Endometriosis and human infertility: a new investigation into the role of eutopic endometrium

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BACKGROUND: Endometriosis is related to infertility even in the absence of mechanical alterations of the reproductive tract. Even though the pathogenesis of this phenomenon is still unclear, an impaired endometrial receptivity has been recently suggested. The aim of the present study was to investigate if endometriotic peritoneal fluids (EPF) could interfere with endometrial stromal cell (ESC) decidualization and if tumor necrosis factor (TNF)-α could be involved in the EPF effect.

METHODS: Eutopic ESC were isolated from patients with or without endometriosis. ESC were treated with 17β-estradiol 10⁻⁸ M and 6a-methyl-17α-hydroxyprogesteroneacetate 2×10⁻⁷ M for 16 days. In vitro decidualization was morphologically and biochemically assessed. We analysed whether ESC decidualization could be affected by EPF or peritoneal fluids from control patients (CPF), with or without soluble TNF-α receptor 1 (sTNFR-1).

RESULTS: Compared with ESC from control patients, eutopic ESC from patients with endometriosis showed an impaired decidualization. Decidualization of normal ESC was morphologically normal but biochemically abnormal in the presence of EPF, which was able to decrease the secretion of decidualization markers. sTNFR-1 was able to partially counteract this effect.

CONCLUSIONS: In endometriosis, the milieu surrounding the uterine cavity may be involved in impaired eutopic ESC decidualization, partially due to increased peritoneal levels of TNF-α.

Keywords: endometriosis; endometrial receptivity; decidualization

Introduction

Endometriosis is a benign estrogen-dependent gynecological disease, which affects ~10% of women of reproductive age, is characterized by the presence of endometrial tissue outside the uterine cavity, and is associated with pelvic pain, dysmenorrhea and infertility (Ulukus et al., 2006). Despite all the research accumulated over the years, the etiology and the pathogenesis of endometriosis are still unclear.

One of the most controversial aspects investigated by several studies is the link between minimal to mild endometriosis and infertility, particularly in patients with no mechanical alteration of the reproductive tract (D’Hooghe et al., 2003). Whether the endometriosis-related reduction of fertility is due to suboptimal oocyte/embryo quality or to a compromised endometrium remains a controversial issue (Kim et al., 2007). Several studies have shown that gene expression in the endometrium of women with endometriosis is aberrant, making the endometrium suboptimal for an implanting blastocyst (Giudice et al., 2002; Kao et al., 2003). Very recently, a role for eutopic endometrium in endometriosis-related infertility has been also suggested by Klemmt et al. (2006), who reported a reduced decidualization capacity in endometrium from women with endometriosis. Nevertheless, other authors did not confirm this observation (Kim et al., 2007).

Since decidualization is a critical process for the successful establishment and maintenance of pregnancy, we wanted to further investigate whether eutopic endometrial stromal cells (ESC) obtained from women affected by endometriosis (eutopic endometriotic ESC) have a normal capacity to differentiate in vitro to the decidual phenotype in presence of hormonal stimuli. In vivo decidualization is a complex differentiative process characterized by morphological and functional changes under the influence of progesterone during the secretory phase of the menstrual cycle.

In particular, in the present study we compared the decidualization performance of eutopic endometriotic ESC with ESC obtained from healthy subjects (normal ESC). Typical morphological changes were assessed and the levels of two important
biochemical decidualization markers were measured in the culture media: prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1) (Bell et al., 1991; Daly et al., 1983).

Beyond the intrinsically altered properties of eutopic endometriotic ESC, the suggested impaired receptivity may be due to a negative influence exerted by the uterine cavity milieu. In order to address this issue, normal ESC were incubated with peritoneal fluids from endometriotic patients (EPF) or from control subjects (CPF), and decidualization was morphologically and biochemically assessed. In fact, the endometrial cavity milieu is supposed to be compromised by peritoneal fluid diffusion through the tubes (Harada et al., 2001) and similar immunological activation and hormonal conditions have been observed in both the endometrial and peritoneal cavities (Selam and Arici, 2000). Moreover, EPF is characterized by high levels of cytokines, growth factors and adhesion molecules which have been related to infertility (Arumugam, 1994; Curtis et al., 1993; Tummon et al., 1988). In particular, tumor necrosis factor (TNF)-α is secreted at increased amounts in EPF (Rana et al., 1996) and a clear positive association between its content in peritoneal fluids and the severity of endometriosis has been observed (Richter et al., 2005). In order to verify whether TNF-α could be responsible for EPF effects, decidualization of normal ESC was performed in presence of peritoneal fluids with or without the soluble form of TNF-α receptor-1 (sTNFR1), a TNF-α inhibitor.

