2005; Cilt: 2 Sayı: 2 Sayfa: 85-91

TENASCIN-C EXPRESSION IN ENDOMETRIOSIS

Murat ULUKUŞ*, E. Cagnur ULUKUŞ, Ege N. Tavmergen GOKER***, Osman ZEKİOĞLU****, Gursen GUNDEM*, Mustafa ULUKUŞ*, Erol TAVMERGEN*****

* Ege University Faculty of Medicine Department of Obstetrics and Gynecology, Izmir, Turkey
** 9 Eylul University Faculty of Medicine Department of Pathology Izmir, Turkey
*** Ege University Family Planning Infertility Research and Treatment Center, İzmir, Turkey
**** Ege University Faculty of Medicine Department of Pathology Izmir, Turkey

SUMMARY

Objective: *To investigate the expression of Tenascin-C (TN-C) in endometrium and endometriosis.*

Study Design: *Comparative immunohistochemical study.*

Setting: *Academic medical center.*

Patients: *Ectopic (n = 32) and homologous eutopic endometrium (n = 20) from women with endometriosis and endometrium from women without endometriosis (n = 17) were included in the study.*

Interventions: *Tissue sections were immunostained with monoclonal TN-C antibody.*

Main outcome measures: *Microscopic evaluation to assess the presence and localization of TN-C throughout the menstrual cyle in both eutopic and ectopic endometrial tissues of women with endometriosis and compare it to the normal endometrium.*

Results: *No statistical difference of TN-C expression was found neither between both groups of endometrial samples and nor between the eutopic and ectopic endometrial samples of women with endometriosis.*

Conclusions: *Expression of TN-C seems to be restricted in both normal endometrial tissues and endometrial tissues of women with endometriosis. Our findings are partially controversial with the studies suggesting that this ECM glycoprotein have a regenerative role on the endometrium. However, it seems to have a role on the implantation process and it may be related with the infertility mechanisms involved in endometriotic disease.*

Key words: *endometriosis, eutopic endometrium, tenascin-C.*

ÖZET

Endometriyoziste Tenascin-C (TN-C) Ekspresyonu

Objektif : *Endometriyoziste ve endometriyumda TN-C ekspresyonunun araştırılması*

Planlama: *Kıyaslamalı immünhistokimyasal çalışma*

Ortam: *Akademik medikal bir merkez*

Hastalar: *Otuziki adet endometriyozili hastanın ektopik endometriyal dokuları ve bu hastaların 20'sinin karşılık endometriyumları ile endometriyozisi olmayan 17 hastanın endometriyal dokuları çalışmaya dahil edilmiştir.*

Girişim: *Tüm dokular monoklonal TN-C antikoru ile immünhistokimyasal olarak boyanmıştır.*

Değerlendirme parametreleri: *Menstrüel siklus süresince endometriyozisi olan ve olmayan hastaların ötopik ve ektopik endometriyumlarındaki TN-C'nin varlığının ve lokalizasyonunu değerlendirmek ve karşılaştırmak amacıyla mikroskobik değerlendirme*

Adress for correspondence: Ege Nazan Tavmergen GOKER. Ege University Family Planning Infertility Research and Treatment Center 35100 Bornova, Izmir, TURKEY
Fax: (232) 3430546
e-mail:egenazan@medege.edu.tr
Alındığı tarih: 29. 04. 2005, kabul tarihi: 16. 05. 2005

yapılmıştır.

Sonuç: *Endometriyozisi olan ve olmayan hastaların ötopik endometriyumları arasında ve endometriyozisli hastaların ötopik ve ektopik endometriyumları arasında TN-C ekspresyon açısından anlamlı bir fark izlenmemiştir.*

Yorum: *TN-C ekspresyonu hem endometriyozisde hem de endometriyozisi olmayan bireylerin endometriyumunda azalmış olarak bulunmuştur. Bulgularımız bu ekstraselüler matriks glikoproteininin endometriyum üzerinde rejeneratif rolü olabileceğini ileri süren çalışmalarla kısmen çakışmaktadır. Bununla birlikte, implantasyon prosesinde rolü olabileceği ve endometriyozis nedenli infertiliteyle ilişkili olabileceği düşünülmüştür.*

Anahtar kelimeler: *endometriyozis, ötopik endometriyum, tenascin-C.*

INTRODUCTION

Endometriosis is a benign reproductive age disease which affects between 5% and 40% of normal women and up to 60-80% of women with pelvic pain and/or infertility[1]. It is characterized by the presence of endometrial glands and stroma outside the uterine cavity. Pelvic pain and infertility are the most common problems accompanying endometriosis. Although mild or moderate endometriosis can cause infertility in about 30-40% of patients, the exact mechanism by which endometriosis affects fertility is unknown[2,3]. Adhesions as mechanical factors, peritoneal fluid and its content as toxic factors, impaired folliculogenesis and fertilization, and implantation defects seem to be the most accurate reasons for the explanation of the infertility[4,5].

