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ORIGINAL ARTICLE

Nuclear factor-κB expression in the endometrium of normal and overweight women with polycystic ovary syndrome

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ABSTRACT

The aim of this study was to investigate whether the expression levels of endometrial NFκB p65 differ between normal weight and overweight PCOS women and to compare them with BMI-matched control subjects without PCOS. The study group comprised 20 normal weight (BMI: 18.5–24.9 kg/m^2) and 15 overweight PCOS women (BMI: 25–29.9 kg/m^2) with infertility. Healthy fertile women without PCOS were recruited as the control group. The patients in the normal weight PCOS group and control group were age and BMI-matched. Endometrial samples were obtained during the mid-luteal phase for immunohistochemical staining. The H-Score method was used to evaluate NF-κB p65 (Rel A) expression. Both normal and overweight PCOS women demonstrated significantly higher endometrial NF-κB p65 expression than the women without PCOS. The H-scores of endometrial NF-κB p65 expression were similar in both groups of PCOS women. NF-κB p65 was positively correlated with serum insulin, HOMA-IR and total testosterone levels in PCOS women. By leading to pathological inflammation, an increase in NF-κB p65 expression in the endometrium of normal and overweight PCOS women may contribute to PCOS-related subfertility.

KEYWORDS

PCOS; nuclear factor κB; endometrial inflammation

IMPACT STATEMENT

- **What is already known on this subject:** Although the pathogenesis of PCOS has not yet been clarified, low-grade chronic inflammation is gradually being established as an important pathogenetic factor. Increased levels of inflammatory cytokines such as IL-6 and TNF-α have been reported in women with PCOS. Causes of pathological endometrial inflammation may arise from either a local endometrial disease or linked to diseases which are located in a distant reproductive tissue. Nevertheless, possible role of endometrial NF-κB, basic cellular regulatory of inflammation, in the pathophysiology of PCOS related implantation defect has not been elucidated yet.
- **What do the results of this study add:** This study provides first and novel insights into the relationship between PCOS related infertility and pathological endometrial inflammation. We demonstrated that there is a close association between PCOS and pathological endometrial inflammation. Moreover, we clearly showed that pathological endometrial inflammation occurs in both normal and overweight women with PCOS. Further, endometrial NF-κB p65 (Rel A) expression were found to be positively correlated with serum insulin levels and hyperandrogenism in overweight PCOS women.
- **What are the implications of these findings for clinical practice:** If we can analyse pathological endometrial inflammation by measuring endometrial NF-κB p65 (Rel A) expression, treatment could be directed towards eliminating the source of pathological endometrial inflammation.

Introduction

Polycystic ovarian syndrome (PCOS)-related infertility is not only derived from chronic anovulation but implantation defects can also further complicate the process of becoming pregnant (Giudice 2006). Decreased fecundity rates and impaired endometrial receptivity have been reported in PCOS women with infertility (Donaghay and Lessey 2007). Although the presence of PCOS does not predict miscarriage, PCOS and hyperandrogenism may be related with repeated miscarriages (Tulppala et al. 1993). The endometrium of PCOS women has been reported to exhibit defective steroid hormone/steroid receptor expressions and resistance to progesterone (Apparao et al. 2002; Savaris et al. 2011). Although ovulation can be restored with different stimulation protocols, implantation rates have been reported to remain lower than those of fertile control subjects, together with increased early miscarriage rates (Sagle et al. 1988; Tulppala et al. 1993). Suboptimal progesterone action in PCOS women culminates in unopposed oestrogen action in the endometrium (Giudice 2006). Even in ovulatory PCOS women some receptivity markers, such as integrin and homeobox genes have been

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observed to be decreased (Apparao et al. 2002). Similarly, exogenous testosterone administration leads to a decline in endometrial HOXA10 expression, suggesting a role for androgen reduction in the endometrial receptivity of PCOS women (Cermik et al. 2003). Moreover, while the endometrium of PCOS women exhibits high androgen receptors, steroid receptor coactivators and transcriptional intermediary factor 2, PCOS endometrium fails to down-regulate oestrogen receptor-alpha expression (Apparao et al. 2002; Gregory et al. 2002).

