A physiological approach for treating endometriosis by recombinant pigment epithelium-derived factor (PEDF)

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STUDY QUESTION: Is pigment epithelium-derived factor (PEDF) expressed in the rodent endometrium and can it be utilized to treat endometriosis without negatively affecting reproductive parameters?

SUMMARY ANSWER: PEDF is dynamically expressed in rat endometrium throughout the estrous cycle in a reciprocal manner to vascular endothelial growth factor (VEGF); it possesses potent therapeutic properties for endometriosis that do not compromise the reproductive parameters.

WHAT IS KNOWN ALREADY: Endometriosis pathogenesis depends mainly on neovascularization, with a high local level of VEGF. PEDF, a 50 kDa secreted glycoprotein with a potent anti-angiogenic activity, negates several strong pro-angiogenic factors, such as VEGF.

STUDY DESIGN, SIZE, DURATION: Rat endometrial samples were collected at various days of the estrous cycle (n = 5 rats/day) and mRNA of VEGF and PEDF was determined. Endometriosis was induced by transplanting uterine pieces onto the inner surface of the abdominal wall of recipient rats, resulting in proliferation of the endometrial transplants. Recipient rats were randomly injected intravenously (IV), every third day for the next 3 weeks, with either Tris (‘control’; n = 7) or recombinant PEDF (rPEDF; 2 mg/kg/day; ‘PEDF prevention’; n = 7), while others were IV injected every third day starting from Day 9 after grafting until the end of 3 weeks, with rPEDF (2 mg/kg/day; ‘PEDF treatment’; n = 6). The effect of rPEDF on the duration of the estrous cycle and on the number of ovulated oocytes was evaluated in rats that were randomly divided into four groups and were injected with either Tris or rPEDF every third day for 3 weeks: naive rats (n = 6); rPEDF-treated rats (n = 5); endometriosis-induced rats (n = 5); or endometriosis + rPEDF rats (n = 6).

MATERIALS, SETTING, METHODS: Reproductive parameters: the estrous cycle was evaluated by daily vaginal smears, and the number of ovulated oocytes in the oviductal ampullae of estrus rats was counted. The efficiency of endometriosis induction and treatment was evaluated on the third week after endometrial transplantation, on the day of pro-estrus. Endometrial transplants were isolated and weighted. PEDF and VEGF were monitored by quantitative PCR and immunohistochemistry using confocal microscopy.

MAIN RESULTS AND THE ROLE OF CHANCE: PEDF mRNA and protein were dynamically expressed in the endometrium all throughout the estrous cycle, reciprocally to VEGF; VEGF was highly expressed during estrus while PEDF expression was low, and vice versa at metestrus II. The weight of the endometrial transplants was significantly reduced after PEDF administration (13% of control for ‘PEDF treatment’ rats; 7% of control for ‘PEDF prevention’ rats; P < 0.001). Histology of the transplants’ remnants showed a complete loss of their endometrial characteristics. Furthermore, the level of VEGF mRNA in the transplants of PEDF-administered rats was significantly lower (P < 0.05) than in transplants of control rats. Administration of rPEDF had no effect on the estrous cycle or ovulation rate of naive rats, while it had a significantly beneficial effect on the low ovulation rate of endometriosis-induced rats (P < 0.05).

LIMITATIONS, REASONS FOR CAUTION: The experiments were performed in a rat model.

† Equal contribution.
**Introduction**

Endometriosis is one of the most common health problems among women, associated with dysmenorrhea, dyspareunia, chronic pelvic pain and infertility. Endometriosis is characterized by the presence of endometrial tissue (lesions) outside the uterus, most commonly on the peritoneum and ovaries. It affects ~10% of reproductive-age women and up to 50% of infertile women (Cramer and Missmer, 2002; De Hondt et al., 2005; Gupta et al., 2008; Mansour et al., 2010). The pathophysiology of endometriosis-related infertility is still unknown, although various mechanisms have been suggested (Norenstedt et al., 2001). Severe endometriosis is associated with pelvic adhesions and distorted pelvic anatomy, leading to possible mechanical or anatomical disturbances (Barnhart et al., 2002). Since the presence of only few endometrial lesions may be associated with marked subfertility (Guizick, 1997), even mild stages of endometriosis may have a direct negative effect on oocytes quality,embryogenesis and implantation (Barnhart et al., 2002). Indeed, the reproductive success of patients with endometriosis-related infertility undergoing IVF is significantly decreased, resulting in almost one half of the pregnancy rate achieved among women with other indications for IVF (Barnhart et al., 2002).