Tenascin-C (TN-C) is an extracellular matrix (ECM) glycoprotein which was first discovered from fetal chicken tendon and muscle[6]. Myotendinous antigen, glioma mesenchymal protein, cytotactin, J1 glycoprotein, hexabrachion and neuronectin are the other names given to TN-C as it was obtained independently from different sources by several groups [7]. Contrary of the other ESM glycoproteins such as fibronectin and laminin, expression of TN-C is restricted during embryogenesis and oncogenesis. However, in various normal adult tissues it can be reexpressed by the reperative-hyperplastic processes or by the pathologic tissue formation such as carsinogenesis and involution[8].

Although the precise function of endometrial TN-C is unknown, it has been postulated that it may have a role in cell adhesion[9]. In addition, it has been shown that, the expression of TN-C was high in the proliferative phase endometrium compared to secretory phase, which suggests that it may also have a role on the regeneration of the endometrium. This finding indicates that, endometrial TN-C expression may be regulated by the stage of the menstrual cycle in response to ovarian steroid hormones[10-12]. However, it has been demonstrated that this regulation is lost in endometriosis and cancer tissues[10-13]. The loss of its regulation in the endometriotic tissue may have a critical role in the pathophysiology of endometriotiosis[10].

The prevalance of endometriotic disease is not as high as the prevalance of retrograd menstruation which occurs approximately 80-90% of all woman[14]. Therefore, in addition to the peritoneal fluid and its content, the factors which cause the endometriotic disease may also be found in the endometrium itself, as some authors hypothesize[15]. TN-C could be one of these factors that affect the eutopic endometrium and even it can be related with infertility problem in women with endometriosis by influencing the receptivity of the endometrium.

In the light of these findings we had three main purposes to design this study. The first is to investigate the expression of TN-C in the normal endometrial tissues. The second; is to compare the characteristics of this expression with the eutopic endometrial tissues of women with endometriosis, and the third; to compare the TN-C expression between eutopic and ectopic endometrial tissues of women with endometriosis.

MATERIALS AND METHODS

This retrospectively designed study was made in the Family Planning Infertility Research and Treatment Center of Ege University. Two groups of infertile women were included in the study. The first group consists of 32 patients with grade IV endometriotic disease as determined according to the revised American

Fertility Society Classification[16] and had endoscopic cystectomy. At the same time, homologous eutopic endometrial tissues were collected from 20 of these patients. Age of these women ranged from 24 to 40 years with an average of 31,2 years. The second group, as a control group consists of 17 women with a normal pelvis and their infertility problem was related to andrological factors. The endometrial tissues of these women were also collected. Age of these women ranged from 24 to 39 years with an average of 32,1 years. Endometriotic disease was confirmed by the histological examination of the biopsy materials. The day of the menstrual cycle was established from the women's menstrual history and was confirmed by endometrial dating using the criteria of Noyes et al[17]. All endometrial samples were grouped according to the menstrual cycle phase: proliferative (days 1-14 of the cycle) and secretory phase (days 15-28 of the cycle). Among eutopic endometrium of women with endometriosis 11 were in the proliferative and 9 were in the secretory phase, whereas in the control group 9 were in the proliferative and 8 were in the secretory phase. The menstrual phase of the majority of ectopic endometrial tissues were not be able to identify histopathologically. All patients had regular 21-35 day menstrual cycles and no one had received hormonal therapy in the last three months.

