Abstract. Polycystic ovary syndrome (PCOS), a hyperandrogenic disturbance commonly found in women of reproductive age (6%), can disturb normal fertility. The symptoms of PCOS include hyperandrogenism, oligomenorrhea, chronic anovulation, and hyperinsulinemia. Hyperandrogenism causes oocytes to be of poor quality (immature). Meanwhile, granulosa cell apoptosis could also affect oocyte quality. This study aimed to identify differences in the expression of the pro-apoptotic gene BCL-2-associated X (BAX) and anti-apoptotic gene B-cell lymphoma-2 (BCL-2) between patients with PCOS and healthy controls. In this cross-sectional study, 40 respondents (20 women with a confirmed diagnosis of PCOS and 20 controls) were recruited at the Yasmin IVF Clinic of Cipto Mangunkusumo Hospital (Jakarta, Indonesia). These respondents provided informed consent. BAX and BCL-2 levels were assayed using real-time PCR. There were no significant differences in terms of BAX (p = 0.38) or BCL-2 levels (p = 0.223) between the PCOS and control groups. The BAX/BCL-2 ratio was also not significantly different between the groups (p = 0.31). In conclusion, BAX/BCL-2 gene expression did not significantly differ between the patients with PCOS and control subjects. Further studies using larger sample sizes are warranted to confirm these findings.

1. Introduction
Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women of reproductive age, affecting 6%–8% of women in this group [1]. PCOS is characterized by hyperandrogenism, chronic anovulation, and polycystic ovaries on ultrasound. However, the etiology and underlying mechanisms of disease development remain unclear [2]. Hormonal disturbances in patients with PCOS may cause anovulation, leading to menstrual disorders and infertility. In patients with PCOS, these disturbances involve the gynecologic system and include amenorrhea or oligomenorrhea, alopecia, hirsutism, acne vulgaris, obesity, and infertility [3]. Irregular menstruation
is the most frequent symptom and one of the earliest signs of PCOS. In addition, hyperandrogenism is an important symptom in patients with PCOS, as are acne and hirsutism [4]. According to the Rotterdam criteria [5], there are three main points for establishing a diagnosis of PCOS, namely oligo/anovulation, clinical and biochemical signs of hyperandrogenism, and ultrasonographic confirmation of polycystic ovaries. At least two of these criteria are needed to establish a diagnosis, with caution that all the disease caused by hyperandrogenism are evidently not associated with current patients complaints.

In prior research, Wei et al. demonstrated that patients with PCOS had a 2-fold higher risk of changes in follicular quantity in all phases of development [6]. Although large numbers of small follicles are present in the ovaries of patients with PCOS, these women lack matured follicles due to dysfunction of the hypothalamus–pituitary–ovary axis and possible dysregulation of ovarian functions, including follicle selection and follicular atresia [7].

Cell apoptosis plays an important role in follicular development. In reproductive-age women, apoptosis mainly occurs in follicular granulosa cells, indicating possible dysregulation of cell apoptosis in patients with PCOS [6]. B-cell lymphoma-2 (BCL-2) is involved in regulating the apoptotic process [6]. In particular, BCL-2 prevents apoptosis (intrinsic pathway) by inhibiting the release/activation of caspase-9, thus decreasing mitochondrial permeability and inhibiting apoptosis. BCL-2-associated X (BAX) is a pro-apoptotic protein that can increase outer mitochondrial membrane permeability and stimulate the release of apoptotic molecules, including cytochrome c and DIABLO, thus promoting the apoptotic process. Considering the potential role of apoptosis in PCOS, we compared BAX/BCL-2 between patients with PCOS and healthy controls.

