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Original Research

Regulation of endometrial NF-κB expression in patients with PCOS undergoing total embryo freezing

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Abstract

Background: To investigate Nuclear Factor kappa B (NF-κB) expression pattern in the endometrial samples taken on the day of egg collection in women with polycystic ovarian syndrome (PCOS)scheduled for total embryo freezing due to the risk of ovarian hyperstimulation syndrome (OHSS). **Methods**: Forty women with PCOS scheduled for total embryo freezing due to the risk of ovarian hyperstimulation syndrome were included in the study. Twenty-five infertile women who decided to freeze all of their embryos for any reason other than PCOS were utilized as the control group. Endometrial sampling was performed from all patients in both groups immediately following the egg collection. Five fertile women with at least two children constituted the second control group. Endometrial sampling was performed from fertile patients during the mid-luteal phase. After immunohistochemical staining of endometrial samples with NF-κB/p65, the intensity of endometrial NF-κB/p65 expression was measured utilizing the H-score method. **Results**: NF-κB/p65 immunoreactivity was detected in both luminal and glandular endometrial cells from all samples. The mean H-score of endometrial NF-κB/p65 expression in the PCOS group was significantly increased compared to age and Body mass index (BMI) matched control group and fertile controls. NF-κB/p65 immunoreactivity of the control and fertile groups were found to be similar. There was no statistically significant difference in the mean H-score of endometrial NF-κB/p65 expression between the control and fertile groups. A positive and significant correlation was found between H-score values of NF-κB and E2 (estradiol), endometrial thickness, total oocyte count and total follicle stimulating hormone (rFSH) dose on human chorionic gonadotropin (hCG) day. Similarly, a strong positive correlation was found between serum testosterone, insulin levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values and NF-κB values. In addition to progesterone values on hCG day, no significant correlation was found between other parameters and NF-κB. **Conclusions**: Increased endometrial NF-κB/p65 expression may contribute to the diminished reproductive potential of women with PCOS undergoing total embryo freezing.

Keywords: PCOS; NF-κB/p65; Total embryo freezing; Endometrium; OHSS

1. Intrdouction

In addition to endocrine and metabolic problems, subfertility is a common problem in patients with polycystic ovarian syndrome (PCOS). There is no single cause of reduced fertility in PCOS patients and it may occur as a result of the cumulative effect of the following factors: (i) phenotype of patients; (ii) impaired peripheral and central peptide synthesis; (iii) failed receptivity gene expression; (iv) pathological endometrial inflammation; (v) oocyte competence varies depending on the patients phenotype and other comorbidities accompanying the syndrome [1–6]. In addition to these endometrium of PCOS patients differs from both healthy non-PCOS controls and fertile subjects at the molecular level. In addition to dsyregulated receptivity genes and sex steroid receptor expression insulin resistance may adversely affect glucose utilization in endometrial cells [6,7]. Moreover, systemic chronic low-grade inflammation may cause implantation defect in the endometrium of PCOS patients. These abnormal changes at the metabolic and genomic level seen in the endometrial cells of PCOS patients may cause failed trophoblast invasion and placentation resulting in both subfertility and increased miscarriage rates [7]. It has been reported that one of the possible causes of pregnancy complications in PCOS patients is increased by 2–3 times compared to healthy controls, and one of the possible causes is impaired endometrial microenvironment [8].

Total embryo freezing is a widely used preventive method in patients who are scheduled for Invitro fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI) due to PCOS but who are also at risk of ovarian hyperstimulation syndrome (OHSS) [9]. Women suffering PCOS constitute the main patient group that are at risk for OHSS. The leading measure of life-threatening complications of OHSS is freezing of all embryos and transfer back during a subsequent cycle. Although physiological endometrial inflammation is required for a healthy implantation, the state of endometrial inflammation on the day of egg collection is an unknown entity [10–12]. A study by Koc *et al*. [5] has

shown that both obese and non-obese patients with PCOS have an increased amount of pathologic inflammation in their endometrium during the mid-luteal phase. Nevertheless, the state of inflammation in the endometrium on the day of egg collection in women with PCOS undergoing total embryo freezing has not been investigated.

