration of the cyst content and the removal of the cyst wall by means of a stripping technique. In all cases, we were able to remove the endometriotic cyst wall from the ovarian tissue. We did not electrolyze the superficial peritoneal endometriotic implants accompanying the endometrioma in any of the cases. The adhesions were removed by hydrodissection and/or scissors. All of the participants were required to meet the inclusion criteria: 1) presence of pathologic diagnosis of endometrioma or other benign ovarian cyst in women who underwent laparoscopic ovarian cystectomy; 2) no hormonal medication and intrauterine contraception use within the past 6 months before enrollment in the study; and 3) absence of any systemic and/or rheumatologic disease that may lead to an inflammation. Excluded cases were the ones with: 1) malignant ovarian tumor diagnosed with the pathologic evaluation of the specimen; 2) previous medication use; 3) previous endometrial pathology such as Asherman syndrome, endometrial polyp, and submucous fibroids; 4) diagnosis of pelvic inflammatory disease, deep endometriosis, or hydrosalpinx at the time of the study; 5) previous abdominal surgery, especially to ovaries; 6) history of habitual abortion; 7) infertility etiology other than endometrioma; 8) women with out-of-date endometrium according to pathologic evaluation; and 9) requirement of electrolysis, in addition to stripping, of the endometriotic cyst wall owing to densely adherent cyst wall to the underlying ovarian tissue.

Endometrial Biopsy

All patients, regardless of group, were selected for the present study on the basis of consistent histologic findings, menstrual history, and serum P levels. Endometrial biopsies were taken with the use of a Pipelle from all participants before surgery in the midluteal phase of the cycle. The endometrial tissue was divided into two sections, one of which was fixed in 10% formalin and embedded in a paraffin block, and the other washed three times with a sterile saline solution to remove blood, transferred into RNA stabilization buffer, and stored at −80°C for later analysis. The ones with endometrioma or nonendometriotic ovarian cyst underwent a second endometrial biopsy 3 months after the surgical removal of the cyst during the WOI. The midluteal phase was calculated as 7–9 days after the ultrasonographic confirmation of ovulation and was confirmed by endometrial histologic dating and serum P levels. All menstrual cycles studied in the present study were ovulatory according to midluteal serum P >10 ng/mL. Endometrial dating was performed by an independent pathologist experienced in gynecologic pathology. Paraffin-embedded sections of 4 mm were stained with hematoxylin and eosin and periodic acid–Schiff stains. Then these specimens were evaluated according to the histopathologic criteria of Noyes et al. (43). An out-of-date biopsy was defined as a lag of ≥3 days between the chronologic and the histologic day (44).

RT-PCR

Sample preparation. The tissue samples (≥30 mg) were immediately transferred into RNA stabilization buffer (RNA Later; Qiagen) to stabilize and protect RNA in tissue and subsequently stored at −80°C until used. Tissue samples were homogenized in RNA Later with the use of a Tissuelyser (Qiagen).

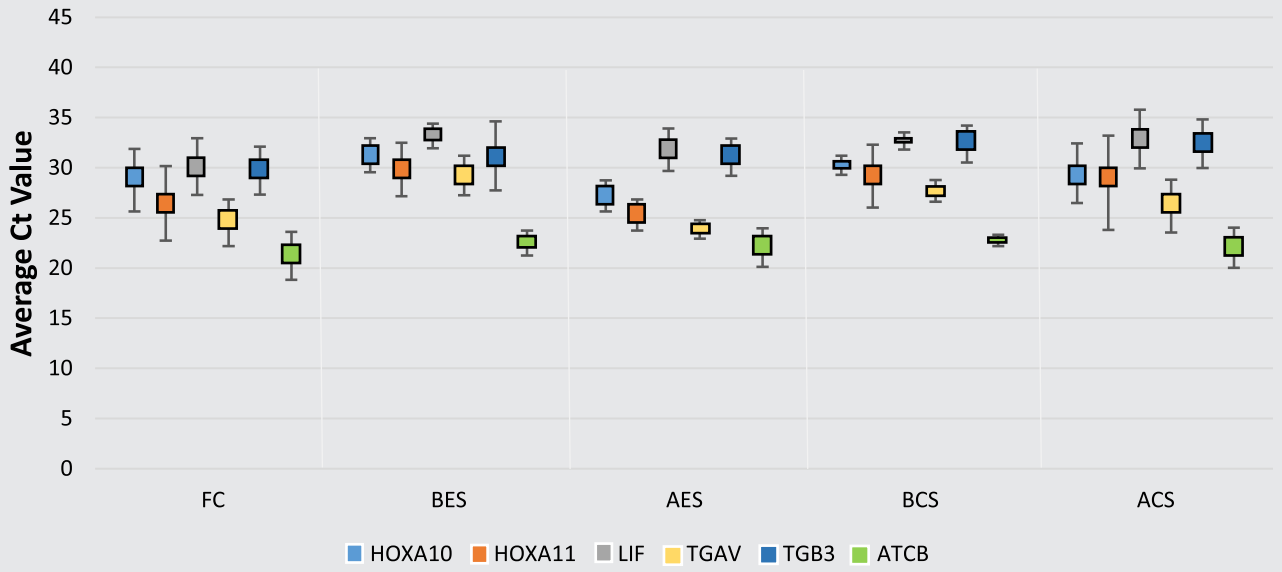
Total RNA isolation. Total RNA was isolated with the use of the RNeasy Mini Kit (Qiagen) from the homogenized tissue samples. From each sample, RNA quantity and purity was measured spectrophotometrically with the use of the Maestronano (Maestrogen).