Immunohistochemical Staining

The endometrial and endometriotic tissues retrieved from archival paraffin blocks were originally fixed in formalin. The blocks were sectioned at poly-L-lysin-coated slides. The avidin-biotin-peroxidase method was performed using the primary monoclonal antibody against TN-C protein (ready-to-use, Neomarkers, Fremont, Canada). The sections were deparaffinized in xylene, rehydrated, immersed in distillated water and endogenous peroxidase activity was blocken. using a 0.3% solution of hydrogen peroxidase in TRIS pH 7.2. After antigen retrieval by heating in 1 mmol EDTA buffer (pH=8.0), primary antibody was applied for 60 minutes at room temperature and washed in TRIS. Biotinylated secondary antibodies and streptavidin-peroxidase complex (Lab Vision Corp., Fremont, Canada) were added consecutively for 10 minutes at room temperature and washed in TRIS. The peroxidase activity was visualised with 0.03% 3-3' diaminoben-zidine tetrahydrochloride (Sigma Chemical, St Louis, MO, USA) applied for 5 minutes. After rinsing in deionized water and counterstaining in hematoxyline, the slides were dehydrated and mounted. Tonsil tissue was used as a positive control.

Semiquantitative Scoring

The selection of the area for the analysis was based on avaibility of sufficient endometrial and endometriotic tissue for immunohistochemical scoring. Brown cytoplasmic staining was considered as positive. The degree of positive staining for TN-C protein was evaluated by scoring on a scale of 0 to 2. Tissues with equal to 0 (-) were no staining, with 1 (+) were considered weakly positive, and those with 2 (++) were strongly positive

Fisher-Exact and Chi-square tests were used for the statistical analyze of the data and $P<0.05$ value was accepted for the statistical significance.

RESULTS

Immunohistochemical detection of TN-C on the endometrial samples of 20 patients with endometriosis showed 6 weakly focal positive (+) staining; 4 on the proliferative and 2 on the secretory stage. The staining patterns of the samples of proliferative stage revealed 2 stromal, 1 glandulary pattern and 1 sample showed both patterns (Figure-1A). The staining patterns of the samples of secretory stage showed 1 glandulary and 1 stromal pattern. 14 endometrial samples showed no staining (-) with TN-C antibody.

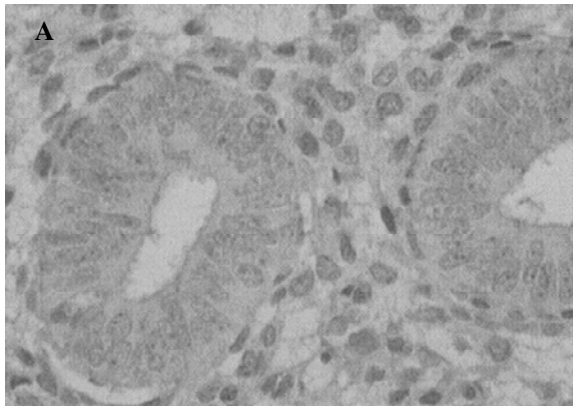
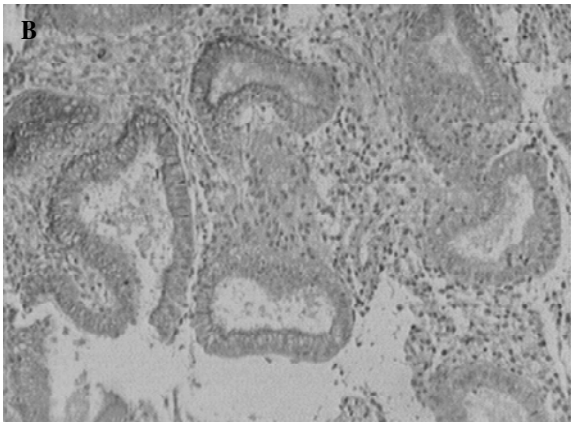
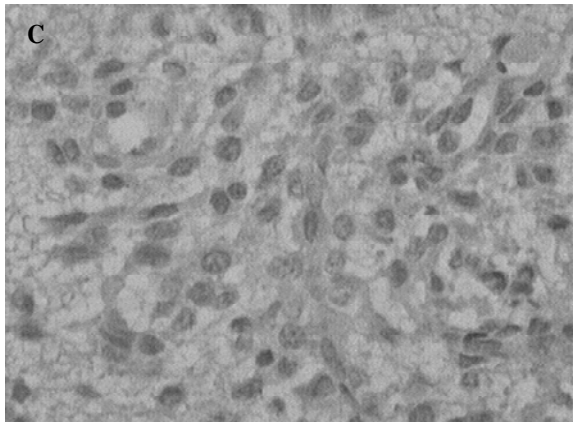


Figure: *Representative micrographs of TN-C immunohistoc-hemistry staining in endometrial tissues of women with and without endometriosis.*