Nuclear Factor kappa B (NF-κB) is the main cellular regulator of endometrial inflammation [1,5,12]. It is also involved in cell proliferation, apoptosis, invasion, and angiogenesis of the developing endometrium [11–13]. It is a molecule consisting of five different subunits: p50/p105, p52/p100, p65 (RelA), c-Rel, and RelB. The subunits are bound to the inhibitory protein IκBα and block the nuclear translocation of NF-κB. Following internal or external stimuli, IκBα is phosphorylated and the release of NF-κB occurs. NF-κB dimers migrate to the nucleus where they activate many genes related to inflammation [11–13]. Since PCOS is a syndrome characterized by subclinical and chronic inflammation, NF-κB expression may change in the endometrium of patients with PCOS undergoing controlled ovarian stimulation [1,5]. Concordantly, pathologic increase in NF-κB expression was found in endometrial samples of patients with PCOS [5]. There are no studies investigating NF-κB levels on the day of egg collection in the endometrium of patients with PCOS in whom total embryo freezing is planned due to the potential risk of OHSS. This study was designed to detect NF-κB expression pattern in the endometrial samples taken on the day of egg collection in women with PCOS scheduled for total embryo freezing.

2. Materials and methods

Forty patients scheduled for IVF/ICSI due to PCOS were included in the study. Participants were selected from the patients who applied to Istanbul IVF-Center with complaints of infertility. Women in the PCOS group were selected from among women with PCOS having a normal Body mass index (BMI: 18.5–24.9 kg/m^2). Patients with a BMI above 25 kg/m^2 were not included in the study. Some of these patients had previous unsuccessful IVF attempts and some had a history of OHSS. In the preliminary interview with the patients, the decision of total embryo freezing was made. The women in the control group consisted of 25 patients who were scheduled for total embryo freezing for reasons other than PCOS. They were matched with the PCOS group in terms of BMI and age. Endometrial samples taken from five fertile cases were selected as the second control group. Age and BMI of the patients in the fertile group were similar to those in the PCOS and control groups. The fertile women enrolled as the control group had at least two children and had no history of primary or secondary infertility. 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. In order to be included in the control group, the individual must not have any of the Rotterdam criteria. Women in the control group who met at least one of these three criteria were not included in the study. PCOS patients were not separated according to their phenotypes. However, all participants in the PCOS group had clinical and laboratory findings of phenotype A: hyperandrogenism (HA) + ovulatory dysfunction (OD) + polycystic ovarian morphology (PCOM). Since phenotype B: HA + OD, phenotype C: HA + PCOM, and phenotype D: OD + PCOM are very rare, we were not able to group patients according to phenotype.

Excluded cases were the ones with: (1) previous endometrial pathology such as Asherman syndrome, endometrial polyp, submucous fibroids, uterine septum and other congenital uterine anomalies; (2) diagnosis of pelvic inflammatory disease, deep endometriosis, or hydrosalpinx; (3) diagnosis of endometrioma or other benign ovarian cysts; (4) hormonal medication and intrauterine contraception use within the past 6 months before study enrollment; and (5) diagnosis of systemic and/or rheumatologic disease that may lead to systemic inflammation and receptivity defect; (6) previous ovarian surgery; (7) history of habitual abortion; (8) subfertility etiology other than PCOS; (9) history of hypo/hyper trodism and other endocrine disorders, such as diabetes mellitus.

