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KISS1/KISS1R Expression in Eutopic and Ectopic Endometrium of Women Suffering from Endometriosis

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Abstract. *Background: The KISS1/KISS1R system has been implicated in the physiology of reproduction and many studies have documented the stimulatory effect of kisspeptin on Gonadotropin-releasing Hormone (GnRH) and gonadotropin secretion. In addition, the KISS1/KISS1R system has been implicated in several pathophysiological processes, including cancer. Materials and Methods: We examined the pattern of KISS1 and KISS1R expression in eutopic and ectopic endometrium tissues which were obtained from 24 women suffering from endometriosis and 16 control women who underwent laparoscopic excision for other benign gynecological diseases. Results: Significant KISS1R expression was detected in 10 out of the 24 samples of eutopic endometrial biopsies of women suffering from endometriosis, while their matched biopsies of ectopic endometrial lesions did not reveal any KISS1R expression. KISS1R expression was not detected in the endometrial biopsies of control women. In addition, KISS1 expression was not detected in practically any the endometrial tissues of either control women or women with endometriosis. Conclusion: The expression of KISS1R in 10/24 samples of human endometrial biopsies of women suffering from endometriosis and the loss of its expression in the samples of matched ectopic endometrial tissues, suggests that the KISS1/KISS1R system may play a role in the pathophysiology of endometriosis only for a particular group of patients. Since KISS1 is not expressed by the endometrium and endometriotic tissue, it is conceivable that the activation of KISS1R in this particular group is mediated by KISS1 expression by non-endometrial tissues (endocrine action).*

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KISS1 gene, located on human chromosome 1, was initially discovered by Lee *et al*. in 1996 as a metastasis suppressor gene in malignant melanoma cell lines (1). The primary translation product of the KiSS1 gene by proteolytic processing results in shorter peptides with 54, 14, 13 and 10 amino acids (2, 3), which comprise the family of kisspeptins. Kisspeptins act as endogenous ligands to a G protein-coupled receptor, previously defined as an orphan receptor, named as KISSR1 (4-6).

Until 2003, most of the attention drew by the newly identified KISS1/KISS1R system, focused on its potential role in tumor progression and dissemination. Growing evidence from molecular and clinical studies in different cancer types, including melanoma, choriocarcinoma and bladder, gastric, esophageal, thyroid, epithelial ovarian, endometrial and pancreatic cancer, demonstrated a metastasis suppressing role for these molecules (7). However, in late 2003, a previously unsuspected and totally unnoticed role for the KISS1/KISS1R system was revealed when two independent groups (8, 9) reported KISS1R-inactivating mutations in humans and mice suffering from hypogonadotropic hypogonadism, a syndrome characterized by the absence of sexual maturation and low gonadotropin levels. Since then, intense research has revealed the indispensable role of the kisspeptins in the physiology of reproduction. Compelling experimental evidence strongly supports the contention that the KISS1/KISS1R system exerts its effects on the Hypothalamic-Pituitary-Gonadal (HPG) axis by acting primarily at hypothalamic (central) levels (10, 11). It has been shown that kisspeptin producing-neurons directly innervate Gonadotropin-Releasing Hormone neurons (GnRH), relaying humoral and environmental signals. Kisspeptin producing-neurons accumulate at hypothalamic nuclei known

to be essential in the regulation of GnRH secretion. Kisspeptins have since been recognized as one of the most potent secretagogues of luteinizing hormone (LH) known in mammals so far (10, 12-15).

Despite the widely accepted conception of 'centrally-acting' kisspeptins at the hypothalamus level of the HPG axis, recent data also implicate peripheral involvement of the KISS1/KISS1R system. Expression of *KISS1* and *KISS1R* genes in human, fish and rat ovary, with ovarian expression of KISS1 fluctuating in a cyclic-dependent manner (16), has recently been demonstrated. Furthermore, testis also expresses both *KISS1* and *KISS1R* genes (4), with evidence of potential direct action on LH-induced testosterone release from the testis (17, 18). Thus a possible role of the KiSS1/KISS1R system in the local regulation of gonadal function (19, 20) is implicated, further expanding the sites and the complex mode of action of kisspeptins in the reproductive axis.