Reverse transcription cDNA synthesis. Complementary DNA (cDNA) was obtained with the use of the Quantitect Reverse Transcription Kit (Qiagen). This system consists of two major steps: reverse transcription (RT) and removing genomic DNA. Genomic DNA is eliminated by incubating each RNA sample in Wipeout buffer at 42°C. Following that, RT mix (1 μL Quantiscript Reverse Transcriptase, 4 μL Quantiscript RT Buffer, 1 μL RT Primer Mix) and target RNA at 1 μg concentration in 20 μL total volume were prepared on ice. The reaction mix was incubated at 42°C for 15 minutes and then was kept at 95°C for 3 minutes to inactivate RT. The Quantiscript Reverse Transcriptase used in that system has high affinity to RNA and was optimized for RNA at a range of 10 pg–1 μg concentrations.

Expression of HOXA-10, HOXA-11, LIF, ITGAV, and ITGB3 genes according to in-house RT-PCR. Primers used in the study were designed with the use of Primerdesign. In addition, standard positive control samples for each gene were synthesized with the use of Primerdesign to analyze polymerase chain reaction (PCR) reaction efficacy. β-Actin gene (ACTB) was used as housekeeping gene. The mRNA level of each sample was normalized to that of the β-actin mRNA level. Real-time PCR reaction was performed with the use of Quantitect Probe PCR Kit (Qiagen) and the RotorgeneQ (Qiagen) real-time PCR device according to the manufacturer's instructions. Real-time PCR results are expressed as Ct (cycle threshold), ΔCt, and ΔΔCt. For calculation of average Ct values, each endometrial sample was studied three times (Fig. 1). Sequences and accession numbers of all primers designed to be used as forward and reverse primers for RT-PCR were: HOXA-10 (NM_018951): F 5′-GGT TTG TTC TGA CTT TTT GTT TCT-3′, R 5′-TGA CAC TTA GGA CAA TAT CTA TCT CTA-3′; HOXA-11(NM_005523): F 5′-AGT TCT TTC TTC AGC GTC TAC ATT-3′, R 5′-TTT TTC CTT CAT TCT CCT GTT CTG-3′; LIF (NM_002309): F 5′-GGA GGT CAC TTG GCA TTC AG-3′, R 5′-GG AAG AGA ACG AAG AAC CTA CC-3′; ITGAV (NM_002210): F 5′-AAA CAG AAT TTG TAA GTT GGC AGA T-3′, R 5′-GGT GAC ATT GAG ATG GGT AGT G-3′; ITGB3 (NM_000212): F 5′-ACC ATC TCT TTA CCT CCT AAT TCC-3′, R 5′-CTG GCT CTA CAA TAG CAC TCT C-3′; ACTB (NM_001101): F 5′-GCA AGC AGG AGT ATG ACG AGT-3′, R -5′-CAA GAA AGG GTG TAA CGC AAC TAA-3′.

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

FIGURE 1



Average cycle threshold (Ct) values for HOXA-10, HOXA-11, LIF, ITGAV, ITGB3, and ATCB genes. The boxes indicate the mean Ct values and the bars indicate standard deviations.

Celik. Endometrioma surgery and implantation. Fertil Steril 2015.

Analysis

The relative gene expression was determined by means of the $2^{-\Delta\Delta Ct}$ comparative method with the use of RT^2 Profiler PCR Array Data Analysis version 5.5 (SA Biosciences). All data were normalized according to ACTB gene (β-actin) mRNA content. Fold change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta\Delta Ct}$) in the patient group divided the normalized gene expression ($2^{-\Delta\Delta Ct}$) in the fertile control group. Fold change >3 indicates positive or up-regulation, and the fold regulation is equal to the fold change. Fold change <2 indicates negative or down-regulation, and the fold regulation is the negative inverse of the fold change.

Comparative RNA expression analysis in all groups versus the fertile control group (calibrator) was performed as follows:

- Fertile control group: $\Delta Ct = Ct$ (target gene) $- \Delta Ct$ (housekeeping gene ACTB)
- Patient group: $\Delta Ct = Ct$ (target gene) $- \Delta Ct$ (housekeeping gene ACTB)
- $\Delta\Delta Ct = \Delta Ct$ (patient group) $- \Delta Ct$ (fertile control group)
- Fold ratio $= 2^{-\Delta\Delta Ct}$

Statistical Analysis

The normality distribution of data was tested with the use of the Kolmogorov-Smirnoff test, and all variables were skewed normally. The continuous variables were analyzed by means of analysis of variance test with post hoc Tukey procedure and Mann-Whitney *U* test. The categoric data were analyzed by means of the Pearson chi-square test. A *P* value of $<.05$ was considered to be significant. The results are expressed as mean $\pm$ SD. Fold increases were considered to be positive for transcript overexpression when the corresponding mRNA level was ≥ 3-fold higher than that of initial transcript expression, negative if <2-fold, and borderline if between 2- and 3-fold. The data was analyzed with the use of the Statistical Package for Social Sciences software 19.0 for Windows package software (SPSS).