Both groups of participants underwent routine laboratory and radiological examination to diagnose the underlying factors for infertility. After 3–7 days of abstinence, semen analysis was performed from all male partners. Those with abnormal semen paramaters were excluded from the study. Hysterosalpingography was performed in all participants and patients with bilateral tubal patency and absence of intrauterine mass were included in the study. In addition to demographics characteristics of women in PCOS and the control group, age, body mass index (BMI) (kg/m^2), total testosterone, fasting glucose, insulin, serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured. Serum estradiol and progesterone levels on the day of human chorionic gonadotropin (hCG) administration, the number of total oocytes, Metaphase II (MII) oocytes and frozen embryos were recorded. Homeostatic model assessment (HOMA-IR) Formula was used for calculating insulin resistance. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee.

Same protocol was used for ovarian stimulation in PCOS and control groups. Recombinant follicle stimulating hormone (Gonal-F, Merck Pharmaceutical Group Inc, Istanbul, Turkey) and/or human menopausal gonadotrophin (Merional, IBSA Pharmaceutical Group Inc., Istanbul, Turkey) was started as the initial dose on the second or third day of the menstrual cycle. Serial vaginal ultrasonography was used for monitoring the ovarian response. In order to prevent premature luteinization, 0.25 μg GnRH antagonist

(Cetrotide 250 μg, Merck Serono, Istanbul, Turkey) was added daily when the leading follicle reached a diameter of 14 mm. When the mean diameter of two or three leading follicles reached 17 mm or more, triptoreline acetate (Gonapeptyl 0.1 mg/mL, Ferring, Istanbul, Turkey) was used to trigger ovulation. In the control group, a single dose of recombinant hCG was used to induce ovulation. The oocyte pick-up was carried out after trigger success, at a minimum of 35 and a maximum of 36 hours after administration. After ICSI was performed on suitable oocytes, all embryos obtained were subjected to total freezing. Following egg collection and while the patient was under anesthesia, endometrial sampling was performed with a pipelle cannula. The collected endometrial tissue was fixed in 10% formalin and embedded in a paraffin block.

3. Immunohistochemical staining of oocyte retrieval day endometrial samples for NF-κB/p65

Four micrometer paraffin sections of endometrial samples obtained on the day of oocyte collection were cut and placed on poly-l-lysine coated slides. The slides were de-waxed in xylene, rehydrated in ethanol, and incubated for 10 minutes in 3% hydrogen peroxide. The sections were incubated for 8 to 10 minutes following washing with PBS. The immuno-staining was performed by using NF-κB/p65 Ab-1 antibody. Following washing with PBS, the poly-l-lysine coated slides were incubated with horseradish peroxidase kit (NeoMarkers, Labvision Corp, Fremont, CA, USA). To achieve a negative control, endometrial tissues were incubated with rabbit serum with depleted immunogenic properties. All slides were exposed to 3-Amino-9-ethylcarbazole chromogen with hematoxylin and mounted with an aqueous mount. Human placental samples were accepted as the positive control for NF-κB staining. To evaluate the intensity of endometrial NF-κB/p65 expression, the H-score measurement method was used. This is an immunohistochemical and semiquantitative method. It consists of the percentages of positively stained endometrial cells multiplied by a weighted intensity of staining: H-score = ΣPi (I + 1), where Pi is the percentage of stained endometrial cells in each intensity step (0%–100%), and i is the intensity showing weak (i = 1), moderate (i = 2), or strong (i = 3) staining.

4. Statistical analysis

All data analysis was performed using the Statistical Package for Social Sciences software 21.0 for Windows package software (SPSS, Inc., Chicago, IL, USA). All parameters studied in the PCOS and non-PCOS group showed normal distributions, which were confirmed by the one sample Kolmogorov-Smirnov test. Comparisons between the two groups were made using an independent samples *t* test or Mann-Whitney U test. The relationship between the H-score values of NF-κB and other demographic, hormonal and reproductive parameters was evaluated by Spearman's correlations analysis. Data are presented as the means $\pm$ SD. A p value of <0.05 was considered statistically significant.