Materials and Methods

Chemicals

Collagenase type IA, penicillin-streptomycin solution, 17β-estradiol (E₂), 6α-methyl-17α-hydroxyprogesteroneacetate (MAP) and sTNFR-1 were purchased from Sigma Aldrich S.r.l. (Milano, Italy). Bradford reagent was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Dulbecco Modified Essential Medium (DMEM) and trypsin were from Invitrogen Inc. (Milano, Italy). Fetal bovine serum was from Euro Clone (Milano, Italy). PRL enzyme-linked immunosorbent assay (ELISA) kit was purchased from Radim SpA (Roma, Italy) and IGFBP-1 ELISA kit was from Diagnostic Biochem Canada Inc. (Ontario, Canada).

Samples

Participants (n = 58) were women of reproductive age (23–40 years) undergoing laparoscopy during the secretory phase of the menstrual cycle (19–24 days). All patients had a history of regular menstrual cycles and did not receive any hormonal preparation in the 3 months preceding laparoscopy. Women with endometrial pathology or with any hormonal alteration were not included. At the time of laparoscopy, pelvic organs were carefully examined for endometriosis, which was staged according to the American Society for Reproductive Medicine (1997).

Endometriosis was excluded in 25 patients. At the time of laparoscopy, these women underwent peritoneal fluid sampling (n = 10) or uterine curetting in order to obtain eutopic endometrial specimens (n = 15).

There were 33 patients affected by endometriosis (6 with stage I disease, 14 with stage II disease, 7 with stage III disease and 6 with stage IV disease). In the present study, only patients with stage I and II disease were included (minimal to mild endometriosis). At the time of laparoscopy, these women underwent peritoneal fluid sampling (n = 10) or uterine curetting in order to obtain eutopic endometrial specimens (n = 10).

Peritoneal fluids and endometrial biopsies were obtained from different cohorts of patients for either the normal or endometriotic groups.

Written informed consent was obtained from all participants and the present protocol was approved by the institutional review board of Università Cattolica del Sacro Cuore.

Peritoneal fluid preparation

Once collected, peritoneal fluids were centrifuged (1000 rpm, 10 min, 4°C), aliquoted and stored at −80°C (Rong et al., 2002). All peritoneal fluids, 10 from women with endometriotic (endometriotic peritoneal fluid, EPF) and 10 without endometriosis (CPF) were separately pooled shortly before cell culture treatments.

Isolation and primary culture of normal and eutopic endometriotic ESC

Menstrual secretory phase eutopic endometrium for each normal (n = 15) and endometriotic (n = 10) specimen was confirmed by histological criteria of Noyes et al. (1975).

The separation of ESC from endometrial epithelial cells was obtained following the method described by Satyaswaroop et al. (1979) with minor modifications (Pierro et al., 2001).

Purified ESC were suspended in DMEM (supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/ml penicillin-streptomycin), plated into 25 cm² tissue culture flasks and cultured until confluence was reached (37°C, 5% CO₂).

Cell viability was assessed by Trypan Blue exclusion (~85.8%).

Once grown to confluence (2–3 days later), both normal and endometriotic ESC were detached by using trypsin treatment, seeded into 24-well plates (10⁵ cells per well) and used for the in vitro decidualization experiments described below.

In vitro decidualization of normal and eutopic endometriotic ESC

Either normal or eutopic endometriotic ESC were seeded in 24-well plates, grown until confluence (1–2 days) and treated with E₂ 10⁻⁸ M and MAP 2 × 10⁻⁷ M for 16 days in order to obtain in vitro decidualization as previously described (Han et al., 1999; Irwin et al., 1989; Kasahara et al., 2001). Each culture of ESC was carried out in triplicate. On day 16, cells were counted in order to assess cell viability.