The mechanisms contributing to the establishment of endometrial tissue outside the uterus are still a matter of debate; however, it is clear that angiogenesis is necessary for the development and maintenance of endometrial lesions (Soares et al., 2011). The regulation of endometrial vasculature involves canonical pathways of angiogenesis, including the action of vascular endothelial growth factor (VEGF) that appears to be the main factor involved in endometriosis progression (Laschke et al., 2011). The inhibition of angiogenesis in general and of VEGF in particular may impair endometriosis.

However, since endometriosis affects women of reproductive age, a period that is characterized by the occurrence of many reproductive angiogenic processes (follicle maturation, corpus luteum function, eutopic endometrial proliferation, etc.; Reynolds et al., 2002), there is a need for a selective angiogenesis inhibitor that will exert a specific activity against endometriosis without affecting other reproductive functions. The commercial anti-VEGF drugs available today induce severe side effects that rule out their use for endometriosis treatment in young, otherwise healthy women (Eremina et al., 2008; May and Becker, 2008). Practically, the most commonly suggested therapies for endometriosis in the clinics are: (i) induction of an amenorrheic—hypoestrogenic state, by suppressing ovarian estrogen secretion, and therefore is not recommended to women desiring pregnancy; and (ii) surgery that could cause additional damage to an already compromised ovarian function (Garcia-Velasco and Somigliana, 2009; de Ziegler et al., 2010).

Pigment epithelium-derived factor (PEDF) is a 50 kDa secreted glycoprotein that, among others, possesses a potent anti-angiogenic activity (Dawson et al., 1999) by negating several strong pro-angiogenic factors, such as VEGF in various tissues (Kanda et al., 2005; Cai et al., 2006). The anti-angiogenic effect of PEDF was extensively investigated in the eye and in several tumors, demonstrating its role in decreasing abnormal angiogenesis (Bell, 2011; Manalo et al., 2011).

We have previously characterized the regulation of PEDF in the ovary (Chuderland et al., 2013). We found that PEDF is secreted from the granulosa cells of rodents and humans and is a physiological negative regulator of ovarian angiogenesis. We further showed that gonadotrophins, as well as steroid hormones, regulate the secretion of PEDF.

Since endometriosis is crucially dependent on neovascularization, owing to high local levels of VEGF, and in light of previously observed reduction in PEDF levels in human serum, peritoneal fluid and ectopic endometrial lesions (Chen et al., 2011, 2012; Fu et al., 2012), we hypothesized that PEDF could serve as a physiological therapy for endometriosis. We demonstrate in the current study the physiological expression of endometrial PEDF throughout the estrous cycle and utilized its anti-angiogenic properties to alleviate endometriosis.

**Materials and Methods**

**Animals**

Wistar-derived female rats (5–6 weeks old; Harlan Laboratories, Jerusalem, Israel) were housed in the air-conditioned, light-controlled animal facilities of the Sackler Faculty of Medicine. Animal care was in accordance with institutional guidelines and was approved by the Institutional Animal Care and Use Committee.