RESULTS

The demographic characteristics of the groups were similar. Each group of patients had normal body mass index. Infertility duration in both groups was similar. The mean ages of endometrioma and nonendometriotic ovarian cyst patients were 32.0 $\pm$ 6.4 and 33.5 $\pm$ 4.7 years, respectively. All of them had regular menstrual patterns every 27–32 days. The fertile women enrolled as the control group were aged 34.8 $\pm$ 9.1 years and had at least two or three children each. No differences in serum concentrations of P were detected among groups. The mean size of endometrioma was similar to the mean size of nonendometriotic cysts. In the endometrioma group, the cysts were bilateral in eight women (40%) and unilateral in twelve women (60%). All cysts were unilateral (100%) in the nonendometriotic cyst group.

HOXA-10, HOXA-11, LIF, ITGAV, and ITGB3 expression levels were lower in eutopic endometrium of infertile patients with endometrioma before the removal of cyst compared with healthy fertile control subjects (Table 1). However, the differences failed to show statistical significance, except for IT GAV. Decline in ITGAV mRNA expression reached statistical significance ($P<.01$).

Compared with fertile subjects, surgical removal of endometriomas up-regulated endometrial HOXA-10, HOXA-11, and ITGAV mRNA expression. However, both endometrial

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

TABLE 1

HOXA-10, HOXA-11, LIF, ITGAV, and ITGB3 mRNA expression in the BES, AES, BCS, and ACS groups compared with fertile control subjects.

Gene	Group	Average ΔCt	Average $2^{-\Delta Ct}$	Fold change	95% CI	*P* value	Regulation
HOXA10	BES	8.76	0.0023	0.43	0.14–0.72	.14	Down
	AES	5.16	0.0279	5.23[a]	1.39–9.07	.02[b]	Up
	BCS	7.54	0.0055	1.03	0.36–1.70	.76	Up
	ACS	7.42	0.0058	1.09	0.0001–2.46	.50	Up
HOXA11	BES	7.34	0.0061	0.23	0.0001–0.59	.18	Down
	AES	3.23	0.1065	3.99[a]	0.0001–8.93	.08	Up
	BCS	6.41	0.0115	0.43	0.0001–1.30	.79	Down
	ACS	6.47	0.0116	0.42	0.0001–1.34	.50	Down
LIF	BES	10.67	0.0006	0.29	0.0001–0.61	.09	Down
	AES	9.75	0.0011	0.55	0.15–0.95	.14	Down
	BCS	9.92	0.0010	0.49	0.15–0.84	.13	Down
	ACS	10.37	0.0005	0.26	0.0001–0.56	.07	Down
ITGAV	BES	6.74	0.0093	0.09	0.0001–0.18	.01[b]	Down
	AES	1.82	0.2828	2.75	0.08–5.42	.20	Up
	BCS	4.95	0.0323	0.31	0.11–0.52	.03[b]	Down
	ACS	4.16	0.0557	0.54	0.10–0.99	.25	Down
ITGB3	BES	8.69	0.0002	0.87	0.0001–2.77	.61	Down
	AES	9.02	0.0019	0.69	0.0001–1.79	.39	Down
	BCS	9.61	0.0012	0.45	0.0001–1.19	.23	Down
	ACS	10.37	0.0007	0.27	0.0001–0.67	.16	Down

Note: ACS = after nonendometriotic cyst surgery; AES = after endometrioma surgery; BCS = before nonendometriotic cyst surgery; BES = before endometrioma surgery; CI = confidence interval.
[a] Fold change value >3 was accepted as positive regulation for the genes studied.
[b] $P<.05$.

Celik. Endometrioma surgery and implantation. Fertil Steril 2015.

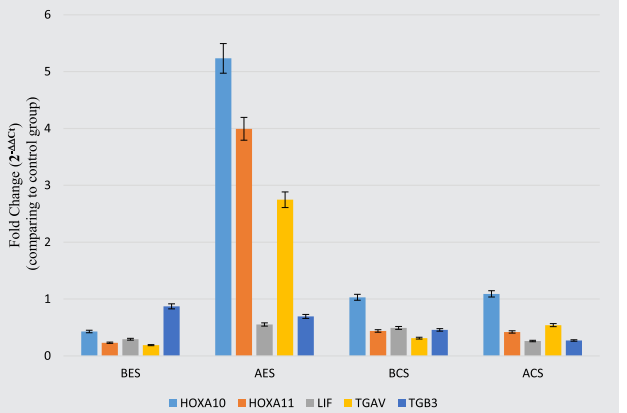
LIF and ITGB3 mRNA expression were not changed significantly after endometrioma surgery. Compared with fertile control subjects, fold changes of HOXA-10, HOXA-11, and ITGAV mRNA after endometrioma surgery were found to be 5.23, 3.99 and 2.75, respectively (Fig. 2; Table 1). Only fold change increase in HOXA-10 mRNA was found to be statistically significant ($P<.02$). Although fold change increase in HOXA-11 mRNA was >3 (3.99-fold change) this positive regulation was insignificant ($P<.08$). Similarly, fold change of ITGAV integrin was found to be 2.75, indicating insignificant and borderline ITGAV mRNA expression after surgery ($P<.20$).

