

# Fibroblast growth factor-1 expression in the endometrium of patients with repeated implantation failure after *in vitro* fertilization

M.E. SAK, T. GUL, M.S. EVSEN, H.E. SOYDINC, S. SAK\*\*, A. OZLER, U. ALBALIK\*

Department of Obstetrics and Gynecology, and \*Department of Pathology; School of Medicine, Dicle University, Diyarbakir, Turkey

\*\*Department of Obstetrics and Gynecology, Diyarbakir Maternity and Children Hospital, Diyarbakir, Turkey

**Abstract. – BACKGROUND:** An examination of the alterations in Fibroblast Growth Factor-1 (FGF-1) expression in a group of repeated implantation failure after *in vitro* fertilization (IVF) patients, when compared to fertile patients.

**PATIENTS AND METHODS:** Study group consisted of 24 patients with repeated implantation failure and 29 fertile control patients. Endometrial samples received at the luteal phase were exposed to immunohistochemical staining for the fibroblast growth factor-1 (FGF-1) with antibodies.

**RESULTS:** In the study group all patients have primary infertility (n = 24), and the average duration of infertility was  $3.9 \pm 1.3$  years. The average recurrent IVF failure was  $2.6 \pm 0.6$  attempts. There were no significant differences in the histological data according to the Noyes classification ( $p = 0.226$ ) and age ( $p = 0.231$ ) between the patients in the study and control groups (n=29). The control group was found to have more severe expression of FGF-1 ( $< 0.001$ ) than the study group when endometrial glandular epithelial cells, stromal cells and vascular endothelial cells were evaluated.

**CONCLUSIONS:** Endometrial glandular epithelial cells, stromal cells and vascular endothelial cells of the control and study group were evaluated and it was found that the control group displayed a stronger expression of the FGF-1 ( $< 0.001$ ). The expression of FGF-1 in the IVF implantation failure group is less than in the fertile group, which suggests that growth factors such as FGF-1 are important maternal factors effecting implantation.

*Key Words:*

Implantation failure, IVF, FGF-1.

## Introduction

Despite expanding global experience in advanced reproductive technologies, the majority of *in vitro* fertilization (IVF) attempts do not result

in a successful pregnancy, this is most often a result of implantation failure<sup>1</sup>. Failed implantation takes place due to incompatible conditions in maternal and embryonic factors. The associated maternal factors are anatomic, endometrial, thrombophilic, immunological, and genetic. The disruption in the development of the embryo, and male factors can be included in the embryological factors<sup>2</sup>.

The process of the implantation of the embryo is a highly dynamic and precise control of molecular and cellular events, and it is not fully understood. Endometrial receptivity for implantation of the blastocyst (the implantation window) is limited, and corresponds to about 6-8 days after ovulation, lasting for about 4 days. This limited time presents itself in the form of low implantation rates in natural cycles. The development of assisted reproductive techniques is one of the most important factors affecting the success in treating low implantation rates<sup>2-4</sup>.

Successful implantation in the endometrium depends on the window period, and on autocrine and paracrine regulation of the developmental steps of the embryo and the endometrium, which are induced by complex synchronized molecular and cellular events. According to the classical criteria of Noyes et al<sup>5</sup>, endometrial sampling is commonly used to determine the day of endometrially synchronized changes due to ovulation. However, fertile and infertile women's endometrial biopsies may present non-phase endometrium<sup>6</sup>. Integrin, vascular endothelial growth factor, mucin, and most of the cytokines were identified as important factors in implantation. Endometrial biopsy specimens and products of advanced microassay technologies have determined the endometrial receptivity and implantation in the expression of many genes.

Fibroblast Growth Factors (FGFs) are a family of multifunctional mitogenic polypeptides that induce endothelial cell migration and promote the formation of blood vessels and the patterning of early branching events<sup>7</sup>. Some factors promote the expression of decidual fibroblast growth factors (FGFs). Some of them are novel and irrelevant since the stroma is composed of fibroblasts<sup>8</sup>. FGFs affect embryo implantation and support improved endometrial trophoblastic interaction<sup>9,10</sup>. No data exists, to our knowledge, regarding the role of FGF-1 expression in patients with implantation failure after repeated IVF attempts. Our goal was to compare the expression of FGF-1 in patients with repeated IVF failure to the normal fertile population.