In order to confirm decidual transformation of ESC, morphological characteristics of decidualization were checked by inverted microscope (Axiovert 200, Carl Zeiss S.p.A.) in all cell groups (Cornillie et al., 1985; Wynn, 1974). Biochemical markers of ESC differentiation were also assessed (Bell et al., 1991; Daly et al., 1983). To this aim, duplicate samples of culture media from all groups of ESC were assayed on days 7, 10, 13 and 16. Commercially available ELISA was used to measure levels of either PRL (inter- and intra-assay coefficients of variation below 6.99 and 8.24%, respectively, sensitivity of the assay 1.5 ng/ml) or IGFBP-1 (inter- and intra-assay coefficient of variation below 3.4 and 7.4%, respectively; sensitivity of the assay 0.5 μg/l) in the culture media according to the manufacturer’s instructions.

PRL and IGFBP-1 levels were normalized to the amount of total proteins assessed by Bradford reagent in each culture medium.

In vitro decidualization of normal ESC in presence of CPF and EPF

Normal ESC seeded in 24-well plates were grown until confluence (1–2 days).
In order to evaluate the effect of CPF and EPF on decidualization, a first group of ESC was treated with E2 $10^{-8}$ M and MAP $2 \times 10^{-7}$ M for 16 days as described in the previous paragraph (BASAL group). Similarly, a second and a third group of ESC were cultured for 16 days in presence of E2 and MAP together with 10% (Rong et al., 2002) of CPF or EPF, respectively (CPF and EPF groups). Finally, a fourth group of ESC was cultured for 16 days without E2 and P (CONTROL group). Each group of ESC was carried out in triplicate. On day 16, cells were counted in order to assess cell viability. Each culture from an individual donor was tested simultaneously for sensitivity to the CONTROL, BASAL, CPF or EPF treatments.

As described in the previous paragraph, all groups of ESC were morphologically and biochemically (PRL and IGFBP-1 levels) characterized in order to assess decidual transformation. PRL and IGFBP-1 levels were normalized to the amount of total proteins.

Prior to cell treatment, EPF and CPF were assayed for IGFBP-1 and PRL content.

**In vitro decidualization of normal ESC in presence of sTNFR-1**

In order to evaluate the effect of sTNFR-1 on stromal decidualization, each group of normal ESC from the same donor was cultured in presence of sTNFR-1 ($1 \text{ ng/ml}$). The concentration of sTNFR-1 was chosen in order to largely exceed TNF content of both CPF and EPF (Richter et al., 2005). sTNFR1 was dissolved in PBS with 0.1% BSA and the solvent was tested on ESC as a negative control.

On day 16, at the end of the decidualization protocol, cell viability was checked and culture media from all groups of ESC were collected in order to assess total proteins, PRL and IGFBP-1 levels as previously described.

**Statistical analyses**

Statistical analysis was performed using ANOVA followed by the ‘Tukey–Kramer’ test for comparisons of multiple groups or by paired Student’s *t*-test for comparison of data derived from two groups. Values with *P* < 0.05 were considered statistically significant.

**Results**

**In vitro decidualization of normal ESC**

As expected, the incubation of normal ESC with E2 $10^{-8}$ M and MAP $2 \times 10^{-7}$ M for 16 days (BASAL group) was able to induce stromal differentiation. In particular, in the presence of E2 and MAP, ESC were transformed into large polygonal cells resembling *in vivo* decidual cells, as previously described (Kasahara et al., 2001).

Moreover, ESC decidualization was evaluated by biochemical markers assays. Fig. 1 shows PRL (panel A) and IGFBP-1 (panel B) secretion in the BASAL group of normal ESC on days 7, 10, 13 and 16 of treatment with E2 and MAP (*in vitro decidualization*)

PRL and IGFBP-1 are expressed in ng/mg total protein. Each value represents the mean $\pm$ SEM of 15 independent experiments.

*

***$P$ < 0.001 respect to day 7; $0.001 < P < 0.001$ respect to day 10; $++ P < 0.001$ respect to day 13

Figure 1: Time dependent secretion of PRL (panel A) and IGFBP-1 (panel B) in BASAL group of normal ESC on days 7, 10, 13 and 16 of treatment with E2 and MAP (*in vitro decidualization*)

**In vitro decidualization of eutopic endometriotic ESC**

We wanted to test whether eutopic endometriotic ESC retain the capacity for differentiation in response to E2 $10^{-8}$ M and MAP $2 \times 10^{-7}$ M for 16 days as assessed by morphology and measurement of PRL and IGFBP-1 secretion.