Although the pathogenesis of PCOS in normal and overweight women has not yet been clarified, low-grade chronic inflammation is gradually being established as an important pathogenetic factor (Escobar-Morreale et al. 2011). PCOS is a subclinical inflammatory disease. Increased levels of inflammatory cytokines such as IL-6 and TNF-α have been reported in women with PCOS (Sirmans et al. 2012). The anti-inflammatory peptide, adiponectin, has also been reported to be down-regulated in women with PCOS (Toulis et al. 2009). Any inflammatory condition located in the abdomino-pelvic region may alter endometrial receptivity (Weiss et al. 2009). The condition leading to pathological endometrial inflammation may arise either from a local endometrial disease or secondary to disease located in distant reproductive tissue. For example, PCOS, hydrosalpinges and ovarian endometrioma alter the endometrial inflammation irrespective of their location (Daftary et al. 2007; Celik et al. 2013; Piltonen et al. 2013). Currently, the nuclear factor-κB (NF-κB), pathway, as a basic cellular regulator of inflammation, is the most studied process in the pathogenesis of reproductive disorders. Nevertheless, the role of the endometrial NF-κB pathway in the pathophysiology of PCOS-related implantation defects has not yet been elucidated. NFκB is one of the most important molecules regulating immunity and inflammation in many species (Renard and Raes 1999) and as such, this pathway has been indicated to be active in the normal endometrium of healthy women (Laird et al. 2000; King et al. 2001).

NF-κB comprises homodimers or heterodimers from five subunits: p50/p105 (NF-κB1), p52/p100 (NF-κB2), p65 (Rel A), c-Rel and RelB. NFκB dimers are located in the cell cytoplasm bound to its inhibitory protein IκBα, which blocks the nuclear translocation of NFκB. In response to an extracellular signal, the NFκB cascade is activated and IκBα is phosphorylated allowing the release of NF-κB (Renard and Raes 1999; Chen and Greene 2004) dimers capable of binding to DNA, which translocate to the nucleus and activate the transcription of several target genes (Karin et al. 2004; Hoffmann and Baltimore 2006). The aim of the present study was to investigate the expression levels of endometrial NF-κB p65, a celluar determinant of inflammation, in normal and overweight women with PCOS and compare these results with BMI and age-matched healthy participants without PCOS. Moreover, whether NF-κB p65 (Rel A) was correlated with demographic, metabolic and hormonal characteristics of the studied participants was also evaluated in an attempt to investigate the role of pathological endometrial inflammation in the pathogenesis of PCO-related infertility.

Materials and methods

Patient selection

The NF-κB p65 (Rel A) subunit expression was evaluated in endometrial tissues obtained during the mid-luteal phase from 20 normal weight infertile PCOS patients (Group 1, BMI:18.5–24.9 kg/m^2) and 15 overweight infertile PCOS patients (Group 2, BMI: 25–29.9 kg/m^2). In addition, endometrial tissues were obtained during the mid-luteal phase from 15 fertile women with normal pelvic cavities who underwent laparoscopic tubal ligation or reversal of tubal sterilisation before surgery (Group 3, BMI: 18.5–24.9 kg/m^2). The patients in the normal weight PCOS group and control group were age and BMI-matched (BMI:18.5–24.9 kg/m^2). Participants in the control group were healthy volunteers with regular menses and no history of hirsutism and had normal biochemical and hormonal profiles, thereby excluding the diagnosis of PCOS. The fertile women enrolled as the control group had at least two children.

Since a previous gravidity or parity could influence the results of NF-κB expression and endometrial receptivity cases with the previous history of infertility or habitual abortion were not included. Patients were diagnosed as PCOS based on the revised Rotterdam criteria, which require two of the following three manifestations: (1) oligo- and/or anovulation, (2) clinical and/or biochemical hyperandrogenism and (3) polycystic ovaries determined by ultrasonography (Rotterdam ESHRE consensus 2004). The ultrasound criteria used for diagnosis of polycystic ovary were the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (>10 mL). Participants in the control group had no evidence of hyperandrogenic skin manifestations or polycystic ovaries on ultrasound.