**Induction of endometriosis**

Various methods of endometriosis induction in rodents have been described. We have surgically induced endometriosis using a modified method described by Rudzitis-Auth et al. (2012). Donor rats were injected subcutaneously for 3 consecutive days with 2 mg of 17-β-estradiol (Sigma Chemical Co., St Louis, MO, USA, in propandiol) to ensure a proliferative endometrium. Rats were sacrificed on the fourth day and their uterus were removed into a petri dish filled with sterile warm (37°C) Dulbecco’s modified Eagle’s medium (Biological Industries, Beit-Ha’emek, Israel). The uterine horns were slit open along their anti-mesenteric axis to expose the endometrium. Uterine fragments (4 mm in diameter, ~7 mg) were punched-out, using a biopsy punch (Acu-Punch®, Acuderm inc., Ft Lauderdale, FL, USA).

Recipient rats were anesthetized by an intraperitoneal (IP) injection of a mixture of ketamine hydrochloride (60 mg/kg; Kepro, The Netherlands).
and xylazine hydrochloric (10 mg/kg; VMD, Belgium). The abdominal cavity was opened through a midline incision and the pieces of uterine tissue were transplanted onto the inner surface of the abdominal wall by suture of monofilament non-absorbable 5-0 Prolene suture (Ethicon, USA), with their endometrial surfaces facing the lumen of the abdominal cavity. Abdominal layers were closed with a 3-0 black silk suture (Ethicon) and metal wound clips (Autoclips, 11 mm, Clay-Adams, USA) before the rats were allowed to recuperate. After surgery, rats were treated with intravenous (IV) injection of recombinant PEDF (rPEDF; 2 mg/kg) or with Tris. Rats were randomly divided into three groups: Group I—“control”, injected with the vehicle (Tris) every third day for the next 3 weeks; Group II—“PEDF treatment”, IV injected with rPEDF (2 mg/kg/day) every third day from 9 days after grafting until the end of 3 weeks; and Group III—“PEDF prevention”, IV injected with rPEDF (2 mg/kg/day) every third day for the next 3 weeks (Fig. 3A).

Reproductive parameters

Estrous cycle and ovulation

Estrous cycle and ovulation were monitored daily by vaginal smears. Ovulated, cumulus-enclosed oocytes arrested at metaphase of the second meiotic division (MII) were isolated at the morning of estrus from the oviductal ampullae into the M2 medium (Sigma). Cumulus cells were removed by a brief exposure to hyaluronidase (400 IU/ml; Sigma) and the number of ovulated oocytes was recorded.

Histological evaluation

Uterine fragments

The rats’ estrous cycle was monitored for at least two cycles, after which the uteri of rats at proestrus and metestrus II were excised, fixed in 4% formaldehyde, paraffinized, sectioned (7 μm) and stained with hematoxylin and eosin (H&E; Bio-optica, Milano, Italy).

Endometrial transplants

After endometriosis induction, rats were monitored and were weighed periodically; vaginal smears were taken daily. Rats were euthanized at the end of the third week following the operation, on the day of estrus. The endometrial transplants were excised, measured (size), weighed, fixed and processed as the uterine fragments. Sections were visualized and photographed by a Leica laser confocal microscope (SP5 Wetzlar, Germany).

Immunohistochemistry

Paraffin-embedded sections of rat endometrial samples were deparaffinized, microwave heated while being subjected to an antigen retrieval agent (H-3300, Vector Laboratories Inc., Burlingame, CA, USA). Sections were then cooled on ice to room temperature, rinsed in PBS, incubated for 1 h with PBSTg (0.2% Tween and gelatin in PBS), washed with PBS, blocked for 10 min in blocking solution (Cell Marque Corporation, CA, USA) and incubated overnight with anti-PEDF antibody. At the following day, sections were washed in PBSTg and PBS before and after applying the appropriate secondary antibody (rabbit Alexa Flour488-conjugated antibody), together with a nuclear marker (Hoechst 3342), rinsed, mounted with moviol (Sigma), visualized and photographed by a Leica laser confocal microscope (SP5 Wetzlar, Germany).

