



## Gonadotropin Stimulation's Effects on Folliculogenesis in Mild and Severe Endometriosis

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### Keywords

Folliculogenesis; mild endometriosis; number of mature follicles; severe endometriosis

### Abstract

Infertility in endometriosis has been linked to a number of theories, including a disruption in the folliculogenesis process. Further research is needed to determine whether this issue is linked to the stage of endometriosis or gonadotropin stimulation. In a cross-sectional study design, the study comprised 28 women with moderate endometriosis and 25 women with severe endometriosis who had had in vitro fertilization. The data includes the total number of follicles, mature follicles, and ovarian responses. Bivariate and multivariate analysis were used to examine the data. In bivariate analysis, the t-test and chi-square were utilized. There was no significantly difference in number of follicles total between the two groups (15.2 vs. 10.6;  $p > 0,05$ ). In comparison to the severe endometriosis group, the moderate endometriosis group had considerably more mature follicles (2.891.62 vs. 1.761.17;  $p < 0,05$ ). In the severe endometriosis group, insufficient ovarian response was more likely (RR 1.26 ;  $p < 0.018$ ). The number of follicles in total is the same in mild and severe endometriosis, but mild endometriosis has more developed follicles, and severe endometriosis has a higher rate of inadequate ovarian response.



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### 1. Introduction

Endometriosis and unexplained infertility occur in  $\pm 25\%$  of infertile couples without menstruation problems, tubal factors, or sperm factors. The exact mechanism by which endometriosis impacts fertility has yet to be discovered (Macer & Taylor, 2012). Although Previous research has attempted to answer this topic, the concept that endometriosis causes infertility is still controversial. Several pathways suspected to be involved with infertility in women with endometriosis are described by some publications. Fertility disorders are caused by anatomical distortions caused by pelvic adhesions and the influence of endometriotic implant products. They harm oocyte development, tubal transport, impaired folliculogenesis, impaired follicular steroidogenesis, embryonic development, and cytokines and prostaglandins ultimately affecting the embryo's development. As a result, the implantation process is sped up, resulting in lower implantation and pregnancy rates (Jørgensen et al., 2017; Pahlajani & Falcone, 2010). The reason for decreased implantation rate is still unknown, but it involves some parameters, including the egg and embryo quality. The poor quality of these oocytes is suggested to have started during the folliculogenesis phase before they were released into the abdominal cavity and interacted with peritoneal fluid. This implies a disruption in follicular function and a suppression of the LH surge, resulting in a reduction in

the oocyte's ability to fertilize (Sanchez et al., 2017). Endometriosis causes infertility, independent of the localization and extent of endometrial lesions. In the absence of adhesions to the pelvic cavity, the mechanism of infertility linked with endometriosis is unknown. There are several potential causes, including abnormal folliculogenesis, ovulatory dysfunction, hyperprolactinemia, luteal phase issues, fast ovum transit, sperm phagocytosis, poor fertilization, embryotoxicity during early embryonic development, and implantation abnormalities (Donnez, Binda, Donnez, & Dolmans, 2016; Lin et al., 2020).

Endometriosis infertility management is complicated, fraught with issues, and conflicts in terms of diagnosis, therapy, or long-term complications. Endometriosis infertility can be treated surgically, medically, or with a mix of both. To increase the success of pregnancy can be done by superovulation with gonadotropins followed by Assisted Reproductive Technology (Kotlyar et al., 2017; Soares, Martínez-Varea, Hidalgo-Mora, & Pellicer, 2012; ulvinder & Gautam, 2016). Previous studies have shown that endometriosis reduces fertilization and implantation rates, but it is not clear whether this is related to oocyte quality or not (Harb, Gallos, Chu, Harb, & Coomarasamy, 2013). The relationship between follicular maturity and the stage of endometriosis is still controversial. Although ovarian stimulation and IVF appear to enhance the number of oocytes, the chance of fertilization remains lower than infertility caused by other factors. It remains unclear whether gonadotropin stimulation can improve folliculogenesis disorders in both mild endometriosis and severe endometriosis. We conducted this research to address these issues.

## 2. Materials and Methods

Cross-sectional study design was employed in the investigation. The medical records of endometriosis-related infertility patients who underwent IVF at Permata Hati Clinic, Dr Sarjito Hospital, Yogyakarta, Indonesia were a source of secondary data for our study. Endometriosis was diagnosed based on the findings of a surgical laparoscopy. Endometriosis is classified into four phases by the American Society for Reproductive Medicine (ASRM) depending on the degree and depth of endometriotic lesions. In this study, stages I and II were characterized as mild endometriosis, whereas stages III and IV were labeled as severe endometriosis. In total, 53 infertile patients in all met the inclusion and exclusion criteria for the research. The research excluded patients with endometriosis infertility who had post-laparoscopy hormone treatment, patients who stopped their cycles, and patients who did not get gonadotropin-stimulated ovulation stimulation. There were 28 patients with moderate endometriosis and 25 individuals with severe endometriosis. Endometriosis stage served as the study's independent variable, while the quantity of total and mature ovarian follicles served as the study's dependent variables. The statistical analysis would take into consideration factors including age, the duration of infertility, BMI, basal E2 and basal FSH levels, the kind of stimulation protocol employed, and the needed dosage of gonadotropins. Data were analyzed with t-test and chi-square test. To establish significance, a p-value of 0.05 was employed.