2. Methods

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. This cross-sectional study was conducted at the Yasmin IVF Clinic at Cipto Mangunkusumo Hospital (Jakarta, Indonesia). Patients were diagnosed with PCOS based on their AMH serum levels before undergoing in vitro fertilization. The diagnosis of PCOS was established using the Rotterdam 2003 criteria. Subjects with other ovarian disorders, smokers, and those who used hormonal contraceptive methods were excluded. Prior to the study, all participants provided signed informed consent. Granulosa cells were obtained via ovum pick-up and stored at −80°C. RNA isolation from granulosa cells and quantitative polymerase chain reaction were performed using an RNeasy Mini Kit, according to the manufacturer’s protocol.

3. Results

In total, 20 patients with PCOS and 20 healthy controls were enrolled in this study. The median BAX level in the control and PCOS groups was $5.73 \times 10^{-5}$ and $3.6 \times 10^{-5}$, respectively ($p > 0.05$, Table 1). Similarly, BCL-2 expression was not significantly different between the control and PCOS groups ($1.15 \times 10^{-7}$ vs. $7.8 \times 10^{-8}$, $p > 0.05$). The BAX/BCL-2 ratios in the PCOS and control groups were $1.002 \times 10^{3}$ and $5.8 \times 10^{3}$, respectively ($p > 0.05$).

Table 1. BAX and BCL-2 expression in granulosa cells

|          | PCOS (n = 20) | Controls (n = 20) | P-value |
|----------|---------------|------------------|---------|
| BAX      | $3.6 \times 10^{-5}$ | $5.73 \times 10^{-5}$ | 0.38    |
| BCL-2    | $7.8 \times 10^{-8}$ | $1.15 \times 10^{-7}$ | 0.23    |
| BAX/BCL-2| $1.0 \times 10^{3}$        | $5.8 \times 10^{3}$     | 0.31    |

Mann–Whitney U-test

BAX, BCL-2-associated X; BCL-2, B-cell lymphoma-2; PCOS, polycystic ovary syndrome

According to the aforementioned results, the rate of apoptosis was lower in the patients with PCOS than that in the healthy controls despite the insignificant associations of BAX/BCL-2 expression with PCOS.
4. Discussion
The BCL-2 protein plays an important role in apoptosis [8]. High BCL-2 protein expression is associated with inhibition of cell growth and increased cell death, whereas low BCL-2 expression leads to inhibition of cell apoptosis. Meanwhile, BAX, a member of the BCL-2 family, can inhibit the proto-oncogenic action of BCL-2 by inducing the apoptotic process. In the present study, BCL-2 and BAX mRNA levels were lower in the patients with PCOS than in those in the healthy controls, albeit without significance. This finding contradicts previous observations of elevated apoptosis in follicular granulosa cells, particularly in antral follicles, among patients with PCOS [9].

Anti-apoptotic factors regulate granulosa cell apoptosis during the development of follicles and atresia, which can subsequently suppress the activity of caspase 3 and act as endogenous inhibitors of cell death in mammalian granulosa cells. BAX is reportedly downregulated in women with PCOS, whereas anti-apoptotic factors are overexpressed [8]. Sun et al. demonstrated that the mRNA expression of BAX was significantly decreased in granulosa cells from patients with PCOS compared with that in healthy controls [8].

The ratio between BCL-2 and BAX plays an important role in maintaining the balance of cell survival and death, as high apoptotic activity is always associated with low BAX/BCL-2 ratios [10]. Previous research by Ding et al. demonstrated that the BAX/BCL-2 ratio was significantly lower in patients with PCOS than in healthy controls [11]. An increase in ovarian apoptosis due to imbalances among BCL-2 family members might be involved in the transformation of growing follicles in PCOS [12].

Meanwhile, our inability to identify differences in the BAX and BCL-2 levels between the groups could be due to the low concentration of mRNA used, thus affecting the precision and accuracy of the measurement. In addition, the ovarian stimulation performed in the subjects may have altered BAX/BCL-2 expression.

5. Conclusions
We identified no significant differences in BAX/BCL-2 expression between patients with PCOS and healthy controls. Further studies with larger sample sizes are required to clarify the roles of BAX/BCL-2 in PCOS.

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