In this context, kisspeptins are also involved in the procedure of placentation and suppression of trophoblast invasion into the maternal deciduas, an essential process for fetal development, which so closely mimics the invasion of cancer cells. Kisspeptin plasma levels are dramatically increased during pregnancy, possibly mediating the down-regulation of the HPG axis (21, 22). Moreover, other important facets of the reproductive procedure are linked to kisspeptins, including the photoperiodic control of reproduction in seasonal breeders (23) and the integration between energy status and reproduction with leptin being a major metabolic regulator of hypothalamic KISS1 neurons (24-26). Interestingly, kisspeptin-KISS1R signaling has been suggested to control ovarian cyclicity through regulating the two modes of GnRH release: KISS1 neurons in the anteroventricular periventricular nucleus are thought to trigger GnRH surge and thus mediate estrogen-mediated positive feedback action on GnRH release, while KISS1 neurons in the arcuate nucleus are thought to generate GnRH pulses, mediating the negative feedback action of estrogens on GnRH release (27, 28).

Numerous studies have now established the fundamental role of the KISS1/KISS1R system in key aspects of reproduction, with many researchers regarding it as one of the most significant breakthroughs in neuroendocrinology since the discovery of GnRH in 1970 (19, 29). Given the well-established stimulatory effect of kisspeptin on GnRH and gonadotropin secretion, many hormone-dependent disorders of the reproductive axis currently treated with GnRH analogues could be possibly related to the KISS1/KISS1R system. However, data concerning estrogen-dependent gynecological disorders of the HPG axis, including endometriosis, are still lacking.

Endometriosis is one of the most common benign gynecological conditions, defined as the presence of endometrial stroma and glandular cells outside the endometrial cavity. Endometriosis affects approximately 6-10% of women of a reproductive age (30), with this figure rising to 20% in women presenting with infertility (31). Despite extensive research, the etiology of endometriosis remains unknown. Numerous theories have proposed potential immunological, hormonal and environmental etiologic factors; however, the exact mechanisms are yet to be determined (32, 33). The most widely accepted theory remains Sampson's transplantation theory, initially published in 1921 (34, 35), according to which endometriosis results from retrograde menstrual reflux of endometrial fragments. Despite a large body of research data developed over the years supporting this theory, it still cannot satisfactorily explain the development of endometriosis; while most women with patent fallopian tubes do have retrograde menstruation, only a few develop endometriosis (36, 37), thus implicating additional factors in the implantation and growth of ectopic endometrium. Apparently, the retrograde endometrial cells should be able to adhere to the peritoneum, invade the basement membrane, degrade the extracellular matrix (ECM) and finally develop their own blood supply to survive, a procedure that very closely imitates malignant metastatic disease.

While the system of urokinase type plasminogen activator (uPA)/plasmin/ metalloproteinases(MMPs)/tissue inhibitors of metalloproteinases (TIMPs) is well-recognized as a central player in different models of metastasis (38-40), it is also well-documented that MMPs not only exert a crucial role in the normally cycling endometrium (41, 42), but also seem to be essential in the invasion process of ectopic endometrial implants (43, 44). Interestingly, the KISS1/KISS1R system has been shown to correlate to MMP activity; kisspeptins down regulate the expression of MMPs by forming a stable complex with pro-MMP-2 and pro-MMP-9 (45), and active MMPs cleave the Gly118-Leu119 peptide bond of kisspeptins thus inactivating them (45, 46).

Taking into account all these well-documented observations and given both the 'antimetastatic' and the 'reproductive' dimension of the KISS1/KISS1R system, new frontiers in the potential connection of endometriosis to the KISS1/KISS1R system can now be foreseen. To the best of our knowledge, the identification and expression of *KISS1* and *KISS1R* genes in endometriosis have never been evaluated before. The objective of the present study was to investigate localization and mRNA expression of kisspeptin (*KISS1* gene) and its receptor (*KISS1R* gene) in eutopic and ectopic endometrium of patients with endometriosis. Potential implication of the KISS1/KISS1R system in the pathogenesis of the disease as well as possible therapeutic implications of kisspeptin through suppression of the reproductive axis and subsequent suppression of gonadal estrogens are discussed (47, 48).