## Patients and Methods

The study group consisted of unsuccessful IVF patients admitted to the Dicle University Medical Faculty, Department of the Gynecology and Obstetrics, between december 2009 and june 2012. Ethics Committee approval was obtained before the study. All patients in the study group had primary infertility and implantation failure after IVF. The implantation failure was evaluated by at least one good quality embryo transfer after two or more IVF attempts. All patients in the study group had clarified fallopian tubes opened with hysterosalpingography or laparoscopy. Patients diagnosed with endometriosis were excluded from the study. Patients whose implantation rates were adversely affected by male infertility, intracavitary fibroids, polyps, intrauterine synechiae, and factors such as uterine anomalies were excluded from the study group. Patients with one-sided tubal occlusion were included and anovulatory patients were excluded from the study because ovulatory patients showing a pattern of secretory endometrial biopsies.

Patients in the control group had at least one live birth, were ovulatory, fertile, and had not lost a pregnancy. Each of the two groups' age and endometrium according to the histological criteria of Noyes et al endometrium were verified. The patients in the study group were evaluated for the duration of their infertility and the number of unsuccessful IVF attempts. Hysteroscopic examination was performed in all patients under local or general anesthesia. After hysteroscopic examination, patients in the luteal phase were treated with Silastic suction curettage. Endometrial samples

were examined by a single pathologist for endometrial histological suitability and pathological evaluation.

Endometrial samples were stored in paraffin blocks. Hysteroscopic and pathologic examination revealing polyps, inflammation, hyperplasia (such as leiomyoma), or endometrial pathology excluded patients from the study as these would affect the expression of FGF.

## Immunohistochemical Evaluation

Five micrometer thick sections from paraffin blocks were selected. Slides were stored in a 62°C oven for 60 minutes. For the deparaffinization process, paraffin blocks were soaked for 4 to 5 minutes in a 96% alcohol solution, and for 4 to 5 minutes in xylene. For the purpose of antigen retrieval a citrate solution of pH 6 was added, and then heat shocked at 125°C in a high pressure Biocare Decloaking Chamber. The mixture was allowed to stand for 20 minutes of protein block (Ultra V Blok, Fremont, CA, USA, ScyTek, Logan, VT, USA). As the primary antibody, FGF-1 (Santa Cruz: sc-55520, Santa Cruz, CA, USA) was incubated for 60 minutes, then linked with a biotinylated antibody (ScyTek, Logan, VT, USA) and Streptavidin/HRP solution (ScyTek, Logan, VT, USA). It was then allowed to stand for 20 minutes in instilled AEC (3-amino-9-ethylcarbazole) Single solution and washed for 10 min with distilled water. FGF-1 slides stained with hematoxylin and eosin (H&E) were evaluated with light microscopy (Nikon Eclipse 80i, Melville, NY, USA) by an expert pathologist. Five different areas of each tissue were randomly selected and calculated by evaluating the average score. The results were scored according to endometrial glandular, stromal, and endothelial cells, depending on the severity of staining. The extent of staining was based on the grades of 1-3, grade 0 (negative), grade 1 (poor), grade 2 (moderate), and grade 3 (strong). The common score was given as the addition of two scores.

## Statistical Analysis

The Mann-Whitney U test was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) to compare both groups with regard to age, immunohistochemistry FGF-1 expression scoring and endometrial date according to histological findings. A *p* value < 0.05 was considered significant. All values were expressed as mean ± standard deviation (SD).

## Results

All patients in the study group had primary infertility ( $n = 24$ ). The average duration of infertility was  $3.9 \pm 1.3$  years. Between patients in the study and control groups ( $n=29$ ), there was no significant difference in the histological data based on the Noyes et al classification ( $p = 0.226$ ) (Table I). The IVF and control groups with H&E stained slides were examined, and endometrial tissues were seen in the luteal phase. The FGF-1 stained slides from the study groups were examined and the endometrial glandular epithelial cells, stromal cells, and vascular endothelial cells were found to have a lower expression of FGF-1. In the stained FGF-1 slides, endometrial glandular epithelial cells, stromal cells, and vascular endothelial cells of the control group were evaluated and found to have a stronger expression of FGF-1 ( $< 0.001$ ) (Figure 1). Considering the strength of individual cases of FGF-1 expression in epithelial, stromal, and endothelial cells was found to be correlated with the cases on an individual basis; epithelial, stromal, and endothelial cells were found to be correlated with the strength of the expression of FGF-1.

## Discussion

This study shows the first endometrial samples collected from patients with recurrent implantation failure FGF-1 expression of angiogenic growth factor. In our study, we found FGF-1 expression to be lower in patients without endometrial pathology, with recurrent implantation failure.