Although benign ovarian cysts were not associated with a significant change in these markers of endometrial receptivity, a trend toward decreased endometrial HOXA-11, LIF, ITGAV, and ITGB3 mRNA expression was noted in the nonendometriotic cyst group before surgery compared with the fertile subjects (Table 1). However, only the decline in ITGAV mRNA reached statistical significance ($P<.03$). Expressions of these five markers in the endometrial samples did not change significantly after surgical removal of nonendometriotic benign ovarian cysts (Fig. 2).

Compared with preoperative fold change values, significantly increased HOXA-10 and HOXA-11 mRNA expressions were noted in endometrial biopsies obtained from subjects undergoing endometrioma surgery. Three months after surgical removal of endometrioma, we found a 12.1-fold increase in endometrial HOXA-10 mRNA and a 17.2-fold increase in endometrial HOXA-11 mRNA (Fig. 3). These postoperative increments in endometrial HOXA-10 and HOXA-11 mRNA were statistically significant ($P<.008$ and $P<.035$, respectively). Fold change in endometrial ITGAV mRNA after endometrioma surgery was found to be 30.1, which was >3, indicating positive regulation. However, this fold increase was insignificant ($P<.10$).

Postoperative fold change value of LIF mRNA was found to be insignificant (1.8 fold change; $P<.33$). Insignificant down-regulation in endometrial ITGB3 mRNA (0.79-fold change; $P=.37$) was found after endometrioma surgery (Fig. 3).

FIGURE 2

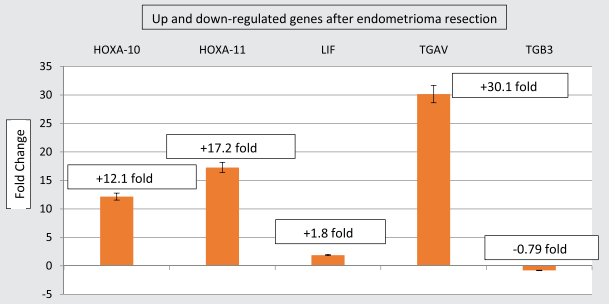


The relative gene expression levels of groups were determined with the use of the $2^{-\Delta\Delta Ct}$ method. All data were compared with the fertile control group and normalized to ACTB gene mRNA content. There were 5.2-fold, 3.9-fold, and 2.7-fold increases in HOXA-10, HOXA-11, and ITGAV genes, respectively, after endometrioma surgery.

Celik. Endometrioma surgery and implantation. Fertil Steril 2015.

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

FIGURE 3



Comparison of preoperative and postoperative fold change values of HOXA-10, HOXA-11, LIF, ITGAV, and ITGB3 transcripts in women with endometrioma. There were significant increases in fold change values for HOXA-10 (12.1-fold; $P<.008$) and HOXA-11 (17.2-fold; $P<.035$) mRNA and positive regulation for ITGAV mRNA (30.1-fold; $P<.10$). Insignificant fold change increase in LIF (1.8-fold; $P<.33$) mRNA and insignificant down-regulation in ITGB3 mRNA were detected after laparoscopic endometrioma resection.

Celik. Endometrioma surgery and implantation. Fertil Steril 2015.

DISCUSSION

According to a review of the literature, controversial results have been obtained in endometriosis research owing to the heterogeneity of women suffering from endometriosis. Most of the studies included patients with different stages of endometriosis, often in the same study group (1). To avoid this handicap, the inclusion of patients in the present study was restricted to infertile patients with uni- and/or bilateral endometrioma. It has been emphasized that appropriate control subjects without the disease are required to study endometrial receptivity in endometriosis (45). This was done in the present study with the use of fertile women. On the other hand, it has been suggested that a normal-appearing hostile endometrial environment in women with endometriosis could be related not to the endometriosis itself, but to the infertility (46). Therefore, we used a third control group of infertile women with nonendometriotic benign ovarian cyst that may have clinical and surgical profiles mimicking those of endometriomas. Despite the decreased functional ovarian reserve after the endometrioma surgery, the pregnancy rate achieved with the use of IVF was found to be similar to IVF cycles conducted for tubal-factor infertility (42, 47). The only prospective randomized study evaluating the effect of surgical removal of endometrioma on pregnancy incidence did not find an augmentation or attenuation in the pregnancy rates (42). Nevertheless, spontaneous conception is possible in ~50% of infertile women after the endometrioma resection, which should not be overlooked (48).

The changes in blastocyst adhesive behavior that occur at the implantation time in infertile women having endometriosis may arise from changes in the expression or distribution of integrin receptors (49). To address this possibility, we examined the expression levels of integrin subunits, $\alpha v\beta 3$, and ITGAV, before and after endometrioma surgery. We observed a significant decline in the expression of ITGAV mRNA in women with endometrioma before surgery compared with control subjects. Our findings are in agreement with the report of Lessey et al., where a significant decrease in the expression of integrin is reported in patients having endometriosis (15). However, other studies did not confirm these findings (50, 51). After the resection of endometrioma, we found a 30.1-fold increase in ITGAV mRNA expression compared with the preoperative values, indicating positive regulation. Our findings are in agreement with the results of Lessey and Young (52), who showed return of normal $\alpha v\beta 3$ levels after treating women with endometriosis by means of laser ablation of implants. Taken together, positive regulation of ITGAV mRNA appears to have functional significance for implantation, and it might be attributed to the surgical resection of endometrioma.