Table I. *Primer sequences for KISS1 and KISS1R genes.*

Gene	Amplicon length (bp)	Sequence
KISS1R	187	Forward 5'-TCG-CTG-GTC-ATC-TAC-GTC-ATC-TGC-3' Reverse 5'-GCT-GGA-TGT-AGT-TGA-CGA-ACT-TGC-3'
KISS1	346	Forward 5'-AAC-TCA-CTG-GTT-TCT-TGG-CAG-CTA-3' Reverse 5'-AGG-AGT-TCC-AGT-TGT-AGT-TCG-GCA-3'
	500	Forward 5'-AAC-TCT-TGA-GAC-CGG-GAG-CCC-A-3' Reverse 5'-AGG-AGT-TCC-AGT-TGT-AGT-TCG-GCA-3'

Materials and Methods

Patients and biopsy collection. Twenty-four patients with endometriosis (mean age 30.1 range 22-41 years) classified as having stage III or IV endometriosis, according to the American Fertility Association (49), who underwent laparoscopic excision of ectopic endometrium lesions, were recruited for this study. All patients had ovarian cysts, with cyst diameter ranging from 22 to 100 mm, as measured by ultrasound before surgery. Seven of the patients were also found to have deeply infiltrating nodules, located in the rectovaginal septum (n=6) and uterosacral ligaments (n=1). Twenty-four ovarian endometriosis tissue biopsies, seven infiltrating-endometriosis biopsies and twenty-four matched eutopic endometrium samples were collected in total. Eutopic endometrial tissue biopsies were performed just after surgery using an endometrial suction catheter (C.C.D. Laboratories France) and were classified as proliferative (cycle days 5-14, n=14) secretory (cycle days 15-28, n=2) and menstruating (cycle days 1-4, n=8), according to the last day of menstruation and established histological criteria (50). All tissue samples, both eutopic and ectopic endometrium, were immediately collected in RNAlater (QIAGEN Ltd, Crawley, West Sussex, UK) and stored at –80˚C until RNA extraction. When the samples were large enough for both diagnostic and research purposes a second portion of the lesion was fixed in 5% formaldehyde and embedded in paraffin for immunohistochemical analysis. We were able to collect four eutopic endometrium samples, two cysts and two nodules from patients with endometriosis to further analyze them by immunohistochemistry.

Eutopic endometrium samples were also collected from 16 patients without endometriosis (mean age=31.6, range 18-44 years) undergoing laparoscopy for other benign gynecological reasons (ovarian cystic lesions n=11, uterine fibromas n=3, diagnostic laparoscopy for chronic pelvic pain n=2). Eutopic endometrial tissue biopsies (12 proliferative and 4 secretory) were performed as for patients with endometriosis, and were immediately collected in RNAlater (QIAGEN Ltd) and stored at –80˚C until RNA extraction. All biopsies were performed by an experienced gynecologist at the *Alexandra* University Hospital, First Department of Gynecology and Obstetrics at Athens University Medical School, Greece. Histological examination of the excised tissues was systematically carried out by the Pathology Department, establishing the presence or absence of endometriosis.

All recruited patients had regular menstrual cycles every 26-32 days and none of the women had received any hormonal treatment for at least 6 months prior to surgery. Written informed consent was obtained from all patients prior to study enrollment, which was approved by the Ethics Committee of the National and Kapodistrian University of Athens.

RNA extraction. Each tissue sample (50 mg approximately) was homogenized using a metallic-blade homogenizer (Ultra-Turrax T25, Fisher Scientific, Cheshire, UK) at 15-20,000 rpm. Total RNA was extracted with TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. Diethylpyrocarbonate treated water was used for the dilution of the RNA pellet. RNA was quantified by UV absorption (BioSpec-Nano spectrophotometer, Biotech, Kyoto, Japan) and its integrity was assessed by visualization of the 18S and 28S ribosomal RNA pattern in a denaturing 1% agarose gel. Placental tissue RNA served as a positive control in our experiments (4, 51).

Reverse transcription (RT) and PCR protocols. In each sample 2 μg of total RNA was reverse-transcribed into cDNA by use of Moloney-murine leukemia virus reverse transcriptase (MMLUV; Finenzymes, Vantaa, Finland). Each reaction was carried out in total volume of 20 μl, containing 10 Mm d-NTPs (deoxyribonucleotide triphosphate; HT Biotechnology Ltd, Cambridge, UK) and 200 U/ml MMLUV in the presence of oligo-dT16 (2.5 mM) and random hexamers (3 mg/ml). RNA was initially denaturated at 70˚C for 5 min and quick-chilled on iced water. The reaction mixture was then added and reverse transcription was performed at 37˚C for 1 h. cDNA samples were stored at –20˚C prior to use as templates in semi-quantitative PCR reaction. Gene-specific cDNAs were also generated from 100 ng of selected RNA samples to more accurately assess the presence of the target genes. The corresponding-gene reverse primer (*KISS1R*-reverse primer or *KISS1*-reverse primer) (10 mM) was added to the RT reaction while both random hexamers and oligo-dTs were omitted.