FGF is a multifunctional mitogenic peptide which provides for endothelial cell migration, blood vessel formation, and early vasculature. So far, 22 different human FGFs have been found and are numbered 1 to 22 sequentially. Many types of FGFs have effects such as angiogenesis, mitogenesis, and chemotaxis on different target cells<sup>9</sup>. To better understand the effects of FGF expression on

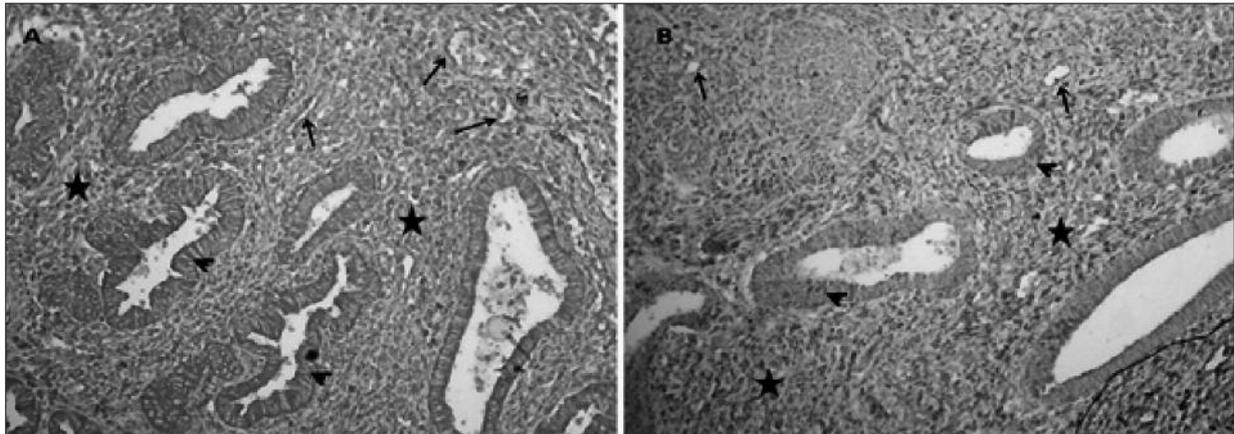
the endometrium, different types of FGFs need to be evaluated. The effects of FGF on the developing human placenta have not been determined as yet. Studies in mice<sup>11</sup> explain the impact of the embryological FGF. Deng et al found that the lack of FGF-1 expression caused *in vitro* and *in vivo* growth restrictions in mice. In studies in mice, VEGF and FGF formed by angiogenesis have shown successful implantation of the embryo.

However, the problem of infertility and recurrent cases of unsuccessful IVF is that there is not enough information about the expression of FGF. However, Kathasambas et al<sup>12</sup> did not find differences in expression of FGF-2 in estrous cycle and early pregnancy; also Gupta et al<sup>13</sup> found the increased expression of FGF-2 at the luminal epithelium and stroma at 10-14 days of pregnancy. In our study, FGF-1 expression in patients with repeated unsuccessful IVF attempts was lower than in the control group, which is especially important for implantation in the luteal phase.

In the pathogenesis of endometriosis, the eutopic endometrium suggests the dysregulation of angiogenic activity. In this study<sup>14</sup>, patients with endometriosis, eutopic endometrium of the secretory phase, and endometrial glandular epithelium expressed a high rate of VEGF around the blood vessels. Jee et al<sup>15</sup> found in his report that patients with recurrent failed IVF luteal phase had decreased expression of VEGF, but there were no differences in the early, mid, and late luteal phases. But this investigation is not case-control study of healthy women who have given birth compared to the expression of VEGF in patients with failed IVF. In our research, the mid luteal phase endometrium was evaluated and FGF-1 expression was also decreased in endometrial glandular epithelial cells, stromal cells, and vascular endothelial cells in those patients with failed IVF. Jee et al<sup>15</sup> connected VEGF expression of glandular epithelium more frequently to the stroma of the glands secreting VEGF. Wollenhaupt et al<sup>16</sup> determined that FGF-2 expression in pig endometrium was higher than in the stroma. Based

**Table I.** Mean clinicopathologic characteristics of study and control group.

	Study group (n=24)	Control group (n=29)	p
Age	31.5 ± 3.0	32.6 ± 2.5	0.231
Histologic dating to Noyes et al criteria	22.2 ± 0.7	21.9 ± 0.7	0.226
Immunohistochemistry FGF-1 expression scoring	3.1 ± 0.9	4.5 ± 1.1	< 0.001
Duration of infertility	3.9 ± 1.3		
Previous failed cycles	2.6 ± 0.6		



**Figure 1.** **A,** Stronger FGF-1 expression in endometrial gland epithelium (*arrowhead*), stromal (*stars*) and endothelial cells (*arrow*) in control group (immunoperoxidase, 200×). **B,** Lower FGF-1 expression in endometrial gland epithelium (*arrowhead*), stromal (*stars*) and endothelial cells (*arrow*) in study group (immunoperoxidase, 200×).

on this, it is accepted that the FGF-2 are stromally derived growth factors. In our study, in endometrial glandular, stromal, and vascular endothelial cells, the FGF-1 expression was equal.