PEDF production

Human rPEDF (NM_002615.4) was expressed in Escherichia coli BL21. Bacteria, allowed to grow at 30°C to OD600nm of 0.5–0.6, were induced for 4–5 h by isopropyl-1-thio-β-D-galactopyranoside (0.5 mmol/l; Sigma), centrifuged and their pellets were lysed. Recombinant protein was purified by ion metal affinity chromatography with Ni-NTA His-Bind resin (Merek KGaA, Darmstadt, Germany) according to the manufacturer’s protocol. Proteins of the eluted fractions were resolved by SDS–PAGE, dyed with GelCode (Blue Stain Reagent, Thermo scientific, IL, USA) or western blotted with a specific anti-PEDF antibody. Elutes with >90% purity were dialyzed against Tris buffer pH 10.

RNA isolation, reverse transcription, PCR and real-time polymerase chain reaction (qPCR)

Total RNA was isolated from endometrium (scraped from the dissected uterus) endometrial transplants using Trizol reagent (Invitrogen, Grand Island, NY, USA), according to manufacturer’s instructions, and quantified with the Nano-Drop spectrophotometer (ND-1000; Thermo Scientific). First-strand cDNA was created by reverse transcription (Maxima™ Reverse transcriptase, Fermentas, MA, USA) from a total of 1 μg RNA, using oligo-dt primers to a total of 20 μl mixture (Fermentas). Changes in the level of expression of mRNA were detected by SYBR green reagent (SYBR® Green PCR Master Mix, ABI, Carlsbad, CA, USA) along with 0.3 μl of the cDNA and specific primers, on an ABI Prism 7900 Sequence PCR machine (Applied Biosystems, Foster City, CA, USA).

Primers for qPCR

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<tr>
<th>HPRT1</th>
<th>VEGF</th>
<th>PEDF</th>
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<tr>
<td>Rat Forward: 5′-CTCATGGACTGATATTAGGACAGGA-3′</td>
<td>Reverse: 5′-GCAGGTCAAGAAAAGATTATAGGC-3′</td>
<td>Forward: 5′-TTCACCAGGAGCAGTGAT-3′</td>
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<tr>
<td></td>
<td></td>
<td>Reverse: 5′-GCCAGGAGCAGTGAT-3′</td>
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Statistics

Results are expressed as mean ± standard error (SEM); evaluated by either functional analysis two-ways analysis of variance (ANOVA), followed by the Tukey post hoc test, ANOVA with repeated measured or Student’s t-test (two-tailed); P < 0.05 was considered statistically significant.

Results

PEDF expression in rat endometrium during the estrous cycle

The angiogenic changes that the endometrium undergoes mandate a balance between pro and anti angiogenic factors; we therefore aimed to study the expression pattern of the physiological anti-angiogenic factor, PEDF. Immunostaining of the rat endometrium indicated that PEDF is expressed in the stroma and more abundantly in the glands (Fig. 1A). The same pattern of expression was found in the human endometrium (unpublished results). The rat estrus cycle, affected by ovarian hormones, consists of four stages: pro-estrus, estrus, metestrus I and metestrus II, during which the endometrium exhibits morphological changes including generation and destructions of endometrial tissue and of blood vessels (Westwood, 2008).

Previous work performed in our laboratory revealed that PEDF expression within the granulosa is dependent on estrogen and progesterone (Chuderland et al., 2013), which led us to hypothesize that
endometrial PEDF is dynamically expressed during the estrous cycle as well. To test this assumption, we compared staining intensity of PEDF in endometrial samples taken from rats at the pro-estrus and metestrus II stages of the estrous cycle. Sections were labeled with anti-PEDF antibody (green, sc-25594) and Hoechst 3342 (blue) as a nuclear marker. Labeled sections were visualized and photographed by a Leica Laser confocal microscope. (B) Fluorescence quantification of the anti-PEDF antibody calculated by the ImageJ analysis tools. Bars are mean ± SEM, presenting fluorescence intensity of endometrium sections taken at the pro-estrus (n = 5) and at metestrus II (n = 5). *Significantly different from pro-estrus values (P < 0.05; t-test).