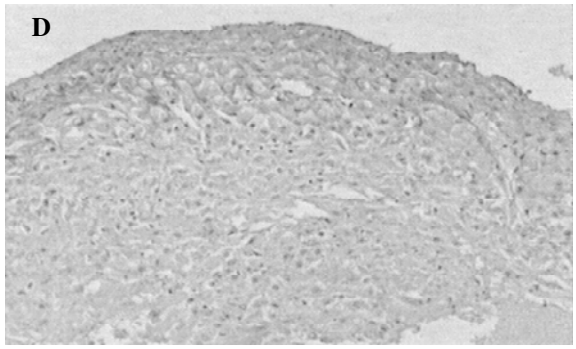
A) Immunostaining for TN-C protein in both stromal and glandulary parts of the proliferative endometrium of a women with endometriosis (TN-C, x 400)



B) Immunostaining for TN-C protein in glandulary part of the secretory endometrium of a woman without endometriosis (TN-C, x 100).



C) Immunostaining for TN-C protein in the stromal part of the endometriotic tissue (TN-C, x 100).



D) Immunostaining for TN-C protein in both epithelial cells of the endometriotic tissue (TN-C, x 100).

Immunohistochemical detection of TN-C on 17 normal endometrial samples revealed 5 weakly focal positive (+) staining; 2 on the proliferative and 3 on the secretory stage. Both positive staining samples of proliferative stage revealed glandulary staining pattern. The staining patterns of the samples of secretory stage revealed 2 glandulary pattern (Figure-1B) and in 1 sample both staining patterns was detected. No TN-C staining (-) was observed on the 12 normal endometrial samples. Immunohistochemical detection of TN-C on the 32 endometriotic cyst samples revealed 7 weakly focal positive (+) and one strongly focal positive (++) staining. 24 samples showed no staining with TN-C antibody and no endometrioma sample was observed which shows both characteristics of staining. The staining patterns of the samples revealed 5 stromal (Figure-1C) and 3 glandulary staining (Figure 1D).

While menstrual stage of the majority of ectopic endometrial samples could not be determined by histopathological examination, these ectopic endometrial samples were examined based on the last menstrual period of the patients.

The characteristics and staining patterns of the eutopic and ectopic endometrial tissues of both groups with TN-C are summarized in Table I.

No statistical difference of TN-C expression was found neither between normal proliferative and secretory

Table I: *The characteristics and staining patterns of the eutopic and ectopic endometrial tissues of both groups with TN-C.*

	Eutopic endometrium of women with endometriosis	Endometrium of women without endometriosis	Ectopic endometrium of women with endometriosis
Tissue number	20	17	32
Proliferative stage	11	9	
Secretory stage	9	8	
Positive staining	6	5	8
Negative staining	14	12	24
Proliferative phase staining	4	2	-
Secretory phase staining	2	3	-
Stromal staining	3	-	5
Glandulary staining	2	4	3
Stromal and glandulary staining	1	1	-

stages endometrial samples, nor between the stages of endometrial samples of patients with endometriosis ($p>0.05$). No statistical difference of total TN-C staining was found between the endometrial samples of both groups ($p>0.05$). In comparison of all stages between each other, no statistical difference of TN-C expression was found between both groups endometrium. There was no statistical difference of TN-C expression between eutopic and ectopic endometrial tissues of patients with endometriosis ($p>0.05$).

DISCUSSION

Endometriotic disease still keeps its mystery about its pathogenesis despite extensive research. Majority of the studies designed to determine the differences between endometriosis and eutopic endometrium, revealed that ectopic endometrium differs from eutopic endometrium with regard to clonality of origin, enzymatic activity, protein expression and histologic and morphologic characteristics. Minority of them has found similarities between both tissues[18].