5. Results

The demographic, hormonal and reproductive parameters of the patients in both groups are shown in Table 1. There was no difference between the groups in terms of age, BMI and duration of infertility. Age (28.7 $\pm$ 0.11) and BMI (24.7 $\pm$ 1.77) of the patients in the fertile group were similar to those in the PCOS and control groups. In addition to serum LH, testosterone and insulin levels, HOMA-IR of the patients in the PCOS group were found to be significantly higher than the control group. Serum FSH evels were similar in both groups. The number of MII oocytes collected and frozen embryos were significantly higher in the PCOS group compared to the control group. Severe OHSS requiring hospitilization did not develop in any of the patients in the PCOS group. Outpatient supportive care was given to those patients with mild OHSS.

Endometrial samples from PCOS, non-PCOS and fertile cases demonstrated adequate staining with NF-κB/p65 antibody. NF-κB/p65 immunoreactivity was detected in both luminal and glandular endometrial cells of all samples. Staining was mostly concentrated in the cytoplasm of endometrial cells. The mean H-score of endometrial NF-κB/p65 expression in the PCOS group was significantly increased compared to the non-PCOS group and fertile controls. NF-κB/p65 immunoreactivity of the non-PCOS and fertile groups were found to be similar (Table 2). There was no statistically significant difference in the mean H-score of endometrial NF-κB/p65 expression between the non-PCOS group and fertile controls. The increase in NF-κB/p65 immunoreactivity in endometrial samples of PCOS cases was evaluated as the level of pathologic endometrial inflammation. Fig. 1 shows in detail the distribution and intensity of NF-κB/p65 immunoreactivity in endometrial samples of PCOS, non-PCOS and the fertile group. A positive and significant correlation was found between H-score values of NF-κB and E2, endometrial thickness, total oocyte count and total rFSH dose on hCG day. Similarly, a strong positive correlation was found between serum testosterone, insulin levels, HOMA-IR values and NF-κB values. In addition to progesterone values on hCG day, no significant correlation was found between other parameters and NF-κB (Table 3).

6. Discussion

Although it is not included in the diagnostic criteria of PCOS, systemic inflammation is an important feature of PCOS [2,3]. On the other hand, the number of studies showing the presence of inflammation at the tissue level are few [1,5]. It has been reported that NF-κB phosphorylation is increased in endothelial cell cultures of women with

Table 1. Demographic, hormonal and reproductive characteristics of PCOS and control groups undergoing total embryo freezing.

	PCOS (n = 40)	Non-PCOS (n = 25)	*p*
Age (y)	28.3 ± 1.23	27.8 ± 1.10	0.45
BMI (kg/m^2)	24.5 ± 0.34	23.8 ± 1.05	0.08
Infertility duration (y)	4.60 ± 0.20	4.89 ± 3.22	0.50
The number of IVF-ET attempts	2.1 ± 0.1	2.4 ± 1.9	0.42
Endometrial thickness (mm)	10.2 ± 2.03	9.88 ± 2.92	0.57
Testosterone (ng/mL)	0.66 ± 1.90	0.42 ± 2.10	0.02
Glucose (mg/dL)	85.3 ± 2.12	79.4 ± 1.01	0.58
LH (mIU/mL)	10.4 ± 0.43	4.98 ± 2.10	0.01
FSH (mIU/mL)	5.33 ± 1.05	4.92 ± 0.45	0.08
Insulin (mU/L)	11.4 ± 1.22	6.90 ± 0.23	0.01
HOMA-IR	3.76 ± 1.02	1.23 ± 1.09	0.01
Total rFSH dose	2102.4 ± 345.3	2450.3 ± 566.4	0.03
E2 on hCG day (pg/mL)	2702.4 ± 980.1	2105.3 ± 665.3	0.001
Progesterone on hCG day	1.43 ± 0.34	0.88 ± 0.11	0.03
Total oocyte	20.2 ± 1.23	13.2 ± 0.33	0.02
MII oocyte	13.5 ± 3.22	9.30 ± 2.01	0.01
The number of frozen embryo	10.2 ± 1.08	6.3 ± 2.81	0.02

Data presented as means ± SD. BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; MII, mature oocyte.