The PCOS participants were selected from a group of PCOS patients with complaints of menstrual irregularity, acne, hirsutism or infertility and the control subjects from the general community. All patients had normal renal, hepatic and thyroid functions. Women taking antiandrogens, antidiabetics, lipid-lowering medications, glucocorticoids or other hormonal drugs were excluded. Due to their negative impact on endometrial NF-κB expression, cases with previous endometrial pathology such as Asherman's syndrome, endometrial polyp, and/or submucous fibroids, diagnosis of pelvic inflammatory disease, mild peritoneal endometriosis, deep endometriosis, and hydrosalpinx at the time of the study, history of habitual abortion, infertility aetiology other than PCOS, out-of-date endometrium on the pathological evaluation of endometrium were excluded. Women with a single ovary, a previous history of ovarian cystectomy, a history of chronic smoking or previous chemotherapy and/or radiotherapy treatment were also excluded.

The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee. Venous blood samples were taken from all subjects for complete hormonal assays, lipid profile, glucose and insulin analysis. The blood of the participants was sampled in the morning following an overnight fast during the early follicular phase (days 2–5) of withdrawal bleeding, whether spontaneous or progesterone

induced. The blood samples were centrifuged and then the plasma aliquots were frozen at −20 °C until assayed. Fasting insulin levels were measured in all PCOS and control participants to estimate insulin sensitivity. Insulin resistance was calculated using the homeostasis model assessment insulin resistance index (HOMA-IR) (Matthews et al. 1985), according to the formula; HOMA-IR = Fasting serum insulin (mU/mL) × Fasting plasma glucose (mg/dL)/405. For each participant, height, weight, BMI, systolic (SBP) and diastolic (DBP) blood pressure were evaluated by standard methods. BMI (kg/m^2) was calculated as the ratio of the weight (kg) to the square of the height (m^2). World Health Organisation (WHO) guidelines were used to define normal and overweight subjects based on BMI. PCOS subjects with a BMI between 18·5 and 24·9 kg/m^2 were defined as normal, and those with a BMI value between 25 and 29.9 kg/m^2 as overweight (WHO 1995).

Endometrial sampling

All participants, independent of group, were selected for the present study on the basis of consistent histological findings, menstrual history and serum progesterone levels. Endometrial biopsy specimens were obtained from all the women with Pipelle endometrial sampling in the mid-luteal phase of the cycle. The endometrial tissue was fixed in 10% formalin and embedded in a paraffin block. Paraffin embedded sections of 4 μm were stained with haematoxylin and eosin and periodic acid-Schiff stain. These specimens were then evaluated according to the histopathological criteria of Noyes et al. (1950). The phase of the natural menstrual cycle was confirmed using ultrasound and histological criteria and by measuring the plasma levels of progesterone. An out-of-date biopsy was defined as a lag of ≥3 days between the chronological and the histological day (Creus et al. 2002; Ordi et al. 2002). PCOS women with anovulatory cycles were subjected to progesterone induced withdrawal bleeding to determine their secretory phases. Women with ovarian endometrioma, hydrosalpinges, peritoneal endometriosis, endometrial polyp, uterine septum, adenomyosis, endometritis, endometrial hyperplasia and systemic diseases were excluded from the study.