## 3. Results and Discussions

### Comparability of research groups:

The study participants' characteristics in terms of BMI, length of infertility, and baseline estradiol levels were similar between the mild and severe endometriosis groups. Meanwhile, there were significant differences ( $p < 0.05$ ) in age, baseline FSH levels and the necessary dosage of gonadotropins. The next section will look at how much age, FSH baseline levels, and gonadotropins affect the number of follicles using linear regression (Table 1).

**Table 1 Characteristics of subjects in the minimal-mild and moderate-severe endometriosis groups**

| Characteristics                | Minimal-mild endometriosis (n = 28) | Moderate- severe endometriosis (n = 25) | P Value |
|--------------------------------|-------------------------------------|---|---------|
| Age (years)                    | 31,64 ± 4,58                        | 34,36 ± 4,49                            | 0,034*  |
| BMI                            | 21,99 ± 2,28                        | 22,98 ± 3,16                            | 0,191   |
| Durationof infertility (years) | 4,75 ± 3,37                         | 6,56 ± 4,28                             | 0,92    |

|                                       |                  |                   |        |
|---------------------------------------|------------------|-------------------|--------|
| Basal E2<br>(pg/mL)                   | 47,14 ± 25,6     | 36,34 ± 17,39     | 0,82   |
| Basal FSH<br>(mIU/mL)                 | 5,92 ± 2,29      | 9,57 ± 8,23       | 0,028* |
| Total<br>gonadotropin count (IU)      | 1909 ±<br>780,03 | 2667 ±<br>1237,32 | 0,01*  |
| Daily amount of<br>gonadotropins (IU) | 224,22<br>±102,3 | 302,5 ± 123       | 0,015* |

**The dependent variable's relationship with the independent variable:** The overall number of follicles was comparable across the two groups, however the proportion of mature follicles differed significantly ( $p < 0.05$ ). The probability of a poor response to gonadotropins is 1,261 times higher in severe endometriosis than in moderate endometriosis. ( $p < 0.05$ ).

**Table 2 Number of follicles in the minimal-mild and moderate-severe endometriosis group**

| Parameter                     | Minimal-mild<br>endometriosis<br>(n = 28) | Moderate- severe<br>Endometriosis<br>(n = 25) | P-Value |
|-------------------------------|---|---|---------|
| Total<br>number of follicles  | 15,21 ± 11,097                            | 10,6 ± 6,0028                                 | 0,07    |
| Number of<br>mature follicles | 2,89 ± 1,618                              | 1,76 ± 1,17                                   | 0,006   |

**Table 3 Relationship of endometriosis stage with ovarian response**

| Group                         | Ovarian response |              | RR<br>(95% CI)   | P-<br>Value |
|-------------------------------|------------------|--------------|------------------|-------------|
|                               | Bad              | Good         |                  |             |
| Endometriosis<br>is moderate  | 25<br>(100%)     | 0<br>(0%)    | 1,261<br>(1,047- | 0,018       |
| Minimal-mild<br>endometriosis | 22<br>(78,6%)    | 6<br>(21,4%) | 1,518)           |             |

**The link between independent factors, external variables and dependent variables:** Multivariate Analysis was used to examine the aggregate relationship between independent variables, external variables, and dependent variables. In the group of women with mild and severe endometriosis, linear regression analysis was used to investigate the relationship between the number of mature follicles and the confounding factors (duration of infertility, age, BMI, baseline estradiol levels, basal FSH levels, and number of gonadotropins). The number of mature follicles exhibited a weak relationship with the basal FSH level, according to the bivariate correlation between the number of mature follicles and other parameters ( $r = 0.32$ ;  $p < 0.05$ ). This demonstrates that the level of FSH in the blood has only a minimal influence on the number of mature follicles (Table 4). In other words, the stimulation response of gonadotropin is more influenced by the stage of endometriosis compared to other factors.