PCOS, while high androgen levels in Ishikawa cell cultures block estrogen-induced receptivity gene expression [4,14]. In the mid-luteal endometrial samples of PCOS cases, a significant increase in NF-κB/p65 expression has been reported [5].

In addition to adequate decidualization and activation of receptivity genes in the endometrium, a physiologic amount of inflammation is also required for successful implantation [12,15]. The physiologic inflammatory response is characterized by coordinate activation of different signaling pathways that regulate expression of both pro- and anti-inflammatory mediators and receptivity genes in endometrium [12]. Inflammation also allows other leukocytes to accumulate at the implantation site, especially the uterine natural killer cell. NF-κB activation is critical for the flawless functioning of this inflammatory process throughout implantation [12,15]. In many tissues, NF-κB is activated through canonical and alternative pathways [13,16]. Endometrial cells may be using canonical or alternative pathways for NF-κB/p65 activation [15]. The canonical pathway is triggered by proinflammatory cytokines such as tumor necrosis factor-α and IL-1 and leading to activation of proinflammatory molecules and receptivity genes [11,15]. Although the exact cause of the process initiating NF-κB activation in the endometrium is unknown, it has been accepted that the morphologic and molecular changes that occur during the decidualization process are the main inductors. Since all the pre-implantation preparation steps of endometrial tissue are hormone-dependent, regulation of the synthesis and release of NF-κB may be regulated by sex hormones, differing from other tissues [11,12,15].

Despite the increase in the number of studies on implantation and NF-κB, the role of NF-κB in embryo implantation has not been extensively studied [5,12]. Our study demonstrated increased pathologic endometrial inflammation in endometrial samples taken on the day of egg collection in PCOS cases with total embryo freezing due to the risk of OHSS. The increased NF-κB expression we detected in the PCOS patient group is reliable evidence of pathologic endometrial inflammation, and it is a known fact that this inflammatory process blocks the release of homeobox genes responsible for basic receptivity [12,17]. Possible causes of increased endometrial inflammation in PCOS cases may be increased estradiol and progesterone levels due to controlled ovarian stimulation. The positive correlation between endometrial NF-κB levels and estrogen levels measured on the day of hCG in our study supports this idea. However, a recent study conducted on patients with PCOS who did not undergo ovarian stimulation reported that there is an intense pathologic endometrial inflammation in mid-luteal samples which weakens our hypothesis [5]. Similarly, the normal endometrial NF-κB levels in our control group patients who underwent ovarian stimulation despite high estrogen levels suggest that estrogens have no effect on pathologic endometrial inflammation. Indeed, we found a positive correlation between endometrial thickness,

Table 2. Endometrial H-Score values of NF-κB/p65 in PCOS and control groups undergoing total embryo freezing.

Groups	Endometrial H-Score of NF-κB/p65
I- PCOS with total embryo freezing (n = 40)	H-score = 3.80 + 1.91
II- Age and BMI matched control with total embryo freezing (n = 25)	H-score = 2.03 + 1.14
III- Fertile control (n = 5)	H-score = 1.90 + 1.02
I vs II	0.001*
I vs III	0.002*
II vs III	0.040

Data are presented as mean and SD. *$p < 0.05$. H-score = ΣPi (I + 1), where Pi is the percentage of stained endometrial cells in each intensity step (0%–100%), and i is the intensity showing weak (i = 1), moderate (i = 2), or strong (i = 3) staining.