Maternal causes (anatomical factors, decreased endometrial receptivity, thrombophilia, immunological factors) and embryonic causes (genetic factors, inadequate development of the embryo, male factor) are included among the causes of recurrent implantation failure after IVF<sup>2</sup>. The fibroblast growth factor family is thought to be involved in the transformation of embryo growth and invasion of the uterine stromal cells in animal studies. FGF-2 is the most common factor known in this family, it occurs in fibroblasts and endothelial proliferation, although widely expressed in adult and fetal tissues. FGF-2 controls mRNA, the estrous cycle, early pregnancy, endometrial epithelium, stroma, and myometrium<sup>9,16</sup>. In the literature no work could be found on the study of the fibroblast family in IVF. In our report, the FGF-1 effect is possible by means of tyrosine kinase. FGF-1 is not expressed strongly enough in the failed IVF patients in the research, which may have caused a lack of endothelial cell migration (which is important for implantation), stopped the process of blood vessel formation, or caused early vascularization of implantation problems.

### Conclusions

The expression of FGF-1 in the IVF implantation failure group is less than in the fertile group,

which suggests that growth factors such as FGF-1 are important maternal factors effecting implantation. Further studies are necessary on the expression of FGF-1, in patients with repeated IVF failure, which can explain the recurrent implantation failure.

### Disclosure of Interest

Authors declare no conflict of interest or financial disclosure for this manuscript.

### References

- 1) KOOT YE, TEKLENBURG G, SALKER MS, BROSENS JJ, MACKLON NS. Molecular aspects of implantation failure. *Biochim Biophys Acta* 2012; 1822: 1943-1950.
- 2) SIMON A, LAUFER N. Repeated implantation failure: clinical approach. *Fertil Steril* 2012; 97: 1039-1043.
- 3) PENZIAS AS. Recurrent IVF failure: other factors. *Fertil Steril* 2012; 97: 1033-1038.
- 4) TURKYILMAZ E, GUNER H, ERDEM M, ERDEM A. NLF2 gene expression in the endometrium of patients with implantation failure after IVF treatment. *Gene* 2012; 508: 140-143.
- 5) NOYES RW, HERTIG AT, ROCK J. Dating the endometrial biopsy. *Am J Obstet Gynecol* 1975; 122: 262-263.
- 6) COUTIFARIS C, MYERS ER, GUZICK DS, DIAMOND MP, CARSON SA, LEGRO RS, MCGOVERN PG, SCHLAFF WD, CARR BR, STEINKAMPF MP, SILVA S, VOGEL DL, LEPPERT PC; NICHD NATIONAL COOPERATIVE REPRO-