Immunohistochemical detection of endometrial NFκB/p65 (Rel a) Ab-1

Paraffin block sections of 4 μm thickness on poly-L-lysine coated slides were used after drying in an oven for 1 h at 60 °C. The sections were de-waxed in xylene, rehydrated in ethanol and then incubated for 10 min in 3% hydrogen peroxide to block endogenous peroxidase. After washing in phosphate buffer saline (PBS), the sections were incubated for 8 min at ultra V block. The immunoreaction was performed for 60 min with ready-to-use NFκB/p65 (Rel A) Ab-1 antibody (NeoMarkers, Labvision corp., CA). After washing in PBS, the slides were incubated with a horseradish peroxidase (HRP) kit. For negative control, the endometrial tissue was incubated with non-immune rabbit serum in place of the primary antibody. Finally, the preparations were developed in 3-Amino-9-Ethylcarbazole (AEC) chromogen, counterstained with haematoxylin and mounted with aqueous-mount. Third trimester human placenta served as the positive control. To evaluate immunohistochemical NF-κB p65 (Rel A) expression in the endometrium, the H-Score method was used (28). This immunohistochemical semi-quantitative method consists of the percentages of positively stained cells multiplied by a weighted intensity of staining: H-Score = ΣPi(i + 1), where Pi is the percentage of stained cells in each intensity category (0–100%), and i is the intensity indicating weak (i = 1), moderate (i = 2) or strong staining (i = 3) (Budwit-Novotny et al. 1986; Lessey 1994).

Statistical analysis

The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test and all variables were skewed normally. The continuous variables were analysed by ANOVA test with the *post hoc* Tukey's procedure and the Mann–Whitney *U* test. The categorical data were analysed with the Pearson Chi-square test. Spearman's Rank Order Correlation coefficients were used to assess associations between the endometrial NF-κB (p65) expression and biochemical, hormonal and demographic parameters. For all comparisons, statistical significance was defined as $p < .05$. The results were expressed as mean ± standard deviation (SD). All data analysis was applied using the Statistical Package for Social Sciences software 19.0 for Windows package software (SPSS, Inc., Chicago, IL).

Results

Table 1 shows the clinical characteristics, biochemical and hormone profiles of normal and overweight PCOS patients and those of the age and BMI-matched control group. There were no significant differences between the normal weight PCOS subjects and the control group in respect of age and BMI. All participants in the normal weight PCOS group and the control group without PCOS had a BMI value of 18–25 kg/m^2. The BMI values of the overweight women with PCOS were 25–29.9 kg/m^2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were found to be similar in all the groups. Fasting insulin, glucose levels and HOMA-IR were significantly higher in both the PCOS groups compared to the control group. The serum levels of total testosterone and circulating LH were significantly higher in both the PCOS groups compared to the control group. There were no significant differences between the PCOS groups in respect of fasting serum glucose, insulin levels and HOMA-IR. Other hormonal and biochemical parameters measured in both PCOS groups were similar. Both normal and overweight PCOS patients demonstrated significantly higher endometrial NF-κB expression levels than those without PCOS. Accordingly, the H-scores of endometrial NF-κB p65 (Rel A) expression in the control group were significantly lower than those of women with PCOS. The H-scores of endometrial NF-κB p65 (Rel A) expression levels were similar in both the PCOS groups

Table 1. Demographic and hormonal characteristics of the normal weight, overweight PCOS and control groups.

	Normal weight PCOS ($n = 20$) Group 1	Overweight PCOS ($n = 15$) Group 2	Control ($n = 15$) Group 3	p Value* Group 1 vs 2	p Value* Group 1 vs 3	p Value* Group 2 vs 3
Age (year)	28.05 ± 3.7	28.1 ± 4.9	27.06 ± 4.06	NS	NS	NS
BMI (kg/m^2)	22.41 ± 1.7	26.9 ± 1.15	22.3 ± 5,54	.012	NS	.010
LH (mIU/ml)	8,43 ± 3.12	8.76 ± 2.12	4.89 ± 0.34	NS	.028	.003
FSH (mIU/ml)	4.64 ± 0.90	5.19 ± 0.22	5.55 ± 7.44	NS	NS	NS
Total testosterone (ng/dl)	78.1 ± 7.06	81.51 ± 0.73	38.3 ± 0.56	NS	.000	.000
Fasting glucose (mg/dl)	98.3 ± 0.39	101.2 ± 6.07	85.3 ± 0.75	NS	.044	.033
Fasting insulin (mU/ml)	20.4 ± 0.29	22.7 ± 5.73	11.4 ± 0.34	NS	.003	.005
HOMA-IR	4.48 ± 1.63	4.67 ± 0.60	2.45 ± 0.65	NS	.004	.005
Systolic BP (mm/Hg)	105.1 ± 6.9	106.6 ± 7.2	103.2 ± 6.4	NS	NS	NS
Diastolic BP (mm/Hg)	81.6 ± 5	84.5 ± 5.4	78.4 ± 5.2	NS	NS	NS

Data are presented as mean and SD. *$p < .05$. NS: not significant.