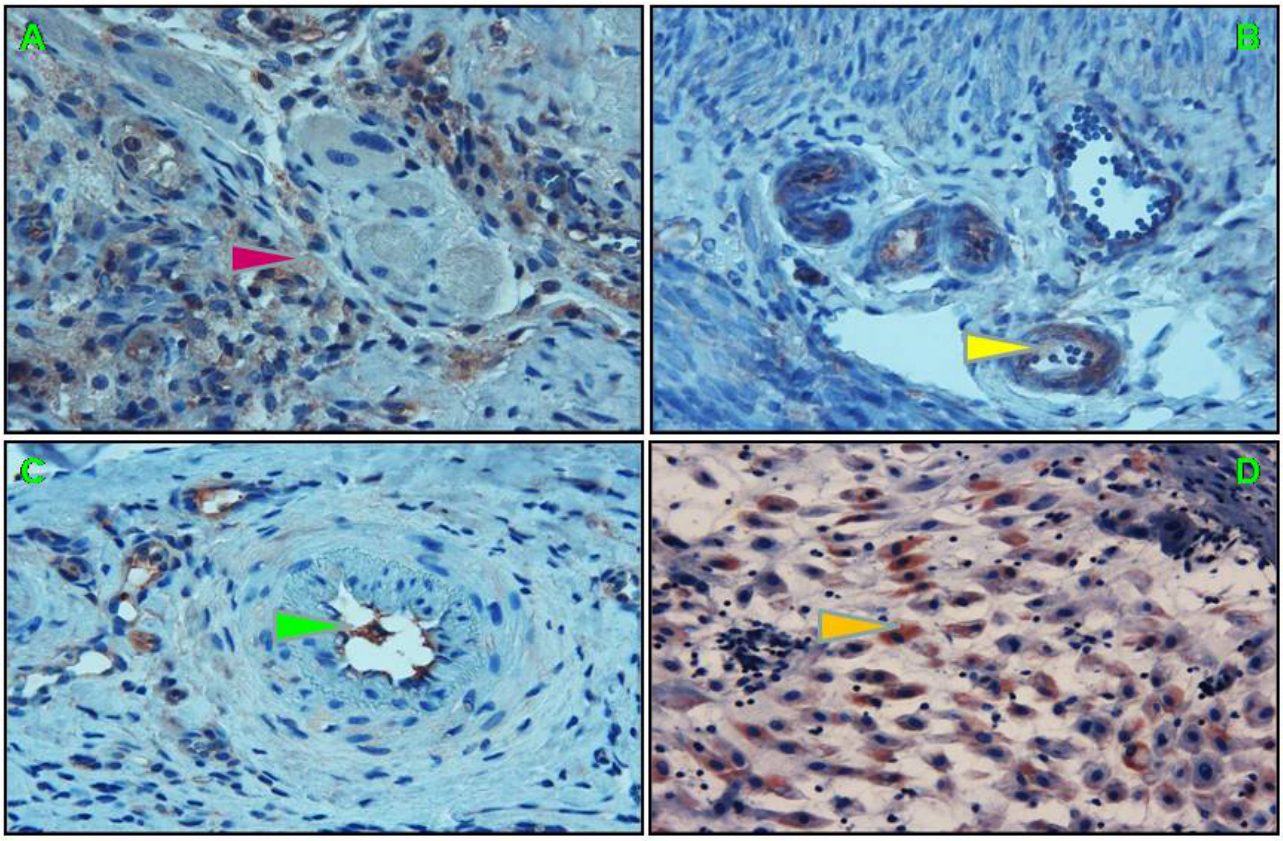

Fig. 1. Immunohistochemical staining of endometrial samples for NF-κB/p65. (A) Increased NF-κB/p65 immunoreactivity in the endometrium collected from women with PCOS (red arrowhead, ×20). (B) Weak NF-κB/p65 staining in the endometrium of non-PCOS controls (yellow arrowhead, ×20). (C) Weak NF-κB/p65 immunoreactivity predominantly localized into the cytoplasm of glandular epithelial cells in fertile control (green arrowhead). (D) 3th trimester human placenta accepted as positive control for NF-κB/p65 (pink arrowhead, ×20).

total oocyte count, rFSH dose and endometrial NF-κB levels which support that estrogens play a role in endometrial NF-κB expression. On the other hand, we found a negative and insignificant correlation between pogesterone and NF-κB levels. Since physiologic levels of estrogen and progesterone regulate the expression of endometrial receptivity genes, a supraphysiological increase in estrogen levels due to ovarian stimulation may contribute to increased endometrial NF-κB expression in patients with PCOS [12,15,18].