To study the relative mRNA expression of the *KISS1R* and *KISS1* genes, in each polymerase chain reaction (PCR) reaction every target cDNA was coamplified with classic 18S internal standard (detected at 489 bp) (Ambion, Austin, TX, USA), and was expressed as ratio to the target cDNA/18S. This procedure compensates for differences in starting amounts of total RNA and in reverse transcription (RT) efficiency.

The amplification program consisted of the following steps: 4 min at 94˚C, 36 cycles of 30 sec at 94˚C, 1 min at 60˚C and 30 seconds at 72˚C, with a final step of 10 min at 72˚C. Ethidium bromide stained 2% agarose gels were captured under ultraviolet light in a Kodak EDAS 290 imaging system (Carestream Health, Rochester, NY, USA), and PCR products were quantified by band densitometry with image software (Scientific Imaging Systems, Kodak ID, New Haven, CT, USA). Values of *KISS1R* and *KISS1* PCR products were normalized to the corresponding ribosomal 18S.

Primer sequences for *KISS1R* gene and *KISS1* gene were designed (Table I). Specificity was confirmed using the NCBI BLAST search tool (http://blast.ncbi.nlm.nih.gov). Two different pairs of KISS1

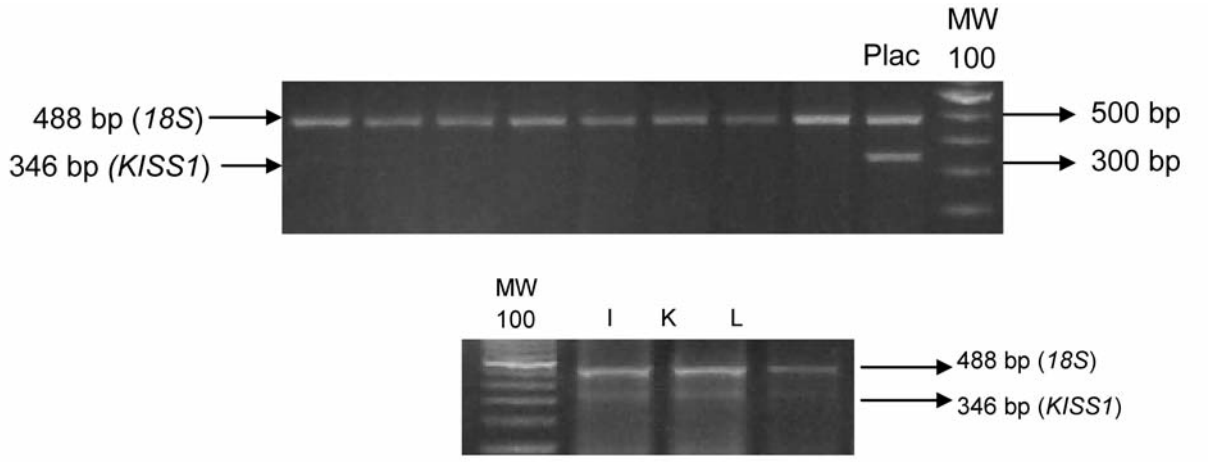

Figure 1. *Gel electrophoresis of KISS1 (346 bp) PCR products with 18S (488 bp) as an internal control in three eutopic endometria (columns A, B, C), three ovarian cysts (columns D, E, F) and two infiltrating nodules (columns G, H) of patients with endometriosis. Plac: placenta serving as positive control. Columns I-L show the three samples that were found to express a very weak KISS1 band (two eutopic endometrial samples in columns I and K and one ovarian cyst in column L).*

primers were designed to determine the presence of *KISS1* mRNA transcripts in the endometrial and endometriotic tissues evaluated. *KISS1*-specific cDNAs were generated for each of the two primers sets. *KISS1R*-specific cDNAs were also generated in selected samples but diagnostic digest with at least three different enzymes (selected through the program NEBcutter V.2; http://tools.neb.com/NEBcutter2) failed to verify the PCR product as *KISS1R* transcript.