In contrast, endometrial $\alpha v\beta 3$ integrin mRNA expression did not change significantly after endometrioma surgery. Although a trend toward down-regulation of $\alpha v\beta 3$ integrin mRNA after surgery was detected, it did not reach statistical significance. It might be speculated that the actual expression of endometrial $\alpha v\beta 3$ mRNA in women with endometrioma after surgery might be suppressed by some unknown factors. It is well known that expression of endometrial integrins are mediated by homeobox genes (15). In a recent study, Daftary et al. demonstrated that HOXA-10 regulates the expression of $\beta 3$ integrin through a consensus HOX binding site located 5′ of the $\beta 3$ integrin gene within its regulatory region (24). Significant increase detected in endometrial HOXA-10 and HOXA-11 mRNA expression but inadequate expression of endometrial $\alpha v\beta 3$ integrin mRNA after endometrioma surgery may seem paradoxic. It is possibly due to the fact that endometrial HOXA-10 mRNA in women with endometriosis may be unable to bind directly to the transcription start site of the $\alpha v\beta 3$ integrin (24, 53). Another possible explanation for this paradox is P resistance seen in endometrioma. $\alpha v\beta 3$ integrin is expressed in the endometrial epithelium during the secretory phase of the menstrual cycle (54). This period is associated with high levels of expression of P and its target genes, such as HOX genes (55). Alteration in the ratio of P receptor A to P receptor B may lead to diminished P response and decreased expression of P-responsive genes in endometriosis (12, 28). In turn, defective expression of HOX-linked endometrial $\alpha v\beta 3$ integrin mRNA might persist after endometrioma surgery. Another possibility is the developmentally regulated expression pattern of $\alpha v\beta 3$ (32). Most of the integrin receptors expressed during preimplantation stages are localized to the interior of the embryo at the late blastocyst stage, while $\alpha v\beta 3$ is also on the external trophectoderm surface suggesting healthy blastocyst is required for complete activation of $\alpha v\beta 3$ (32). All of the above facts to some degree might explain why endometrioma surgery does not increase expression of $\alpha v\beta 3$ mRNA by endometrial cells.

In the present study, infertile women with endometrioma had lower levels of LIF mRNA expression in endometrium compared with fertile women. However, the difference failed to reach statistical significance. Likewise, after endometrioma resection we detected insignificant up-regulation of endometrial LIF mRNA expression. There are controversial reports on endometrial LIF mRNA expression in women with

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

endometriosis (40, 41). Our results were consistent with the results of some studies but incompatible with others. Confirmation of our results comes from study of LIF in endometrium of women with endometriosis. Mikolajczyk et al. (40) assessed LIF expression in uterine flushings and endometrial samples from both infertile patients with endometriosis and fertile control subjects and did not find significant differences between the groups. Conversely, Dimitriadis et al. (41) reported that LIF staining intensity in the endometrium is significantly reduced in patients with endometriosis. Our findings and other results when taken together suggest that in some patients with endometriosis there might be a decreased expression of endometrial LIF, but this defect is not a common feature of patients with endometrioma. It might be speculated that the actual expression of LIF mRNA in endometrium of infertile women with endometrioma might be suppressed by hypermethylation of LIF gene. However, LIF hypermethylation can not be related to the endometrioma itself, but to the infertility. Therefore, insignificant up-regulation of LIF mRNA after endometrioma surgery may not be surprising. Unfortunately, there are no studies evaluating hypermethylation status of LIF gene in patients with endometrioma. Further studies are needed to investigate the effect of endometrioma on methylation patterns of the LIF gene in eutopic endometrium.

The structural and functional changes of the endometrial tissue during the WOI period are regulated by transcriptional factors such as homeobox genes. Women with decreased expression of either of HOXA-10 or HOXA-11 gene during the WOI have lower implantation rates, as seen in endometriosis (13). In the present study, we found that infertile women with endometrioma have lower HOXA-10 and HOXA-11 mRNA expression in the eutopic endometrium before the removal of cyst, compared with fertile subjects. But this difference failed to reach statistical significance. Our findings are in agreement with the reports of Burney et al. and Matsuzaki et al. (56, 57). Those authors did not demonstrated down-regulation of HOXA-10 mRNA expression in patients having endometriosis (56, 57). Conversely, other studies in humans have demonstrated that HOXA-10 expression is down-regulated in the endometrium of patients with endometriosis (13, 58).

On the other hand, we observed a significant increase in HOXA-10 and HOXA-11 mRNA expression in women undergoing endometrioma resection compared with preoperative values. After endometrioma surgery, we observed a 12.1-fold increase in HOXA-10 mRNA and a 17.2-fold increase in HOXA-11 mRNA expressions. Before surgery, eutopic endometrium HOXA-10 transcript levels reached the threshold value (Ct) at the 30th cycle. After endometrioma surgery, HOXA-10 transcript levels reached the threshold value (Ct) at the ~27th cycle, suggesting an increase in HOXA-10 mRNA. ACTB transcript reached the threshold value (Ct) at the 23rd cycle and surgery did not affect its expression. These increases in endometrial HOXA-10 and HOXA-11 mRNA expressions after endometrioma resection were found to be statistically significant. Our findings are in good agreement with the results of a many studies. Improvement of fertility and normalization of some receptivity markers are observed after laser ablation of endometrial implants (51). Werbrouck et al. (59) demonstrated no significant difference between unexplained infertile patients and surgically treated minimal to mild endometriosis after controlled ovarian hyperstimulation/intrauterine insemination treatment. Recently, our group has demonstrated that laparoscopic stripping of endometrioma decreases the endometrial expression of inflammatory cytokines (11).