Given that VEGF is the main pro-angiogenic factor in the uterus (Girling and Rogers, 2005) and since VEGF and PEDF were shown to be inversely regulated in the ovary (Chuderland et al., 2013) as well as in other organs (Cai et al., 2006), we assumed that endometrial PEDF and VEGF are also inversely expressed during the estrous cycle, thus coordinating endometrial angiogenesis (Mahesh and Muldoon, 1987). We tested this assumption by examining the expression level of VEGF mRNA (Fig. 2B) in the same endometrial samples used for monitoring the expression level of PEDF mRNA (Fig. 2A) and found that they were indeed inversely expressed. Namely, VEGF mRNA was highly expressed at the estrous stage concomitantly with low expression level of PEDF mRNA, and vice versa during the metestrus II stage (Fig. 2). When evaluating the association between VEGF and PEDF expression during the estrous cycle using ANOVA with repeated measured, we found a significant difference in the genes, depending on the day of the estrous cycle (P < 0.001). Collectively, the dynamics of PEDF expression in the rat endometrium portrays a mirror-like image of the dynamics of VEGF expression.
In order to evaluate the therapeutic properties of rPEDF, we tested its ability to affect the weight of the developing transplants when administered immediately after grafting (prevention) or when administered 9 days later when endometrial lesions were already established (treatment; data not shown). Rats were randomly divided into three groups: Group I—‘control’, injected with the vehicle (Tris; n = 7) every third day for the next 3 weeks; Group II—‘PEDF treatment’, IV injected with rPEDF (2 mg/kg/day; n = 6) every third day from Day 9 after grafting until the end of 3 weeks; and Group III—‘PEDF prevention’, IV injected with rPEDF (2 mg/kg/day; n = 7) every third day for the next 3 weeks (Fig. 3A). Throughout the entire experimental period after the surgery, all rats were healthy and active; they showed no signs of abdominal swelling or hair loss and no abnormal wound healing. No significant difference in body weight among rats at the various treatment groups was detected throughout the experimental period. Rats were euthanized on the third week after the endometriosis-induction surgery (between Days 21 and 24, on the day of pro-estrus) and the endometrial transplants were examined. The endometrial transplants of Group I grew to an average weight of 23 mg. Following rPEDF treatment, the average weight of Group II transplants was only 3 mg, whereas that of Group III was even lower, almost completely eradicated (1.7 mg; P < 0.001; Fig. 3B–D).

Histology of transplants excised from the control group revealed cyst-like dilated structures with glandular epithelium surrounded by endometrial stroma (Fig. 4A and D). However, after rPEDF treatment, these glandular epithelial structures could not be observed; only randomly distributed epithelial cells were detected (Fig. 4B, C, E and F). Finally, we did not observe a change in the structure of the endometrial glands or stroma as reflected by the histological sections of uteri excised from rats of all treatment groups (Fig. 4G–I).

Taken together, rPEDF treatment inhibited proliferation of the endometrial transplants in the sequential cycles and caused diminution of endometrial remnants.

rPEDF reduces VEGF mRNA level

A well-established endometriosis hallmark is an increase in vasculature caused by elevated local levels of VEGF (McLaren et al., 1996). Since PEDF is a known physiological anti-angiogenic factor that acts, at least in part, through direct negative effect on VEGF, we examined whether rPEDF reduces the size and weight of endometrial transplants by inhibiting the local level of VEGF mRNA. We subjected transplants,
isolated from all three experimental groups, to qPCR analysis and found that rPEDF administration (Groups II and III) reduced the expression level of VEGF mRNA in endometrial transplants, compared with its level in transplants of control rats ($P < 0.05$, Group III; Fig. 5).