Table 2. Comparisons of H-Score of immunohistochemical NFκB/65 (Rel A) expression in the endometrium of normal, overweight PCOS and matched fertile control subjects.

Groups	H-Score of NFκB/65 (RelA) expression
1- ($n = 20$) Normal weight PCOS (BMI:18·5–24·9 kg/m^2)[a]	3.94 + 4.63
2- ($n = 15$) Overweight weight PCOS (BMI:25–29.9 kg/m^2)[b]	3.83 + 2.51
3- ($n = 15$) Fertile control (BMI:18·5–24·9 kg/m^2)	2.65 + 1.39

Data are presented as mean and SD. $p < .05$. NS: not significant.
[a]Insignificant difference between Groups 1 and 2 (*NS*) and significant difference ($p < .001$) between Groups 1 and 3.
[b]Significant difference ($p < .001$) between Groups 2 and 3.

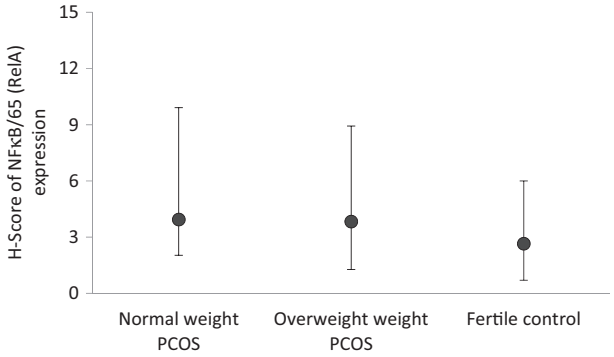

Figure 1. Comparison of mean H-Score of NFκB/65 (RelA) expression among the groups. The circles in the middle of each line indicate the mean, while the top and bottom borders of the line marks are upper and lower limits of 95% confidence interval; respectively.

(Table 2 and Figure 1). The increased endometrial NF-κB p65 (Rel A) immunoreactivity was predominantly localised in the cytoplasm of luminal and glandular epithelial cells in both the PCOS groups (Figure 2). The H-score change in NF-κB p65 (Rel A) was positively correlated with serum insulin, HOMA-IR and total testosterone levels in both PCOS groups (Table 3). A positive correlation, but not of a statistically significant level, was noted between the H-score of NF-κB p65 (Rel A) expression and BMI in both PCOS groups (Figure 3). A negative correlation not of a statistically significant level was determined between H-score of NF-κB p65 (Rel A) expression and serum glucose levels. No significant correlation was determined between demographic, biochemical, hormonal parameters and NF-κB expression levels in the control group participants without PCOS.

Discussion

Despite the progress in reproductive technologies, implantation rates are still relatively low and have not been sufficiently enhanced in women with PCOS. While anovulation is an obvious cause of infertility in women with PCOS, accumulated data suggest that defects in endometrial receptivity may also contribute to PCOS-related infertility (Dor et al. 1980; Gregory et al. 2002). The physiological amount of endometrial inflammation plays a key role in the establishment of successful pregnancy in all women. However, pathological inflammation may lead to a defect in endometrial receptivity and early blastocyst implantation (Weiss et al. 2009; Celik et al. 2013). Controversial results have been reported in the majority of PCOS studies because of the heterogeneity of women with PCOS. In many studies, PCOS patients with different BMI values have been included in the same study group. To avoid this handicap, the PCOS patients in the current study were divided into two groups according to BMI values. Appropriate controls without PCOS are required to study endometrial receptivity in infertile women with PCOS. In the current study, this was achieved with the inclusion of a control group of fertile women without PCOS. Since both endocrine abnormalities and inflammation are more prevalent in women with PCOS (Celik et al. 2011; Escobar-Morreale et al. 2011) it was aimed to determine whether the expression of endometrial NF-κB p65 (Rel A) can give insights into the origin of defective implantation common in lean and overweight PCOS women.