In all of the few published studies which aimed to define the functions of endometrial TN-C, it is found that its expression is usually high in the proliferative stage stromal endometrium and very low or non detectable on the secretory stage endometrium of the menstrual cycle. Therefore, it was attributed a proliferative role to TN-C on the regeneration of the menstrual endometrium as in the other normal adult tissues[10-12]. In our study, despite there was no statistical difference of TN-C expression between the proliferative and the secretory stages of normal endometrial samples, number of the stained samples in the secretory stage was relatively much more than the proliferative stage (2/5 versus 3/5). This finding is in contrast to the other published studies. In our opinion, expression of TN-C is restricted in normal endometrium and it seems that this expression does not alter according to the stage of the endometrium. In our series the fact that the TN-C expression was relatively higher in the secretory stage may indicate that it may have a role related with the implantation process rather than the regenerative role on the endometrium. If TN-C would have a regenerative role on the endometrium, it might be expressed especially higher in the proliferative stage of the stromal endometrium. We did not find either statistical difference of this expression between both endometrial samples of patients with endometriosis. But relatively much more stained samples was revealed on the proliferative stage endometrium (4/6 versus 2/6) contrary of the normal endometrial samples. To our knowledge, Harrington et al's study is the only one published study in the literature in which TN-C expression in endometriosis was investigated. They found that the expression of TN-C was high in the proliferative stage endometrium but low or restricted in the secretory stage endometrium of patients with endometriosis, similarly to their findings in normal endometrial samples[10]. Our findings on the endometrial samples of patients with endometriosis are concordant with theirs. This finding may also suggest the probable role of this ECM glycoprotein on the fertility and it can be supported by the fact that the secretory stage staining was higher in normal endometrial samples than in endometrial samples of patients with endometriosis (3/8 versus 2/9). In fact in our series, endometrial tissues stained with TN-C were very few and weak in both groups (6/20 and 5/17). Therefore all these findings must be revised with larger series.

Harrington et al also investigated fibronectin, laminin, collagen type IV and vitronectin expressions in the ectopic and eutopic endometrial samples in the same study. They found that the expression of these ECM components beside TN-C, were similar in eutopic endometrium and endometriosis. However TN-C expression was found different in ectopic and eutopic endometrial tissues. On the contrary of the eutopic endometrium, TN-C expression was abundant in ectopic endometrial tissues even in secretory stage of the menstrual cycle. Therefore it is postulated that the expression of the endometrial TN-C is regulated according to stage of the menstrual cycle, whereas this regulation is lost in endometriotic tissues[10]. In our series, menstrual stage of the majority of ectopic endometrial samples could not be determined by histopathological examination. Therefore it could not be possible for us to reach a decision about the regulation of TN-C in endometriotic tissues. However we found that this expression is relatively high in the eutopic endometrial samples of patients with endometriosis comparing with the cyst samples (6/20 versus 8/32), which may indicate the possible role of the peritoneal environment. On the other hand we did not confirm the TN-C expression in any eutopic

endometrium in which this expression was shown in all the corresponding endometriotic cyst samples. We did not confirm either this expression in any ectopic endometrial samples in which it was detected in all the corresponding eutopic endometrium. According to us these findings may reveal that this ECM glycoprotein may play a role on the pathogenesis of the endometriotic disease but we do not know yet by which way it works. Expressions of fibronectin, laminin, collagen type IV, vitronectin and TN-C were found similar in the endometrium of women with and without endometriosis and it was suggested that eutopic endometrial TN-C could not have a role on the high levels of TN-C in ectopic stromal endometrial tissue, but peritoneal fluid and its content may influence the levels of ectopic endometrial TN-C[10]. In our series, expression of TN-C was similar in both group endometrial samples (6/20 versus 5/17) but it was relatively less in ectopic endometrium than in the eutopic endometrium (6/20 versus 8/32). Even this difference is not really significant, it may be explained by the peritoneal factors. Implantation defects seem to be one of the most accepted reasons for the explanation of infertility in women with endometriosis. Some studies revealed that implantation rates seem to be lower in women with endometriosis[19-21]. Cell adhesion molecules, integrins, cytokines, growth factors and ECM proteins are the main factors that participate considerable roles on the different phases of implantation. It is demonstrated that their expressions are different in the secretory stage of the menstrual cycle that facilitate the interactions between the decidua and the conceptus [22]. In a mouse study, Julian et al revealed that TN serves as an early marker for uterine receptivity and the attachment phase of implantation and also it may facilitate embryo penetration by disrupting uterine epithelial cell adhesion to underlying basal lamina[23]. Our histopathological findings showed a difference of TN-C expression in the secretory stages of both groups endometrium. Patients with endometriosis have less secretory stage TN-C staining (2/9 versus 3/8). In this group, after endometrioma extirpation, 10 women conceived spontaneously and delivered live babies. This finding may suggest that endometrial secretory stage TN-C staining may have a positive influence on implantation. This might be regulated by the soluble peptid levels which may be regulated and positively influenced after endometrioma extirpation.