However, we do not know whether this increase in endometrial HOXA-10 and HOXA-11 mRNA after endometrioma surgery is associated with removal of endometrioma or a consequence of other factors associated with the disease. Up-regulation of HOXA-10 and HOXA-11 mRNA may not be a direct result of endometrioma resection but rather an outcome of surgery-induced stress. To exclude this, in the present study all endometrial samples were obtained 3 months after endometrioma surgery. Moreover, HOXA-10 and HOXA-11 mRNA expressions did not change significantly after nonendometriotic cyst resection, suggesting surgical stress alone does not affect the expression of endometrial homeobox genes.

One of the possible mechanisms underlying the decreased implantation rates in endometrioma is P resistance. Attenuated P response in eutopic endometrium of women with endometriosis is a well known feature (60). HOXA-10 gene regulates stromal cell responsiveness to P (23), which is essential for the decidualization of endometrial stromal cells and the establishment of endometrial receptivity (61). Disturbed P receptor expression in endometriosis may lead to diminished P response and decreased expression of P-responsive HOXA-10 and HOXA-11 genes (12, 28). In this sense, endometrioma surgery might improve P resistance and may lead to increased endometrial HOXA-10 and HOXA-11 mRNA expression and rehabilitation of endometrioma-related implantation defect.

Another proposed mechanism is the epigenetic change and hypermethylation of the HOXA-10 and HOXA-11 genes, resulting in dysregulation of their expression (62). Studies have demonstrated that the presence of peritoneal endometriosis might induce aberrant methylation of homeobox genes in the eutopic endometrium (60, 63). Wu et al. (64) demonstrated that HOXA-10 was hypermethylated in the endometrium of women with endometriosis. Likewise, in experimental endometriosis models, hypermethylation of the 5′ promoter region of HOXA-10 and decreased expression of this gene were demonstrated in endometrium (62, 63). In addition, chronic inflammation, which is a main component of endometriosis, can lead to epigenetic changes (65). A previous study by our group demonstrated that presence of endometrioma increased nuclear factor κB–related endometrial inflammation and that surgical removal of endometrioma decreased this inflammation (11). Taken together, we can suggest that interruption of pathologic inflammation in endometrium by laparoscopic removal of endometrioma may inhibit hypermethylation of HOXA-10 and HOXA-11 genes and thus increase their expression.

Some inflammatory conditions with decreased implantation rates might benefit from surgery. For example, the presence of hydrosalpinx reduces the receptivity of the endometrium by decreasing the expression of specific factors

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

that are increased following salpingectomy (66). In the presence of hydrosalpinges, the expression of LIF, $\alpha v\beta 3$ integrin, and HOXA-10 genes in endometrium are significantly reduced (25, 66, 67). After salpingectomy, HOXA-10 mRNA levels were similar to those of age-matched fertile control subjects, indicating that salpingectomy restores HOXA-10 expression (25). Thus, laparoscopic removal of endometrioma, similarly to salpingectomy in hydrosalpinx, may improve endometrial receptivity by increasing the expression of HOXA-10 and HOXA-11 mRNA.

Eutopic endometrium of women with endometriosis behaves differently from endometrium of women without the disease, and this would explain reduced implantation rates (46). This hypothesis is supported by the present results that after endometrioma surgery there were significant differences in HOXA-10 and HOXA-11, mRNA expression between patients having endometrioma-associated infertility, infertile women with benign ovarian cyst, and fertile control subjects. If homeobox genes are good markers of endometrial receptivity, then it could be concluded that surgical removal of endometrioma increases endometrial receptivity, and this would be clinically valuable. As opposed to this, studies of oocyte donation demonstrated that endometriosis is not detrimental to embryo implantation in oocyte recipients (9, 68). For this reason, one may suggest that presence of endometrioma does not significantly impair the endometrial microenvironment and that there is no need for endometrioma surgery. But successful implantation after oocyte donation does not mean that the endometrium is certainly healthy. It should be remembered that good-quality oocytes come from healthy donors and may overcome any endometriosis-associated implantation defect. Moreover, pretreatment of recipients having endometriosis with GnRH analogues might improve the subtle endometrial impairment. The study by Lessey and Young supports our hypothesis (52). They demonstrated that treatment with GnRH analogues of women having endometriosis improves fertility (52).

There are some limitations of the present study. The study population was small in the nonendometriotic cyst and fertile control groups. Changes in mRNA expression were not confirmed by protein expression analyses. Moreover, gene expression was analyzed at only a single time point after surgery. The postoperative endometrial biopsies were performed in the third post-cystectomy menstrual cycle. This time interval was chosen because the time needed for the normalization of the endometrium after endometrioma surgery is considered to be 3 months (11). Daftary et al. (25) reported that endometrial HOXA-10 expression was up-regulated to normal levels 4 months after salpingectomy. Performing sequential monthly biopsies to determine the progressive change would have been ideal, but this was not possible owing to ethical, technical, and economic concerns. Therefore, the timing of optimal improvement in endometrial receptivity markers after endometrioma resection remains undetermined.