- DUCTIVE MEDICINE NETWORK 2004. Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertil Steril* 2004; 82: 1264-1272.
- 7) METZGER RJ, KRASNOW MA. Genetic control of branching morphogenesis. *Science* 1999; 284: 1635-1639.
  - 8) DUNN CL, KELLY RW, CRITCHLEY HO. Decidualization of the human endometrial stromal cell: an enigmatic transformation. *Reprod Biomed Online* 2003; 7: 151-161.
  - 9) ANTEBY EY, NATANSON-YARON S, HAMANI Y, SCIAKI Y, GOLDMAN-WOHL D, GREENFIELD C, ARIEL I, YAGEL S. Fibroblast growth factor-10 and fibroblast growth factor receptors 1-4: expression and peptide localization in human decidua and placenta. *Eur J Obstet Gynecol Reprod Biol* 2005; 119: 27-35.
  - 10) BISCHOF P, CAMPANA A. Molecular mediators of implantation. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000; 14: 801-814.
  - 11) DENG CX, WYNshaw-BORIS A, SHEN MM, DAUGHERTY C, ORNITZ DM, LEDER P. Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev* 1994; 8: 3045-3057
  - 12) KATSAHAMBAS S, HEARN MT. Localization of basic fibroblast growth factor mRNA (FGF-2 mRNA) in the uterus of mated and unmated gilts. *J Histochem Cytochem* 1996; 44: 1289-1301.
  - 13) GUPTA A, BAZER FW, JAEGER LA. Immunolocalization of acidic and basic fibroblast growth factors in porcine uterine and conceptus tissues. *Biol Reprod* 1997; 56: 1527-1236.
  - 14) BOURLEV V, VOLKOV N, PAVLOVITCH S, LETS N, LARSON I A, OLOVSSON M. The relationship between microvessel density, proliferative activity and expression of vascular endothelial growth factor-A and its receptors in eutopic endometrium and endometriotic lesions. *Reproduction* 2006; 132: 501-509.
  - 15) JEE BC, SUH CS, KIM KC, LEE WD, KIM H, KIM SH; SEOUL NATIONAL UNIVERSITY COLLEGE OF MEDICINE ASSISTED REPRODUCTIVE TECHNOLOGY (SMART) STUDY GROUP. Expression of vascular endothelial growth factor-A and its receptor-1 in a luteal endometrium in patients with repeated *in vitro* fertilization failure. *Fertil Steril* 2009; 91: 528-534.
  - 16) WOLLENHAUPT K, WELTER H, EINSPANIER R, MANABE N, BRÜSSOW KP. Expression of epidermal growth factor receptor (EGF-R), vascular endothelial growth factor receptor (VEGF-R) and fibroblast growth factor receptor (FGF-R) systems in porcine oviduct and endometrium during the time of implantation. *J Reprod Dev* 2004; 50: 269-278.

**BACKGROUND:** An examination of the alterations in Fibroblast Growth Factor-1 (FGF-1) expression in a group of repeated implantation failure after in vitro fertilization (IVF) patients, when compared to fertile patients. **PATIENTS AND METHODS:** Study group consisted of 24 patients with repeated implantation failure and 29 fertile control patients. Endometrial samples received at the luteal phase were exposed to immunohistochemical staining for the fibroblast growth factor-1 (FGF-1) with antibodies. **RESULTS:** In the study group all patients have primary infertility (n = 24), and the average duration repeated implantation failure (RIF), gene expression, bioinformatics. Comparing the endometrial gene expression profiles between RIF patients and healthy women, and investigating the factors affecting endometrial receptivity and the mechanism of RIF could provide new biomarkers to the diagnosis of RIF patients as insufficient or damaged endometrial receptivity might be one of the main factors related to RIF. Endometrial receptivity refers to the state of the endometrium during the implantation window (WOI), which is considered to be the most receptive period for embryo implantation, preparing a favorable environment in the endometrium[1,9]. Inflammatory reaction, immune response, and angiogenesis all had influence on endometrial receptivity[10,11,12]. Repeated IVF treatment failure is very frustrating to the patients as well as to the clinicians. Even in subsequent IVF/ICSI cycles, lower pregnancy rates can be expected in these patients [1, 2]. Many research efforts have been invested to find the factors that affect the pregnancy rate in these couples, not only to advise patients about treatment success but also to propose methods of treatment that would improve their possibility of conception. However, reduced endometrium receptivity and low embryo quality are thought to be the most important factors [3]. After oocyte fertilization using IVF or ICSI procedure, embryos were cultured in the BlastAssist extended culture media (Origio, Denmark). **Recurrent Implantation Failure** Efstratios M. Kolibianakis, Christos A. Venetis Taylor&Francis 9781138055780 : Implantation is a complex phenomenon, still not thoroughly understood, involving t. \* The psychosocial aspects of recurrent implantation failure \* The role of life-style factors in RIF \* Endocrine causes of recurrent implantation failure \* Congenital uterine anomalies and recurrent implantation failure \* Immunological causes of recurrent implantation failure \* Acquired uterine conditions, reproductive surgery, and RIF? \* Manipulating the endometrium: Can RIF be managed by inducing endometrial? \* Optimizing embryo transfer technique for RIF management \* Proposed management of patients with recurrent implantation failure and directions for Keywords: Repeated implantation failure (RIT); In vitro fertilization-embryo transfer (IVF-ET); Endometrial receptivity; Therapies. **Introduction.** Repeated implantation failure (RIF) implies at least 3 consecutive treatment cycles of failed in vitro fertilization-embryo transfer (IVF-ET), in which 1 or 2 quality embryos are implanted in each treatment cycle. RIF has gradually become the major problem that undermines the pregnancy rate of IVF [1]. A good endometrial. and growth factors in the endometrium, thereby promoting its decidualization and providing a good environment for embryo implantation [8]. As one of the most important antioxidants in the human body, Vitamin E also exhibits the effect of dilating blood vessels.