**Effect of PEDF treatment on ovulation and the estrous cycle**

Endometriosis is associated with reduced ovarian response to gonadotrophin stimulation, fewer mature oocytes and compromised oocytes’ quality (Papaleo et al., 2011). We had thus evaluated the effect of endometriosis in our model system on the length of the estrous cycle and on ovulation outcome. For that, rats were randomly divided into four groups: (a) naive rats ($n = 6$); (b) rPEDF-treated rats ($n = 5$); (c) endometriosis-induced rats ($n = 5$); and (d) endometriosis + rPEDF ($n = 6$). Rats were injected with either Tris or rPEDF every third day for 21 days (Fig. 6A). Induction of endometriosis induced prolongation of the estrous cycle and reduction in the number of ovulated oocyte. Administration of rPEDF to naive rats (not induced for endometriosis) showed no substantial effect (Fig. 6B). However, a significant higher number of ovulated oocytes was recorded at the endometriosis-induced group treated with rPEDF (c) when compared with the endometriosis group treated with Tris [(d); Fig. 6B; $P < 0.05$].

**Discussion**

Under physiological conditions, angiogenesis is tightly regulated by pro- and anti-angiogenic signaling molecules. In the current study, we found that PEDF is expressed in the rat endometrium, and showed that the pro-angiogenic factor VEGF and the anti-angiogenic factor PEDF are reciprocally expressed in it throughout the estrous cycle. The PEDF peak was detected at the day of metestrus II and VEGF at the day of estrus. These observations are compatible with the vasculature fate during the estrous cycle. At metestrus II, the uterus vasculature is relatively reduced; whereas at pro-estrus and estrus, the endometrial vasculature becomes more prominent, and the stroma may show some edema (Westwood, 2008).

The growth and survival of endometrial lesions is attributed to neo-vascularization that is manifested by the presence of immature blood vessels. This phenomenon is significantly more prominent (more than 80%) in the endometrial lesions than in the eutopic endometrium.
This neovascularization is regulated by VEGF, and thus, it is the rationale behind using selective inhibitors of VEGF as an endometriosis treatment (Benjamin et al., 1998, 1999). It was reported that the use of anti-VEGF antibody induced a statistically significant reduction in cell proliferation within endometrial lesions of endometriosis-induced mice model; it increased apoptotic index, reduced vascular density and lesions’ size (Bruner-Tran et al., 2009; Ricci et al., 2011). The ability of anti-VEGF antibody to reduce the lesions’ size was independent of administration time; either immediately after the grafting (Hull et al., 2003; Bruner-Tran et al., 2009) or 2–3 weeks after endometrial lesions had already been established (Nap et al., 2004; Global Industry Analysts, Inc., 2011; Ricci et al., 2011). However, most of the available VEGF blockers are not offered in the clinic since they are associated with severe systemic side effects, among other impairments to reproductive parameters (Papaleo et al., 2011). Therefore, the search for molecules with high VEGF-specificity is of utmost importance for treating non-life-threatening conditions such as endometriosis.

Herein, we showed that IV administration of rPEDF, either as a preventive agent or as a treatment, virtually eradicated the endometrial transplants (90 or 80% weight decrease, respectively; P < 0.001). Interestingly, the histology of the transplants’ remnants showed a complete loss of the characteristic endometrial morphology. We have

**Figure 5** Reduced levels of VEGF mRNA in endometrial transplants after treatment with rPEDF. qPCR analysis of endometrial transplant excised 3 weeks after surgical transplantation with specific primers for VEGF, calibrated by the endogenous control HPRT1. Rats with transplanted endometrial fragments were injected IV every third day for 3 weeks after surgery: rats in Group I (control; n = 7) with vehicle (Tris); rats in Group II (treatment; n = 6) with human recombinant 8his-PEDF (2 mg/kg/injection) only from Day 9 after surgery onwards; rats in Group III (prevention; n = 7) with human recombinant 8his-PEDF (2 mg/kg/injection). Bars are the level of VEGF mRNA in the endometrial transplants, presented in arbitrary units (mean ± SEM). *Significantly different from control value (P < 0.05; ANOVA).