As previously demonstrated by Klemmt et al. (2006) in response to 8-Br-cAMP, from day 7 onward eutopic endometriotic ESC treated with E2 $10^{-8}$ M and MAP $2 \times 10^{-7}$ M were able to differentiate into polygonal decidual cells, and IGFBP-1 secretion by eutopic endometriotic ESC was significantly lower than from normal ESC (Fig. 2B).

Eutopic endometriotic ESC secreted significantly lower levels of PRL on days 7, 10 and 13 with respect to normal ESC, however from day 16, eutopic endometriotic ESC were still able to secrete higher levels of PRL with respect to normal ESC (Fig. 2A).

PRL and IGFBP-1 values were normalized to the total proteins from cultured cells. At day 7 the mean value was $2.3 \pm 0.4$ mg, and in the following time points the total protein amount didn’t change in a statistically significant manner (data not shown).
IGFBP-1 and PRL content in EPF and CPF. The levels of significant manner. (Fig. 4C and D) release with respect to the CPF group in a significantly decreased only on day 16 by CPF with respect to the BASAL group (Fig. 3D). IGFBP-1 release by stromal differentiating cells, normal ESC independent experiments. ***P < 0.001, **P < 0.01 and *P < 0.05 respect to normal ESC

Effects of CPF and EPF on PRL and IGFBP-1 release during normal ESC decidualization

In order to assess the effects of CPF and EPF on PRL and IGFBP-1 release by stromal differentiating cells, normal ESC were cultured for 16 days in the presence of E2 and MAP (in vitro decidualization). PRL and IGFBP-1 are expressed in ng/mg total protein. Each value represents the mean ± SEM of 15 (normal ESC) or 10 (endometriotic ESC) independent experiments. With this aim on days 7, 10, 13 and 16, PRL and IGFBP-1 were assayed in the culture media of both groups.

On culture day 7, no effect was observed on PRL and IGFBP-1 release for either the CPF or EPF groups with respect to BASAL group (Figs. 3A and 4A).

We demonstrated that CPF was able to significantly reduce IGFBP-1 levels respect to the BASAL group from day 10 onward (Fig. 4B—D). On the contrary, PRL levels were significantly decreased only on day 16 by CPF with respect to the BASAL group (Fig. 3D).

Our results show the ability of EPF to significantly reduce PRL and IGFBP-1 levels with respect to the BASAL group on day 10 (Figs. 3B and 4B), on day 13 (Figs. 3C and 4C) and on day 16 (Figs. 3D and 4D). Moreover, on days 13 and 16, EPF was able to reduce PRL (Fig. 3C and D) or IGFBP-1 (Fig. 4C and D) release with respect to the CPF group in a significant manner.

Preliminary assays were performed in order to assess IGFBP-1 and PRL content in EPF and CPF. The levels of both IGFBP-1 and PRL were under the lower detection limit of the assay in both CPF and EPF.

During in vitro decidualization, normal ESC underwent the typical morphological modifications in both the CPF and EPF groups, despite the above mentioned differences in secretion of biochemical markers.

Effect of CPF and EPF on PRL and IGFBP-1 release during normal ESC decidualization in the presence of sTNFR-1

In order to evaluate the effect of sTNFR-1 on stromal decidualization, sTNFR-1 1 ng/ml was added to the culture media of normal ESC belonging to the BASAL, CPF and EPF groups. At the end of the differentiation protocol (day 16), PRL and IGFBP-1 were assayed in the culture media.

No difference in PRL or IGFBP-1 release was found when sTNFR-1 was added to the BASAL group of ESC (data not shown).

Similarly, no difference in IGFBP-1 release was found when sTNFR-1 was added to the CPF and EPF groups of ESC (data not shown).

On the contrary, sTNFR-1 was able to counteract EPF effects on PRL release with respect to BASAL group (Fig. 5A and B). The addition of sTNFR-1 was able to significantly modify the inhibitory effect on PRL release previously described for the EPF group. No significant effect was observed on CPF-treated cells.