Immunohistochemistry. The Bondmax automated system (Leica Microsystems, New Castle, UK) was used for the immunohistochemical staining of paraffin sections, using the KISS-1 (FL-145) monoclonal antibody and the anti-KISS1R polyclonal antibody at 1:150 and 1:100 dilution, respectively. Villous and extravillous trophoblast of the placenta were used as positive controls for both antibodies. Furthermore, negative controls were performed by omitting the primary antibody. Cytoplasmic expression was evaluated for KISS1 and cytoplasmic/ membranous expression for KISS1R.

Results

KISS1 mRNA was undetectable by the RT-PCR method in the majority of the tissues evaluated. Only a very limited number of samples from the endometriosis group expressed a very weak *KISS1*-specific product (1/24 ovarian cysts, 0/6 infiltrating nodules and 2/24 eutopic endometrial samples) (Figure 1). In the control group of patients without endometriosis, none of the 16 eutopic endometrial samples expressed KISS1 (data not shown). Two different sets of primers (Table I) designed to amplify different KISS1 regions were used. Placental cDNA serving as positive control expressed KISS1 transcripts, as verified by diagnostic digest and sequencing (Figure 1). Furthermore, for each set of primers, KISS1-specific cDNA was produced, but only the positive control and the same few samples (one ovarian cyst, two eutopic endometrium) weakly expressed the *KISS1* product.

Contrary to kisspeptin, its receptor (KISS1R) was significantly expressed in almost half of the eutopic endometrial tissues from the endometriosis group of women. In 10 out of the 24 eutopic endometrial biopsies *KISS1R* PCR product was highly expressed. KISS1R was not detected in any of the ectopic endometrial samples, neither in the ovarian cysts nor in the infiltrating nodules (Figure 2). Again, placental cDNA, serving as positive control, expressed KISS1R transcript, verified by diagnostic digest and sequencing. In the control group (non-endometriosis), only three endometrial samples were found to express, *KISS1R*-specific PCR product (Figure 3), however expression was very weak.

Patients age and menstrual cycle phase were further assessed to evaluate any potential effect on *KISS1R* mRNA expression. The age of the patients found to express the *KISS1R* gene in their eutopic endometrial biopsy (n=10) was not statistically different from the age of the rest of the patients (mean age±(SD): 31.6±4.88 *vs.* 29.07±4.28 years). The 10 eutopic endometrial samples detected as expressing KISS1R were further classified according to the phase of the menstrual cycle, as determined pathologically. Indeed, five endometrium biopsies at the secretory phase (out of the eight totally examined in this group), and five proliferative endometrial biopsies (out of the 14 totally examined in the proliferative-phase group) expressed the *KISS1R* transcript. Interestingly, 6 out of the 7 patients that had both ovarian cyst and a deeply infiltrating nodule, expressed the *KISS1R* transcript in their eutopic endometrium, while only 4 out of the 17 patients bearing only ectopic ovarian lesions, did.

We had a limited number of tissue samples available for immunohistochemical staining (four eutopic endometrial samples, two ovarian cysts and two infiltrating nodules). All four of the eutopic endometrial samples that were analyzed,

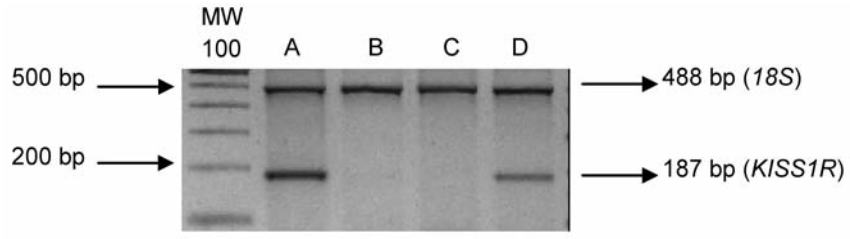

Figure 2. *Electrophoresis of KISS1R (187 bp) PCR products with 18S (488 bp) as an internal control in a patient having both an ovarian endometriotic cyst and a deeply infiltrating endometriotic nodule. Column A: eutopic endometrium, column B: ovarian endometriotic cyst, column C: infiltrating endometriotic nodule, column D: placenta serving as positive control.*