As a result of this retrospectively designed study, expression of TN-C seems to be restricted in both normal endometrial tissue and endometrial tissues of patients with endometriosis. Our findings are partially controversial with the studies suggesting that this ECM glycoprotein have a regenerative role on the endometrium. According to us, it seems to have a role on the implantation process and even it can be related within the infertility mechanisms involved in endometriotic disease. Our histopathological findings did not reveal statistical difference of TN-C expression between the eutopic and ectopic endometriums of patients with endometriosis. But as no one of the corresponding samples showed positive staining of the samples in which this expression revealed, it could also have a role on the pathogenesis of the disease. However to reach a distinct decision about the functions of endometrial TN-C and its role on the pathogenesis of endometriosis, we need more studies with larger series.

REFERENCES

1. Koninckx PR, Kennedy SH and Barlow DH. Endometriotic disease: the role of peritoneal fluid. Hum Reprod Update 1998; 4:741-751.
2. Kistner RW. Endometriosis and infertility. Clin Obstet Gynecol 1979;22:101
3. Mulayim N, Arici A. The relevance of the peritoneal fluid in endometriosis associated infertility. Hum Reprod 1999;14(suppl 2):67-76.
4. Mahutte NG and Arici A. Endometriosis and assisted reproductive technologies: are outcomes affected ? Current Opinion in Obstetrics and Gynecology 2001;13:275-279.
5. Lessey BA. Medical management of endometriosis and infertility. Fertil Steril 2000;73:1089-1096.
6. Koukoulis GK, Howeedy AA, Korhonen M, Virtanen I and Gould VE. Distribution of tenascin, cellular fibronectins and integrins in the normal, hyperplastic and neoplastic breast. Submicrosc Cytol Pathol 1993;25:285-295.
7. Jones PL and Jones FS. Tenascin-C in in development and disease: gene regulation and cell function. Matrix Biology 2000;19:581-596.
8. Koukoulis GK, Gould VE, Bhattacharyya A, Gould JE, Howeedy AA and Virtanen I. Tenascin in normal, reactive, hyperplastic and neoplastic tissues: biologic and pathologic implications. Hum Pathol 1991;22:636-643.
9. Pilch H, Scaffer U, Schlenger K, Lautz A, Tanner B, Hockel M et al. Expression of tenascin in human cervical cancer-association

of tenascin expression with clinicopathological parameters. Gynecologic Oncology 1999;73:415-421.

10. Harrington DJ, Lessey BA, Vikram Rai, Berqvist A, Kennedy S, Manek S et al. Tenascin is differentially expressed in endometrium and endometriosis. J Pathol 1999;187:242-248.
11. Sasono H, Nagura H, Watanabe K, Ito K, Tsuiki A, Sato S et al. Tenascin expression in normal and abnormal human endometrium. Mod Pathol 1993;6:323-326.
12. Yamanaka M, Taga M, Minaguchi H. Immunhistological localization of tenascin in the human endometrium. Gynecol Obstet Invest 1996;41:247-252.
13. Vollmer G, Tan MI, Wünche W and Frank K. Expression of tenascin-c by human endometrial adenocarcinoma and stroma cells: heterogeneity of splice variants and induction by TGF-_. Biochem Cell Biol 1997;75:759-769.
14. Halme J, Hammond MG, Hulka JE, Raj SG and Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64:151-154.
15. Lessey BA, Castelbaum AJ, Sawin SW et al. Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab 1994;79:643-649.
16. The American Fertility Society. Revised American Fertility Society classification of endometriosis:1985. Fertil Steril 1985; 43:351-2.
17. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.
18. Redwine DB. Was Sampson wrong ? Fertil Steril 2002;78:686-693.
19. Arici A, Oral E, Bukulmez O et al. The effect of endometriosis on implantation: results from the Yale University in vitro fertilization and embryo transfer program. Fertil Steril 1996;65:603-607.
20. Simon C, Gutierrez A, Vidal A et al. Outcome of patients with endometriosis in assisted reproduction: results from in vitro fertilization and embryo transfer success rates. Hum Reprod 1994;9:725-729.
21. Yovich JL, Matson PL, Richardson PA and Hilliard C. Hormonal profiles and embryo quality in women with severe endometriosis treated by in vitro fertilization and embryo transfer. Fertil Steril 1988;50:308-313.
22. Giudice LC. Potential biochemical markers of uterine receptivity. Hum Reprod 1999;14(suppl 2):3-16.
23. Julian JA, Chiquet-Ehrismann R, Erickson HP and Carson DD. Tenascin is induced at implantation sites in the mouse uterus and interferes with epithelial cell adhesion. Development 1994; 120:661-671.