Finally, our results indicate that there is a receptivity defect within the eutopic endometrium of women with endometrioma that affects fertility regardless of other causes of infertility, such as defective folliculogenesis, poor oocyte quality, adhesions, etc. Further studies are needed to clarify the importance of endometrioma surgery in infertility outcome and to determine whether endometrioma surgery may be effectively used to improve pregnancy rates in women having different-sized endometriomas.

Acknowledgments: The authors are most grateful to all the patients who kindly participated in the present study.

REFERENCES

1. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril 2008;90:247–57.
2. 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.
3. Trinder J, Cahill DJ. Endometriosis and infertility: the debate continues. Hum Fertil (Camb) 2002;5:S21–7.
4. Tummon IS, Maclin VM, Radwanska E, Binor Z, Dmowski WP. Occult ovulatory dysfunction in women with minimal endometriosis or unexplained infertility. Fertil Steril 1988;50:716–20.
5. 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.
6. Bukulmez O, Yarali H, Gurgan T. The presence and extent of endometriosis do not effect clinical pregnancy and implantation rates in patients undergoing intracytoplasmic sperm injection. Eur J Obstet Gynecol Reprod Biol 2001;96:102–7.
7. Barnhart K, Dunsmoor SR, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril 2002;77:1148–55.
8. 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.
9. Díaz I, Navarro J, Blasco L, Simón 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.
10. Gashaw I, Hastings JM, Jackson KS, Winterhager E, Fazleabas AT. Induced endometriosis in the baboon (Papio anubis) increases the expression of the proangiogenic factor CYR61 (CCN1) in eutopic and ectopic endometria. Biol Reprod 2006;74:1060–6.
11. Celik O, Celik E, Turkcuoglu I, Yilmaz E, Ulas M, Simsek Y, et al. Surgical removal of endometrioma decreases the NF-κB1 (p50/105) and NF-κB p65 (Rel A) expression in the eutopic endometrium during the implantation window. Reprod Sci 2013;20:762–70.
12. Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. J Clin Endocrinol Metab 1999;84:1129–35.
13. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. Hum Reprod 1999b;14:1328–31.
14. Lessey BA. The use of integrins for the assessment of uterine receptivity. Fertil Steril 1994;61:812–4.
15. 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;79:643–9.
16. Wei Q, St Clair JB, Fu T, Stratton P, Nieman LK. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. Fertil Steril 2009;91:1686–91.
17. Odagiri K, Konno R, Fujiwara H, Netsu S, Ohwada M, Shibahara H, et al. Immunohistochemical study of osteopontin and L-selectin in a rat endometriosis model and in human endometriosis. Fertil Steril 2007;88:1207–11.
18. Ordi J, Creus M, Ferrer B, Fábregues F, Carmona F, Casamitjana R, et al. Mid-luteal endometrial biopsy and alphavbeta3 integrin expression in the evaluation of the endometrium in infertility: implications for fecundity. Int J Gynecol Pathol 2002;21:231–8.
19. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. Nature 1995;374:460–3.