The data obtained in the current study clearly shows that in normal and overweight women with PCOS, pathological endometrial inflammation develops in response to the metabolic sequeale of PCOS. An increase in expression of NFκB p 65, the major cellular signal of inflammation, was evident in both PCOS groups compared to the age and BMI-matched control group. By leading to pathological inflammation, an increase in NF-κB p65 (Rel A) expression in the endometrium of normal and overweight PCOS women may limit both spontaneous and IVF pregnancy rates and contribute to subfertility in PCOS subjects. In the current study, both PCOS groups demonstrated significantly higher endometrial NF-κB expression than the control group without PCOS. In order to determine the contribution of basic metabolic and demographic parameters to the endometrial inflammatory state seen in PCOS, each parameter was correlated with NF-κB p65 (Rela A) expression. Increased NFκB (p65) expression was seen to

be positively associated with insulin resistance and testosterone levels, suggesting that insulin resistance and hyperandrogenism may be involved in the pathological endometrial inflammation. This finding was consistent with previous reports that insulin resistance and androgens are closely linked to low-grade inflammation in PCOS (González et al. 2006, 2012). Nevertheless, an insignificant association was observed between BMI and endometrial inflammation suggesting that endometrial inflammation in PCOS women occurs regardless of body composition.

The overweight PCOS group in the current study displayed a higher endometrial NF-κB p65 (Rel A) expression associated with increased insulin resistance. Furthermore, the H-scores of endometrial NF-κB p65 (Rel A) expression were found to be positively correlated with serum insulin levels in the overweight PCOS group. Similar to findings in individuals with overweight PCOS, it has been reported that NF-κB p65 (Rel A) expressions are correlated with insulin levels and HOMA-IR in normal weight women with PCOS. It is well-known that women with PCOS tend to exhibit an adverse metabolic problem associated with increased insulin resistance (Escobar-Morreale and San Millan 2007). Moreover, studies of lean women with PCOS have reported that they are as equally insulin-resistant as overweight women with PCOS, suggesting that insulin resistance is independent of body composition (Dunaif et al. 1989; Marsden et al. 2001). Similarly, in the current study there were positive correlations between insulin levels, HOMA-IR and H-score of endometrial NF-κB expression in normal and overweight PCOS women. The increased expression of endometrial NF-κB p65 (Rel A) depends on metabolic sequelae of PCOS that may contribute to the PCOS-related implantation defect observed in women with

Table 3. Spearman's correlation coefficients (*r*) between H-score for NF-kB p65 (Rel A) expression levels and measured parameters in normal and overweight PCOS participants.

	Normal weight PCOS H-score for NF-kB p65 (Rel A)		Overweight PCOS H-score for NF-kB p65 (Rel A)	
Independent variable	*r*	*p*	*r*	*p*
BMI	+.344	.452	+.257	.512
Insulin	+.441	.002	+.530	.001
HOMA-IR	+.410	.001	+.311	.002
Glucose	−.213	.124	−.123	.342
T-testosterone	+.378	.012	+.289	.032

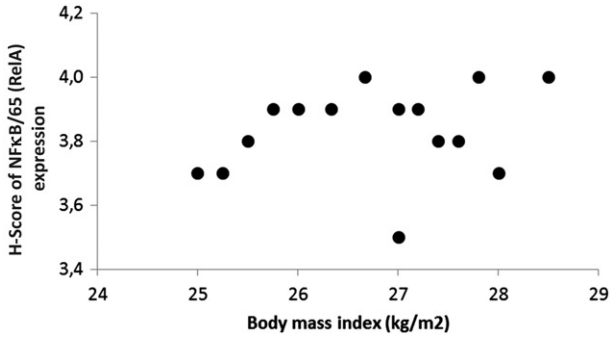

Figure 3. The scatter plot between H-Score of NFκB/65 (RelA) expression and body mass index in overweight PCOS group.