Discussion

Decidualization is a complex differentiative process characterized by morphological and functional changes under the influence of progesterone during the secretory phase of the menstrual cycle (Hess et al., 2007). It is well known that decidualization is critical for the successful establishment and maintenance of pregnancy (Kim et al., 2007). Very recently, a reduced decidualization capacity has been reported in endometrium from women with endometriosis (Klemmt et al., 2006). Kim et al. (2007) did not confirm this observation, invoking differences in experimental design as possible explanations for the conflicting results. Klemmt et al. elicited in vitro decidualization using 8-Br-cAMP alone without including progesterone, i.e. known to be critical in the decidualization process. Indeed, the use of cAMP alone has been described as being able to ‘prime’ cells for decidualization, but unable to up-regulate all the genes required for optimal differentiation without progesterone cooperation (Brosens and Gellersen, 2006; Kim et al., 2007). Moreover, endometrial cells used in the Klemmt study were obtained randomly at different stages of the menstrual cycle (Kim et al., 2007).

In order to contribute to debate on impaired decidualization in endometriosis, we compared in vitro decidualization of eutopic endometriotic ESC with normal ESC by using E2 and MAP as previously reported (Han et al., 1999; Irwin et al., 1989; Kasahara et al., 2001). Either normal or eutopic endometriotic ESC were obtained exclusively from women during the secretory phase of the menstrual cycle, when cells are appropriately primed to respond to a...
Figure 3: Effect of CPF and EPF on PRL released by decidualizing normal ESC on day 7 (panel A), on day 10 (panel B), on day 13 (panel C) and on day 16 (panel D)

Results are expressed as percentage of PRL released by a BASAL group set equal to 100. Each value represents the mean ± SEM of 15 independent experiments. ***P < 0.001 and **P < 0.01 respect to BASAL; *P < 0.01 respect to CPF.

Figure 4: Effect of CPF and EPF on IGFBP-1 release by decidualizing normal ESC on day 7 (panel A), on day 10 (panel B), on day 13 (panel C) and on day 16 (panel D)

Results are expressed as percentage of IGFBP-1 released by a BASAL group set equal to 100. Each value represents the mean ± SEM of 15 independent experiments. ***P < 0.001 respect to BASAL; *P < 0.05 respect to CPF.
progesterone and E₂ receptors has been previously described
dysregulation in the response of eutopic endometriotic ESC
differentiate when they are rescued from negative environ-
ably reflecting the ability of eutopic endometriotic ESC to
endometriosis (Attia et al., 2007).

Kim et al., 2007). Our results
demonstrated a reduced decidualization capacity for
eutopic endometriotic ESC, since PRL and IGFBP-1 levels
released were significantly lower with respect to normal
ESC starting from day 7 until day 13. These data seem to
suggest that the decidualization process of eutopic endome-
trium could be impaired in endometriosis, potentially affect-
ing endometrial receptivity, as previously suggested by
Klemmt et al. (2006).

However, contrary to Klemmt results, in the present study on
day 16 PRL levels were significantly higher in eutopic endome-
triotic cells than in normal ESC. The PRL increase on day 16
could suggest a sort of hyper-response to E₂ and MAP, prob-
ably reflecting the ability of eutopic endometriotic ESC to
differentiate when they are rescued from negative environ-
mental influences after several days of hormonal stimulation.
Alternatively, the PRL surge on day 16 could be due to a
dysregulation in the response of eutopic endometriotic ESC to
hormonal stimuli. Indeed, an aberrant distribution of
progesterone and E₂ receptors has been previously described
in eutopic endometrial tissue obtained by patients affected by
endometriosis (Attia et al., 2000; Bulun et al., 2006; Jackson
et al., 2007).

Interestingly, our results demonstrated that IGFBP-1
secretion by eutopic endometriotic ESC was significantly
lower than from normal ESC even on day 16, when PRL
levels were higher than from normal ESC. We can hypothesize
that in eutopic endometriotic ESC, the amount of PRL secreted
by cells after 16 days could autocrinally determine the lack of
IGFBP-1 surge. Very recently, Eyal et al. (2007) demonstrated
an autocrine mechanism by which PRL could regulate the
extent of differentiation in human uterine fibroblast cells by
negatively affecting the expression of IGFBP-1, as well as of
other genes known to be induced in decidualization.

On the basis of these in vitro results, it is not possible to con-
clude whether the impaired decidualization of eutopic endome-
triotic ESC reflects a negative environmental influence or an
intrinsic cell dysregulation. In order to investigate whether
endometriotic milieu surrounding the uterine cavity could
affect ESC differentiation, we compared the effects of EPF
to CPF on normal ESC decidualization.