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

20. 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 mullerian duct by the synthetic estrogen diethylstilbestrol (DES). Dev Biol 1998;197:141–54.
21. 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.
22. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. Gene Ther 2000;7:1378–84.
23. Lim H, Ma L, Ma WG, Maas RL, Dey SK. Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. Mol Endocrinol 1999;13:1005–17.
24. 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.
25. 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;87:367–72.
26. Nikas G, Aghajanova L. Endometrial pinopodes: some more understanding on human implantation? Reprod Biomed Online 2002;4:18–23.
27. Gendron RL, Paradis H, Hsieh-Li HM, Lee DW, Potter SS, Markoff E. Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. Biol Reprod 1997; 56:1097–105.
28. 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;101:1379–84.
29. Sarno JL, Kliman HJ, Taylor HS. HOXA10, Pb×2, and Meis1 protein expression in the human endometrium: formation of multimeric complexes on HOXA10 target genes. J Clin Endocrinol Metab 2005;90: 522–8.
30. Skrzypczak J, Wirstlein P, Mikołajczyk M. Could the defects in the endometrial extracellular matrix during the implantation be a cause of impaired fertility? Am J Reprod Immunol 2007;57:40–8.
31. Haouzi D, Mahmoud K, Fourar M, Bendhaou K, Dechaud H, de Vos J, et al. Identification of new biomarkers of human endometrial receptivity in the natural cycle. Hum Reprod 2009;24:198–205.
32. Sutherland AE, Calarco PG, Damsky CH. Developmental regulation of integrin expression at the time of implantation in the mouse embryo. Development 1993;119:1175–86.
33. Damsky C, Sutherland A, Fisher S. Extracellular matrix 5: adhesive interactions in early mammalian embryogenesis, implantation, and placentation. FASEB J 1993;7:1320–9.
34. Bader BL, Rayburn H, Crowley D, Hynes RO. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. Cell 1998;95:507–19.
35. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct alpha v integrins. Science 1995;270:1500–2.
36. Bowen JA, Burghardt RC. Cellular mechanisms of implantation in domestic farm animals. Semin Cell Dev Biol 2000;11:93–104.
37. Senturk LM, Arici A. Leukemia inhibitory factor in human reproduction. Am J Reprod Immunol 1998;39:144–51.
38. Gearing DP, Gough NM, King JA, Hilton DJ, Nicola NA, Simpson RJ, et al. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J 1987;6:3995–4002.
39. Stewart CL, Kaspar P, Brunet LJ. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359: 76–9.
40. 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.
41. Dimitriadis E, Stoikos C, Stafford-Bell M, Clark I, Paiva P, Kovacs G, et al. 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.
42. Demirol A, Guven S, Baykal C, Gurgan T. Effect of endometrioma cystectomy on IVF outcome: a prospective randomized study. Reprod Biomed Online 2006;12:639–43.
43. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
44. Creus M, Ordi J, Fábregues F, Casamitjana R, Ferrer B, Coll E, et al. alphavbeta3 integrin expression and pinopod formation in normal and out-of-phase endometria of fertile and infertile women. Hum Reprod 2002;17:2279–86.
45. Garrido N, Navarro J, García-Velasco J, Remohí J, Pellicer A, Simón C. The endometrium versus embryonic quality in endometriosis-related infertility. Hum Reprod Update 2002;8:95–103.
46. Garcia-Velasco JA, Arici A. Is the endometrium or oocyte/embryo affected in endometriosis? Hum Reprod 1999;14:77–89.
47. Loo TC, Lin MY, Chen SH, Chung MT, Tang HH, Lin LY, et al. Endometrioma undergoing laparoscopic ovarian cystectomy: its influence on the outcome of in vitro fertilization and embryo transfer (IVF-ET). J Assist Reprod Genet 2005;22:329–33.
48. Alborzi S, Momtahan M, Parsanezhad ME, Dehbashi S, Zolghadri J, Alborzi S. A prospective, randomized study comparing laparoscopic ovarian cystectomy versus fenestration and coagulation in patients with endometriomas. Fertil Steril 2004;82:1633–7.
49. Sutherland AE, Calarco PG, Damsky CH. Expression and function of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. J Cell Biol 1988;106:1331–48.
50. Bridges J, Prentice A, Roche W, Englefield P, Thomas EJ. Expression of integrin adhesion molecules in endometrium and endometriosis. Br J Obstet Gynaecol 1994;101:696–700.
51. Creus M, Balasch J, Ordi J, Fábregues F, Casamitjana R, Quintó L, et al. Integrin expression in normal and out-of-phase endometria. Hum Reprod 1998; 13:3460–8.
52. Lessey BA, Young SL. Integrins and other cell adhesion molecules in endometrium and endometriosis. Semin Reprod Endocrinol 1997;15: 291–9.
53. 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.
54. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest 1992;90:188–95.
55. Cakmak H, Taylor HS. Molecular mechanisms of treatment resistance in endometriosis: the role of progesterone–hox gene interactions. Semin Reprod Med 2010;28:69–74.
56. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, et al. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology 2007;148:3814–26.
57. Matsuzaki S, Canis M, Vaurs-Barriere C, Boespflug-Tanguy O, Dastugue B, Mage G. DNA microarray analysis of gene expression in eutopic endometrium from patients with deep endometriosis using laser capture microdissection. Fertil Steril 2005;84:1180–90.
58. Gui Y, Zhang J, Yuan L, Lessey BA. Regulation of HOXA-10 and its expression in normal and abnormal endometrium. Mol Hum Reprod 1999;5:866–73.
59. Werbrouck E, Spiessens C, Meuleman C, d'Hooghe T. No difference in cycle pregnancy rate and in cumulative live-birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility after controlled ovarian hyperstimulation and intrauterine insemination. Fertil Steril 2006;86:566–71.
60. Metzger DA, Olive DL, Haney AF. Limited hormonal responsiveness of ectopic endometrium: histologic correlation with intrauterine endometrium. Hum Pathol 1988;19:1417–24.
61. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006;12: 731–46.
62. Lee B, Du H, Taylor HS. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biol Reprod 2009;80:79–85.

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

63. 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.
64. Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo SW. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am J Obstet Gynecol 2005;193:371–80.
65. Martin M, Herceg Z. From hepatitis to hepatocellular carcinoma: a proposed model for cross-talk between inflammation and epigenetic mechanisms. Genome Med 2012;4:8.
66. Bildirici I, Bukulmez O, Ensari A, Yarali H, Gurgan T. A prospective evaluation of the effect of salpingectomy on endometrial receptivity in cases of women with communicating hydrosalpinges. Hum Reprod 2001;16:2422–6.
67. Seli E, Kayisli UA, Cakmak H, Bukulmez O, Bildirici I, Guzeloglu-Kayisli O, et al. Removal of hydrosalpinges increases endometrial leukaemia inhibitory factor (LIF) expression at the time of the implantation window. Hum Reprod 2005;20:3012–7.
68. Sung L, Mukherjee T, Takeshige T, Bustillo M, Copperman AB. Endometriosis is not detrimental to embryo implantation in oocyte recipients. J Assist Reprod Genet 1997;14:152–6.

Downloaded for Anonymous User (n/a) at Acibadem University from ClinicalKey.com by Elsevier on March 07, 2023.
For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.