Another possible cause of pathologic endometrial inflammation may be the high androgen and insulin levels we detected in patients with PCOS. Increased levels of inflammatory markers have been reported in most studies from the peripheral blood of PCOS patients [19]. However, there is only one study investigating inflammatory markers in the endometrial tissue of PCOS patients. In that study, Koc *et al.* [5] reported that NF-κB/p65 expression levels in the endometrium of both normal and over-

Table 3. Correlation analysis between endometrial H-Score of NF-κB/p65 and measured parameters.

	H-Score of NF-κB/p65 expression in subjects undergoing total embryo freezing	
	r	*p*
E2 on the day of hCG	0.65	0.01
Progesteron on the day of hCG	–0.33	0.55
Testosterone	0.74	0.02
Insulin	0.66	0.01
HOMA-IR	0.58	0.04
Total oocyte	0.40	0.03
Endometrial thickness	0.32	0.04
Total rFSH dose	0.43	0.01

weight PCOS patients increased significantly compared to non-PCOS control subjects. The authors emphasized that increasing NF-κB levels independent of BMI is due to increased androgen and insulin levels. Many studies have confirmed that chronic inflammation due to PCOS is associated with increased androgen levels and insulin resistance [1–3,14]. While chronic inflammation in PCOS cases induces androgen increase, increasing androgens also increase both androgen synthesis and insulin resistance with a positive feedback effect [1–3,14]. Although our cases were selected from PCOS patients with normal BMI, HOMA-IR levels were found to be high. Previous studies conducted on lean women with PCOS have showed that they are as equally insulin-resistant as overweight women with PCOS, suggesting that insulin resistance is independent of BMI [20,21]. Therefore, it is not surprising that our PCOS cases have high insulin levels despite a normal BMI and is consistent with the literature. In our study, we found a positive and strong correlation between serum testosterone and insulin levels and NF-κB levels within the endometrium. Consistent with our findings, Koc *et al.* [5] also found a significant correlation between increased endometrial NF-κB levels and serum androgen and insulin levels in PCOS patients. Similarly, Cermik *et al.* [4]. reported that increased androgen levels in PCOS patients caused subfertility by decreasing Homeobox10 (HOXA10) gene expression. Gonzales *et al.* [19] reported that intranuclear NF-κB levels increased significantly in PCOS patients with hyperglycemia and that this increase was associated with insulin resistance and hyperandrogenism. Since serum glucose levels of the PCOS group and non-PKOS cases were found to be similar in our patients, we cannot make a clear comment on this issue. However, it has been reported that increased circulating androgen levels of PCOS patients in reproductive age increase the interest of cells in glucose use, leading to an increase in NF-κB expression [15].

There are many experimental and clinical studies showing the relationships between androgens and insulin levels and chronic inflammation in PCOS patients. It has been reported that by reducing phosphorylation of NF-κB/p65, some herbals could reduce hyperandrogenism in an animal model of PCOS [22,23]. Inagreement with this, iridoids can protect patients with PCOS from inflammatory damage by regulating the NF-κB expression [23]. Exogenous application of sera taken from PCOS patients to endothelial cell cultures led to a significant increase in NF-κB activation [24]. Similarly, in mononuclear cell lines obtained from PCOS patients, the percent change in NFκB activation was positively correlated with androgens [15]. Another study demonstrated that the use of exogenous androgens significantly decreased the expression of endometrial receptivity genes in women with PCOS [4]. A recent study reported that serum NF-κB levels were significantly decreased in patients given metformin or cyproterone acetate therapy for PCOS compared with those who were not treated. The reduction of NF-κB levels by antiandrogen or antiprogestin therapy strongly supports the role of androgens in the pathologic inflammation [25]. The positive correlation between serum testosterone and insulin levels and endometrial NF-κB/p65 found in our study is strong evidence of the clear relationship between hyperandrogenemia, HOMA-IR and increased pathologic inflammation.