**Figure 6** The effect of rPEDF on reproductive parameters. (A) Schematic representation of the experimental setting. (a) Control-naive rats were injected IV with Tris every third day for 3 weeks (control; n = 6). (b) rPEDF-naive rats were injected IV with human recombinant 8his-PEDF (2 mg/kg/injection) every third day for 3 weeks (rPEDF; n = 5). (c and d) Endometriosis—rats with transplanted endometrial fragments were injected IV every third day for 3 weeks with either Tris (c; Endometriosis; n = 5), or rPEDF (d; Endometriosis + rPEDF; n = 6). (B) We have evaluated the length of the estrous cycle indicated by daily vaginal smears and the number of ovulated oocytes at the end of the third week. P < 0.05—Significant difference between the values of group (c) and (d) (t-test).
further showed that rPEDF induced a significant reduction in the level of VEGF mRNA within the endometrial transplants, suggesting that one of the PEDF-related mechanisms of action could be attributed to inhibition of VEGF-dependent angiogenesis.

A delicate balance of pro- and anti-angiogenic factors is mandatory for regulation of the cyclic formation of blood vessels in the endometrium (Smith, 1998). Recently, we have found that granulosa cells express and secrete PEDF in an inversed manner to VEGF. We have also found that disturbance of the delicate balance between VEGF and PEDF lies at the core of ovarian hyperstimulation syndrome (unpublished data). Therefore, we assumed that VEGF–PEDF counter-balance may regulate angiogenesis in the normal endometrium, and impairment of this balance may contribute to the onset of endometriosis. Moreover, it was recently reported (Fu et al., 2012) that endometrial lesions in rats model, contained low levels of PEDF; whereas no changes in PEDF level were detected in the uterine endometrium (Smith, 1998). Recently, we have found that granulosa cells express and secrete PEDF in an inversed manner to VEGF. We have also found that disturbance of the delicate balance between VEGF and PEDF lies at the core of ovarian hyperstimulation syndrome (unpublished data). Therefore, we assumed that VEGF–PEDF counter-balance may regulate angiogenesis in the normal endometrium, and impairment of this balance may contribute to the onset of endometriosis. Moreover, it was recently reported (Fu et al., 2012) that endometrial lesions in rats model, contained low levels of PEDF; whereas no changes in PEDF level were detected in the uterine endometrium. These observation, along with previous studies that report low levels of PEDF in the serum (Chen et al., 2012) and peritoneal fluids of women suffering from endometriosis (Chen et al., 2011) may suggest that low PEDF level is attributed to endometriosis induction and survival of endometrial lesions. We therefore suggest that administering rPEDF may actually restore PEDF to its normal level and thus the development of side effects may be avoided, though to date, there is no evidence of rPEDF being used in humans and a comprehensive study is required to evaluate its potential toxicity.

Ovulation is impaired in women suffering from endometriosis, with fewer follicles in the ovaries and lower ovulation rate (Papaleo et al., 2011). To date, the impact of endometriosis per se on the number of oocytes available for fertilization and on their quality is highly debated (Barcelos et al., 2009). A more extensive research on PEDF function in the reproductive system may clarify the increase in the number of ovulated oocytes we observed in endometriosis-induced rats following rPEDF treatment. Nevertheless, in our model system, we did not detect any compromising in reproductive parameters.

In conclusion, we characterized the expression of PEDF in the rat uterus and found it to be regulated reciprocally to VEGF. We have further demonstrated an endometriosis therapeutic-potential of PEDF that is derived, at least in part, from its anti-angiogenic properties of VEGF inhibition. Published data regarding changes in PEDF level in cases of endometriosis, together with our observations that demonstrate the therapeutic potential of PEDF, lead us to suggest a new physiological approach for treating endometriosis.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors’ roles
D.C. and I.B.-A. developed the concept, designed experiments and prepared the manuscript. D.C. and N.H. carried out most of the experiments, data organization and statistical analyses. R.K.-K. and H.G. helped with animal heading. D.C. wrote the manuscript. R.K.-K. helped drafting the manuscript. R.S. conceived the study, participated in its design and coordination, helped drafting the manuscript and supervised the study. All authors read and approved the final manuscript.

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Conflict of interest

References