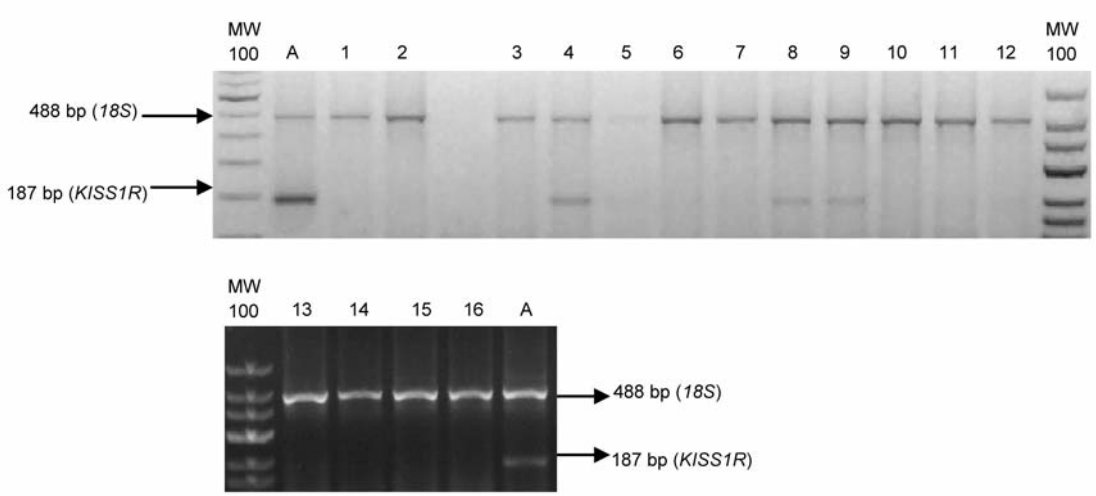

Figure 3. *Electrophoresis of KISS1R (187 bp) PCR products with 18S (488 bp) as an internal control in 16 eutopic endometrial samples from patients without endometriosis (columns 1-16). Column A: Placenta serving as positive control.*

expressed strong *KISS1R* staining (Figure 4F). Contrary to their mRNA expression pattern, the four endometriotic lesions available for immunohistochemistry, expressed KISS1R staining, ranging from mild to strong (Figure 4G and H). KISS1 was not expressed or showed a mild immunohistochemical detection in eutopic endometrial tissues (Figure 4A and B). In ovarian cysts and infiltrating nodules, KISS1 was variably immunoexpressed. Some cases were totally negative and others showed a mild to strong expression (Figure 4C and E).

Discussion

This study addressed the expression of the KISS1/KISS1R system in human endometrium. Both eutopic and ectopic endometrial samples of women suffering from endometriosis were evaluated and their expression levels were compared. While some of the eutopic endometrial samples clearly demonstrated the expression of the *KISS1R* gene, their matched ectopic endometrial locations failed to reveal detectable levels of *KISS1R*, indicating loss of the gene expression in the abnormal ectopic sites. The rest of the patients, in whom KISS1R expression was not detected neither in their eutopic endometrium nor in their corresponding ectopic endometrial locations, did not exhibit any particular epidemiologic or clinical characteristic that could potentially explain this discrepancy.

However, immunohistochemical analysis revealed positive detection of KISS1R protein detection in eutopic and ectopic endometrial samples. The four eutopic endometrial samples that were analyzed with immunohistochemistry, had previously been found to have a strong *KISS1R* expression by PCR. In the four ectopic endometrium lesions (two ovarian cysts and two infiltrating nodules) that stained positively for the anti-KISS1R polyclonal antibody, the RT-PCR method had failed to detect the presence of *KISS1R* mRNA transcripts. This discrepancy remains inexplicable to us. Contrary to the KISS1R expression pattern, *KISS1* gene was not detected in the majority of the endometrial tissues assessed in this study, indicating that the role of KISS1R