**Table 4 Results of bivariate correlation analysis and linear regression between the number of mature follicles and other variables**

| Variable           | Bivariate correlation   |       |
|--------------------|-------------------------|-------|
|                    | Correlation coefficient | P     |
| Age                | -0,1                    | 0,41  |
| BMI                | -0,05                   | 0,72  |
| Infertile duration | -0,16                   | 0,24  |
| Basal E2 level     | 0,07                    | 0,63  |
| Basal FSH level    | 0,32                    | 0,02* |

## Discussion

Study patients in the mild and severe endometriosis groups were similar in terms of mean age, duration of infertility, BMI, and baseline estradiol levels. However, they differed in terms of basal FSH levels and the number of gonadotropins required. The correlation of confounding variables with the number of follicles, followed by multivariate analysis with linear regression, revealed that the baseline FSH level did not affect the number of follicles. The main outcomes in this trial were the number of total follicles, mature follicles, and ovarian response. The number of all follicles in various stages did not differ significantly between mild and severe endometriosis, but the number of mature follicles was lower in severe endometriosis. This condition implies a follicular maturation abnormality, in which the number of developed follicles is smaller than the total number of follicles.

The results of this study almost the same as previous research. The endometriosis group showed inadequate ovarian response to gonadotropins, lower preovulatory E2 levels, and a significantly higher number of mature follicles compared to ordinary women. Ovarian endometriosis and cystectomy are linked to diminished ovarian reserve and ovarian sensitivity to gonadotropin stimulation. However, there was no difference in implantation and pregnancy rates between the two groups (Monsanto et al., 2016).

IVF outcome in patients with stage IV endometriosis following surgical therapy was poorer than in patients with tubal factor infertility of the same age. It means that the low ovarian response is not related to age (Senapati, Sammel, Morse, & Barnhart, 2016). In this study, it appears that the age in the mild and severe endometriosis groups did not differ. Furthermore, the ovaries' reaction, the number of all follicles, and the number of mature follicles were unaffected by age. There are two important markers of deterioration in reproductive function in women who are getting older: a drop in quantitative ovarian response (low responders) and a decrease in ovarian follicle reserves, as shown by low oocyte quality and a significantly reduced potential to produce a pregnancy.

The follicle's ability to respond to ovulation induction decreased in the severe endometriosis group. In comparison to mild endometriosis and tubal factor infertility, the findings of this research support prior studies that states that severe endometriosis leads to poor IVF outcomes. Endometriosis contributes to an unfavorable environment that obstructs the maturation, fertilization, and implantation of oocytes (Pop-Trajkovic et al., 2014). The establishment of endocrine problems and folliculogenesis disorders is one of the pathways linked to infertility in endometriosis. Endometriosis is linked to ovarian function hormonal disorders, such as luteinized unruptured follicle syndrome, luteal phase abnormalities, and aberrant follicular development. In endometriosis woman, there are high amounts of endothelin-1 and low levels of the LH receptor in the granulosa cells during the follicular phase. Furthermore, this condition inhibits the process of steroidogenesis in the follicles (Liu, Han, Liu, Zhu, & Li, 2017; Stille, Birt, & Sharpe-Timms, 2012).

Immunobiologically, the process of hormonal disturbances and follicular growth disorders is related to the production of cell cytokines. IL-6 levels increase in women's natural cycle with endometriosis and decrease in the stimulated cycle of IVF. Increased IL-6 in endometriosis will affect the endocrine system and suppress estrogen production in the proliferative phase (Malutan et al., 2015). Impaired follicular growth in endometriosis patients is associated with increased apoptosis of follicular granulosa cells, which causes granulosa cell damage and impaired synthesis of the hormones estrogen and progesterone (Marquardt, Kim, Shin, & Jeong, 2019). The high apoptotic bodies of granulosa cells in endometriosis patients undergoing ovulation induction were associated with a low quantity and oocyte quality. The level of apoptotic bodies increases with the degree of endometriosis, and the presence of endometriomas increases in number (Clement et al., 2018). Oxidative stress in endometriosis lesions is related to DNA damage. Increased stress oxidative negatively correlated to preovulatory estradiol levels, embryo quality, and fertilization rates (Prieto et al., 2012). The disturbance of follicular maturation in this study may also be through the exact mechanism described above. According to several studies, endometriosis patients had higher peritoneal macrophage activity, increased concentrations of prostaglandins, IL-1, TNF, and proteases (Taylor, Kane, & Sidell, 2015). This fluid abnormality affects gametes, embryos, and tubal function. The conception rate in monkeys with moderate endometriosis is as high as 35%. It is just 12% in late stages, and even if there is ovary adhesion, the pregnancy rate might be 0%. Pelvic adhesions interrupt ovum release, restrict sperm transport to the peritoneal cavity, and inhibit tubal oocyte pickup, tubal motility, and tubal patency, all of which contribute to infertility (Bollig et al., 2023).

## 4. Conclusion

There was no difference in the number of total follicles between mild and severe endometriosis. The number of developed follicles was greater in moderate endometriosis, while ovarian response was lower in severe endometriosis.

Alfaina Wahyuni conceptualizes, designs research, analyzes data and develops results and discussion sections; Ichsanuddin Rizky Verifianto did the data collection. All the authors have read and approved the final manuscript.

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