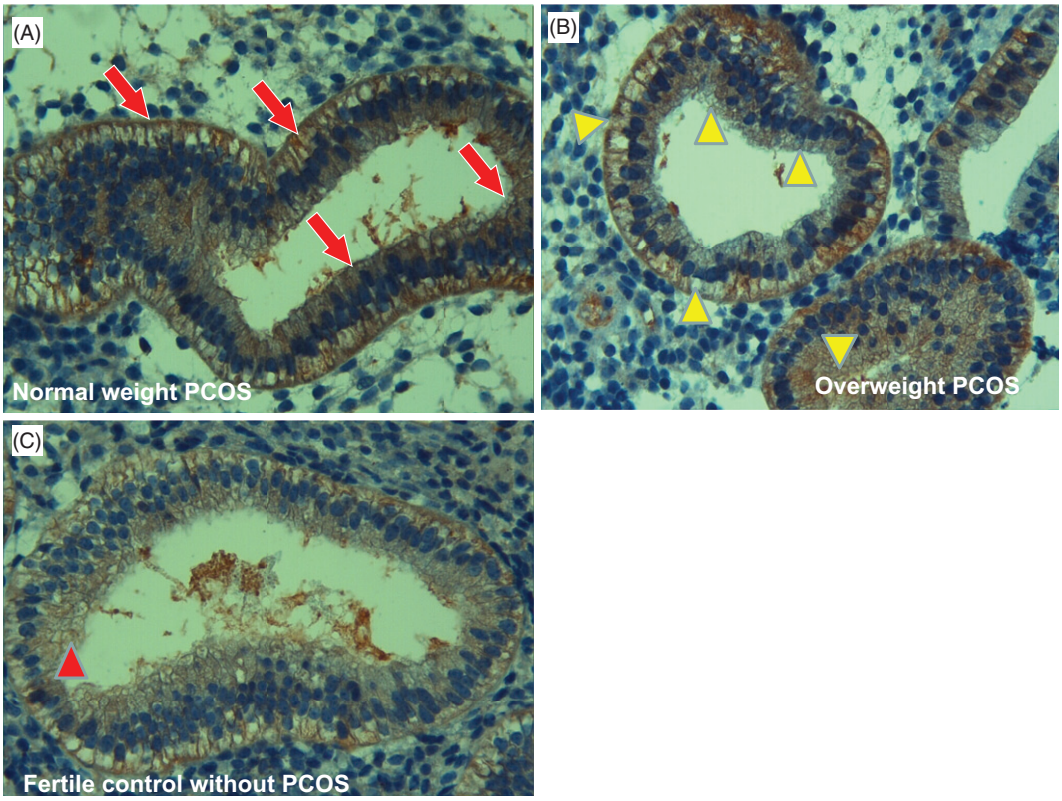

Figure 2. The increased endometrial NF-κB p65 (Rel A) immunoreactivity was predominantly localised in the cytoplasm of luminal and glandular epithelial cells in normal (A, arrows, 40×) and overweight PCOS women (B, arrowheads, 40×). Weak NF-κB p65 (Rel A) staining was detected in the control group (C, arrowhead 40×).

PCOS. It has also been reported that the administration of PCOS serum into human micro-vascular endothelial cell culture in order to evaluate in vitro migration and angiogenesis resulted in a significant increase in both NF-κB function and NF-κB phosphorylation (Tan et al. 2009). Following 6 months of metformin treatment, NF-κB activation was significantly decreased, supporting the importance of the role of insulin and insulin- resistance on NF-κB expression (Tan et al. 2009). Moreover, the results reported above in conjunction with the fact that NF-κB p65 (Rel A) expression did not differ significantly between the normal and overweight PCOS groups in the current study suggests that pathological inflammation and receptivity defect of endometrium of PCOS subjects are independent of BMI status.