We demonstrated that EPF, but not CPF, can negatively
affect decidualization of normal ESC by decreasing PRL
secretion compared with basal decidualization (BASAL
group). Interestingly, IGFBP-1 release was strongly decreased
by both EPF and CPF starting from day 10, even though on day
13 and 16 EPF exerted a higher negative effect on IGFBP-1
than CPF did. We can hypothesize that peritoneal fluids could
interfere with IGFBP-1 determination by affecting post-
secretion IGFBP-1 levels. Indeed, it is well known that either
urokinase-type plasminogen activator (uPA) or metalloprotei-
nases (MMPs) are able to proteolyze IGFBPs and that both
of them are components of peritoneal fluids (Coppock et al.,
2004; Lembessis et al., 2003). Whether or not this IGFBP-1
proteolytic activity is increased in endometriosis is highly
controversial in the literature (Gilabert-Estelles et al., 2003;
Laudanski et al., 2005; Szamatowicz et al., 2002). Neverthe-
less, very recently, the peritoneal fluid from women with endo-
metriosis has been demonstrated to show a significant increase
in uPA and MMP-3 levels compared to that of controls
(Gilabert-Estelles et al., 2007). This last finding could
explain our results, either the low levels of IGFBP-1 in pre-
ence of both peritoneal fluids or the effect exerted by EPF
being stronger than that of CPF.

Despite the difference in biochemical markers secretion, in
our system eutopic endometriotic ESC as well as normal
ESC under all tested conditions (BASAL, CPF, EPF) under-
went the typical morphological modifications. This peculiarity
has been already demonstrated by previous papers, suggesting
that the endometrium from women with endometriosis displays
morphologically normal, but biochemically abnormal, deci-
dualization responses (Giudice et al., 2002; Klemmt et al.,
2006).

Finally, we tested whether sTNFR-1 could modify the effect
of EPF on normal ESC differentiation, since TNF-α is secreted
at increased amounts in EPF (Rana et al., 1996) and is able to
decrease PRL secretion from stromal cells during in vitro
decidualization (Inoue et al., 1994). Our data demonstrated
that the negative influence of EPF on PRL secretion was
limited by adding sTNFR-1, suggesting that TNF-α could be
involved in the effects exerted by EPF. These in vitro results

Figure 5: Effect of CPF and EPF on PRL release by decidualizing
normal ESC on day 16 in the presence (‘+sTNFR-1’) or absence
(‘−sTNFR-1’) of sTNFR-1

Results are expressed as percentage of PRL released by the BASAL
group set equal to 100. Each value represents the mean ± SEM of
15 independent experiments. **P < 0.001 and ***P < 0.01 respect to
BASAL; ‘−sTNFR-1’
may contribute to the current research on the possible therapeutic targets of TNF-R inhibitors on the treatment of endometriosis. Actually, promising animal research using TNF inhibitors provide evidence of the potential effectiveness of such an anti-inflammatory drug in the management of endometriosis and confirm the role of TNF in the development of this disease (D’Antonio et al., 2000; D’Hooghe, 2003).

Moreover, Braun et al. have demonstrated for endometrial cells obtained from patients affected by endometriosis a higher sensitivity to the effects of TNF than for endometrial cells from healthy subjects (Braun et al., 2002). We could speculate that the effects of EPF and sTNFR-1 on eutopic endometriotic ESC could be even stronger if we consider their intrinsically higher ability of utilize factors in peritoneal fluids, such as TNF. New experiments would be very useful to directly test the sensitivity to TNF of eutopic endometriotic ESC during decidual differentiation.

Surprisingly, sTNFR-1 was not able to affect IGFBP-1 secretion when added in combination with either CPF or EPF. We can hypothesize that any possible sTNFR-1 effect on IGFBP-1 levels could be masked by strong post-secretion IGFBP-1 proteolysis in presence of peritoneal fluids, as we previously hypothesized.

In conclusion, on the basis of our results, we can speculate that in endometriosis either intrinsic defects of ESC differentiation or the biochemical environment of the uterine cavity could concur to compromise the normal decidualization required for optimal implantation. In particular, a role for TNF-α is suggested by the capacity of sTNFR-1 to attenuate the inhibitory effects of EPF on PRL secretion by ESC.

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