As in all other tissues, NF-κB has long been accepted as a prototypical proinflammatory signaling pathway in the endometrium. Endometrial NF-κB expression levels measured in the follicular phase in healthy and fertile individuals were reported to be higher than the NF-κB levels detected in both the secretory and menstrual phases [15,26–28]. Unlike the endometrial NF-κB expression patterns of healthy individuals, NF-κB expression patterns in the eutopic endometrium of women with an endometrioma, endometriosis, hydrosalpinx and PCOS is disrupted [26–29]. Implantation rates decrease significantly in women with endometriosis or hydrosalpinx that cause an increase in inflammation in the endometrium [4,12]. Surgical removal of the endometrioma or hydrososalpinx normalized the pathologic increase in endometrial NF-κB levels [1,12]. All of these findings are important in terms of showing that some benign gynecologic diseases located outside the endometrium may trigger pathologic inflammation in the eu-

topic endometrium. Similarly, although PCOS is located outside the endometrium, it can trigger pathologic inflammation in the endometrium due to hormonal changes and chronic inflammation [1–3]. Consistent with our findings, it has been reported that NF-κB expression levels in the endometrium of PCOS patients with normal and high BMI increased significantly compared to healthy controls [5]. Likewise, in accordance with the other studies, expression levels of HOXA10 and HOXA11, which are the main regulator genes of endometrial receptivity, have been reported to be low in women with PCOS [15]. Senturk *et al*. [30] showed that endometrial HOXA10 and 11 levels of PCOS patients were significantly decreased compared to fertile controls and that their expression progressed to normal levels after laparoscopic ovarian drilling. When the results of these studies and our findings are evaluated together, they indicate that the functions of receptivity molecules and NF-κB pathway, which are responsible for implantation and physiologic inflammation, are impaired in women with PCOS.

Our study showed that total embryo freezing not only prevented the development of OHSS, but also delayed embryo transfer, thus preventing an unsuccessful implantation. Delaying transfer by freezing embryos in PCOS cases with a high risk of OHSS may save the clinician time required for the recovery of the endometrium. In the following cycles of these patients, re-preparing the endometrium and performing frozen embryo transfer may allow improvement for successful implantation. However, it is not known how many cycles the pathologic inflammation in the endometrium is decreased in PCOS cases with embryo freezing. Endometrioma and hydrosalpinx studies potentially could reveal clarifying data on this issue. Expression levels of endometrial receptivity genes have been demonstrated to reach fertile levels within 3 to 4 months after endometrioma resection or salpingectomy [11,16]. Similarly, in PCOS cases with laparoscopic ovarian drilling, endometrial receptivity was normalized in control endometrial sampling 3 months following surgery [30].

Our study has three main limitations. First, since the effect of OHSS in the patients in the control group was not excluded, OHSS-related changes in the endometrium of these patients continue. For this reason, there is a need for future studies in which a group of only non-PCOS patients who had OHSS, whose age and BMI are matched, are added to the study. The second limitation is that it was not questioned whether the cases in the fertile group had PCOS findings. The last limitation is that comparisons could not be made in the patient and control groups with similar BMI in our study.

7. Conclusions

In conclusion, endometrium of women with PCOS who underwent total embryo freezing due to the risk of OHSS lack the physiologic inflammation conditions which are favorable for implantation. Freezing the embryo in these patients will both prevent the deterioration of OHSS and save time for the clinician in order to have the endometrium suitable for implantation.

Author contributions

CK, NT and RO conceived, designed and performed the study procedures; CK, NT and RO analyzed the data and contributed reagents and materials; CK wrote the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local Ethics Committee of the Beykent University (Approval number: 2020/11). Informed consent was obtained from all participants at the time of enrollment.

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Conflict of interest

The authors declare no conflict of interest.

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