It has been reported that hyperglycaemia activates NFκB expression and tumour necrosis factor-α release from mononuclear cells in normal-weight women with PCOS (González et al. 2005, 2006). A recent study from the same group (González et al. 2012) evaluated the impact of glucose ingestion on mononuclear cell-derived NFκB activation in normal-weight women with PCOS who had either normal or excess abdominal adiposity. Increased levels of NFκB/p65 protein and decreased levels of inhibitory-κB protein in response to glucose ingestion were reported. It was further noted that a change in MNC-derived NFκB activation was negatively correlated with insulin sensitivity. Nevertheless, in the current study, serum levels of glucose in both the normal weight and overweight PCOS women were similar and there was no significant correlation between the serum glucose levels and endometrial levels of NFκB p65 (Rel A) expression.

Another possible explanation for the increase in endometrial NFκB p65 (Rel A) in both groups of PCOS women might be hyperandrogenism. In PCOS women, inflammation directly promotes hyperandrogenism (González et al. 2012). González et al. (2012) noted that the percentage change in MNC-derived NFκB activation was positively correlated with androgens. However, it is not clear whether androgens have a direct impact on the endometrial inflammatory genes or they act solely as precursors to oestrogens. It is well-known that androgens within the endometrium may not solely serve as substrate for the production of oestrogen, but may also modulate the biological effects of oestrogen in the endometrium in the peri-implantation period (Kowalski et al. 2004). Accordingly, circulating androgen levels including testosterone have been reported to be positively correlated with NFκB activation and p65 protein of mononuclear cells (González et al. 2012). Since many women with PCOS are insulin resistant, it is not known whether increased expression of endometrial NFκB p65 is secondary to hyperandrogenism or hyperandrogenism itself contributes to IR and increases NFκB p65. Whatever the mechanism, PCOS-related hyperandrogenism is correlated with the expression of endometrial NFκB p65.

A limitation of the current study is that there was no evaluation of the DNA binding or IkB phosphorylation of NF-κB in the endometrium of the PCOS and control groups. Therefore, increased expression of endometrial NF-κB 65 (rel A) does not necessarily mean increased NF-κB activity, and it is not possible to comment on whether the elevated NF-κB p65 level and expression reflected altered endometrium gene transcription. Moreover, since the stroma is over-represented in endometrial biopsies compared to epithelium and the proportion of epithelium can vary between different endometrial biopsies, RT-PCR preformed from endometrial biopsies would not give relevant and reliable information on the differences of NF-κB p65 between PCOS and healthy women. In addition, the serum levels of other inflammatory markers were not measured. Another limitation of the study was that the role of fat distribution in endometrial inflammation was not clearly evaluated. Although BMI is the accepted method to categorise obesity, it does not exactly distinguish the fat distribution status of PCOS women with different BMI values and therefore may lead to erroneous comments. Moreover, central fat accumulation and the relationship with chronic inflammation in women with PCOS has been previously reported (Puder et al. 2005).

PCOS is a metabolic complication of metabolic syndrome. In addition to chronic anovulation and suboptimal endometrial progesterone action, decreased expression levels of receptivity markers such as integrin and homeobox genes may lead to PCOS-related infertility (Apparao et al. 2002; Cermik et al. 2003). Nevertheless, PCOS is rarely thought of as a condition that leads to endometrial inflammation. Therefore, it is interesting to note that the current study has shown an association between PCOS and pathological endometrial inflammation which may contribute to defective implantation. The results of the current study clearly suggest that pathological endometrial inflammation occurs in both normal and overweight women with PCOS. The enhanced H-score of NF-κB p65 may mainly depend on the synergistic effect of testosterone, insulin and insulin resistance. These three leading parameters may act together in the development of pathological endometrial inflammation. These results are in line with the majority of the current literature, which reveals insulin resistance and hyperandrogenemia to be the main factors responsible for chronic inflammation in PCOS (González et al. 2012; Papalou et al. 2015). If pathological endometrial inflammation can be detected by the measurement of endometrial NF-κB p65 (Rel A) expression, treatment could be directed towards eliminating the source of pathological endometrial inflammation (Ersahin et al. 2016). This study provides novel insights into the relationship between PCOS-related infertility and pathological endometrial inflammation.

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Disclosure statement

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