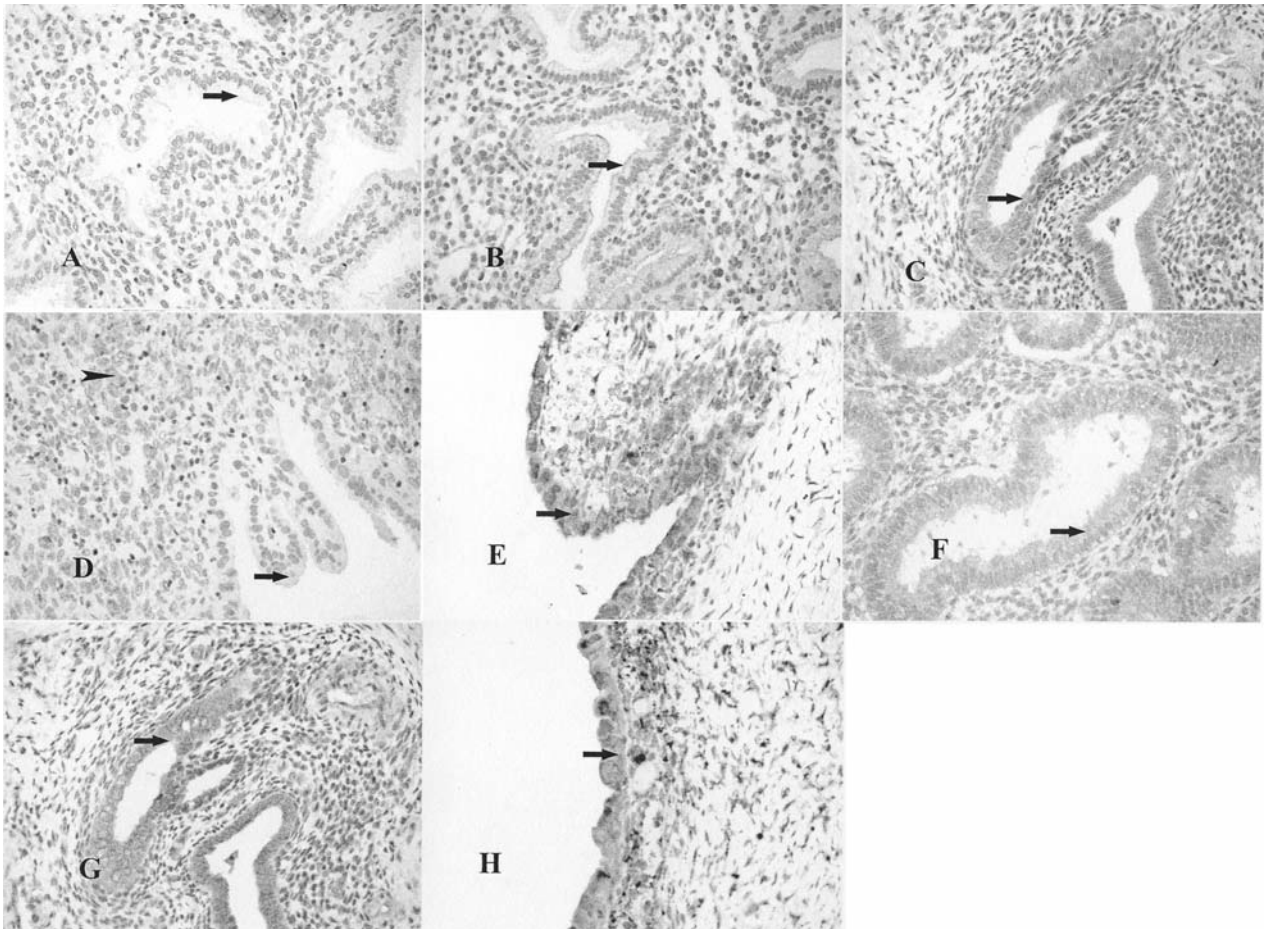

Figure 4. *A: No KISS1 immunohistochemical expression in eutopic endometrium (arrow). B: Mild KISS1 expression in eutopic endometrium (arrow). C: Strong KISS1 expression in a focus of endometriosis (arrow). D: No KISS1 expression in the epithelium of an endometriosis cyst (arrow), hemosiderin appears in macrophages (arrowhead). E: Strong KISS1 expression in the epithelium of an endometriosis cyst (arrow). F: Strong expression of KISS1R in eutopic endometrium (arrow). G: Strong expression of KISS1R in a focus of endometriosis (arrow). H: Strong expression of KISS1R in the epithelium of an endometriosis cyst (arrow). All magnifications are at ×200, except H at ×400.*

activation, if any, is based upon an endocrine KISS1 activity and not on an autocrine action. The three tissues (two eutopic samples and one ovarian cyst) that were detected as having a weak *KISS1* mRNA expression did not have any significant epidemiologic or other clinical differences compared to those in which *KISS1* was not detected. These three tissue samples along with another two eutopic samples, one cyst and two nodules were further analyzed with immunohistochemistry. Only 1/4 eutopic samples were stained positively for the KISS-1 monoclonal antibody, in accordance with the RT-PCR results. One ovarian cyst and two infiltrating nodules showed mild to strong KISS1 protein expression despite the absence of *KISS1* mRNA transcripts. Again this discrepancy between the two techniques (RT-PCR and immunohistochemistry) could suggest that the KISS1 production arises from non endometrial tissue (endocrine action).

The presence of KISS1R receptor in eutopic endometrium of women with endometriosis further corroborates recently published data that implicate a potential peripheral role of the KISS1/KISS1R system. Experimental evidence revealed the expression of *KISS1* and *KISS1R* genes in the ovary of humans (53), rats (16, 54), fish (55) and marmoset monkey (53). KISS1 but not KISS1R expression levels fluctuate in a cyclic-dependent manner, with LH pre-ovulatory surge regulating the ovarian KISS1 expression; the rise in ovarian KISS1 levels, noted in the afternoon of pro-estrus, is impeded by GnRH antagonists that block the LH surge, while it is induced by administration of LH super-agonist (56). It was also noticed that immature rat ovaries do not express detectable KISS1 levels; however, after gonadotropin pre-treatment ovarian KISS1 levels significantly increased. KISS1 and KISS1R immunoreactivity was detected in the

thecal layer of growing follicles and in *corpora lutea* with similar cellular distribution patterns among human and nonhuman ovaries, suggesting a potentially conserved role of the KISS1/KISS1R system (53). All these observations, taken together, strongly suggest that locally produced kisspeptin might have an essential role in ovarian function, including ovulation. Further expanding this suggestion and given our results regarding the KISS1R expression in the endometrium, we could assume that ovarian-produced kisspeptin may, *via* the bloodstream, stimulate the KISS1R receptor on endometrium, thus inducing the KISS1 signaling.

The present study also suggested that for this particular group of patients with endometriosis, there is a loss of KISS1R expression in the ectopic endometrial lesions, *i.e.* from both ovarian cysts and deeply infiltrating nodules, as compared to the eutopic endometrium. Given the well-established metastasis-limiting ability of the KISS1/KISS1R system, the above observations raise the intriguing possibility of a potential role of this system in preventing ectopic implantation of eutopic endometrial fragments into the abdominal cavity and thus impeding the development of endometriosis *via* an anti-invasion action. Interestingly, a potential role of KISS1 in the prevention of ectopic tubal implantation has been suggested, based on the regional and cyclic-dependent pattern of KISS1 expression in the rat oviduct (20). To date, data have analyzed the regulation of kisspeptin secretion and have provided strong evidence for the participation of these molecules in several biological processes. Intracellular signaling by binding of KISS1 to its receptor activates a series of events, including activation of phospholipase C, mobilization of calcium and regulation of collagenase activity, while kisspeptin-54 has been shown to stimulate ERK1 and ERK2 (4-6). In addition, up-regulation of genes involved in the control of cell-cycle progression and apoptosis has been demonstrated (56). Binding of the kisspeptins to KISS1R down-regulates their own expression *via* a feedback mechanism, while the proteolytic products (shorter kisspeptins) which are produced are related to the activity of furin or prohormone convertase (4, 57).

A plethora of bibliographic evidence has now established the crucial role of the KISS1/KISS1R system in the reproductive process in general, including the procedures of placentation and (recently) ovulation; however, it should be mentioned that both procedures can also take place in conditions of defective KISS1R signaling, as shown by analyses in *KISS1R* knock-out mice and humans with *KISS1R* homozygous mutations (8, 58). Interestingly, kisspeptins are also implicated in the suppression of ovulation during lactation (59). Other previously unsuspected pathologies have now been linked to the KISS1/KISS1R system, including polycystic ovarian syndrome (60); the cardiovascular system, where kisspeptins demonstrate a potent vasoconstrictor action (61); diabetes (62, 63); and finally to the menopause, where increased hypothalamic kisspeptin levels were found (11). A wide variety of hormone-dependent disorders, including precocious puberty, prostate cancer and breast cancer, endometriosis and uterine firbromas, are currently being treated with GnRH analogs to selectively and reversibly suppress the HPG axis, thus achieving gonadal steroid suppression. Recently, researchers reported the desensitization of KISS1R by continuous human metastin 45–54 administration in the juvenile male Rhesus monkey (*Macaca mulatta*) (47) and the development of potent and specific kisspeptin analogs and antagonists (48), while administration of human metastin (kisspeptin-45) to healthy male volunteers significantly increased plasma LH, FSH, and testosterone concentrations without any significant toxicity (64).

In conclusion, our present data provide evidence for the potential involvement of the KISS1/KISS1R system in the pathophysiology of endometriosis, at least in a subgroup of women with endometriosis, that should be thoroughly investigated in future studies.

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