Apoptotic Pathways and Anti-Müllerian Hormone affects the Ovarian Tissue Damage during Endometrioma Cystectomy

Tanzil al Hair [1*], Wachyu Hadisaputra [2], Soegiharto Soebijanto [2], Primariadewi Rustamadji [2]

Abstract

Background: Endometriomas are cysts caused by endometriosis that can impair ovarian function and fertility. Anti-Müllerian hormone (AMH) is an important marker for ovarian reserve. This study aimed to determine the optimal timing of operative management for endometrioma by evaluating the impact of cyst size on ovarian tissue damage and hormone levels. Methods: The study included 32 endometrioma patients who underwent laparoscopic cystectomy between February 2018 and December 2019 at Women and Children's YPK Hospital, Jakarta. Patient characteristics were recorded, including age, parity, BMI, and cyst diameter. Serum AMH levels were measured using ELISA before and one month after cystectomy. Immunohistochemical examination assessed apoptotic factor gene expression on the inner surface epithelium of endometriomas. Results: AMH levels declined post-cystectomy in both endometriomas ≤4 cm and >4 cm, with significant differences in the latter group. Additionally, there was increased Bax expression in >4 cm endometriomas. Correlation tests revealed a strong positive relationship between TNFR1 and Caspase-3 in both groups. Multivariate analysis suggested a connection between apoptotic factor gene expression and reduced AMH levels. Conclusion: TNF-α appears to initiate apoptosis in endometriomas through the intrinsic pathway. It is advisable to perform endometrioma surgery when the diameter is ≤4 cm and before the age of 30 to achieve optimal outcomes.

Keywords: AMH, apoptosis, caspase-3, caspase-8, caspase-9, gene expression, endometrioma, intrinsic pathway, P53, Bcl-2/Bax ratio, cytokine TNF-α.

Significance | Early laparoscopic cystectomy for endometriomas, especially before 4 cm diameter, preserves ovarian function, preventing significant AMH decline and reducing long-term fertility risks.

*Correspondence. Tanzil al Hair, Department Obstetrics and Gynecology, Faculty of Medicine Universitas Indonesia, Indonesia.
E-mail: tanzilalhair11@gmail.com

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Introduction

Endometriosis, a medical disorder characterized by the growth of endometrial tissue outside the uterus, causes an endometrioma, an ovarian cyst. Endometriomas, also known as brown cysts, are cysts in the ovaries that contain menstrual blood or endometrial tissue (Benagiano et al. 2016). Endometriosis, a disorder characterized by the growth of endometrial tissue outside the uterus, is the primary cause of endometriomas. The World Health Organization (WHO) aims to advocate for and support the adoption of effective strategies and measures to tackle endometriosis worldwide, with a specific emphasis on low- and middle-income nations. WHO (2023), will work together with a range of partners, such as educational institutions, non-state organizations, and other research-focused groups, to identify effective approaches for preventing, diagnosing, treating, and providing care for endometriosis. Roughly 10%

Author Affiliation.
[1] Department Obstetrics and Gynecology, Faculty of Medicine Universitas Indonesia, Indonesia
[2] Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia

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Endometriosis and endometriomas, prevalent disorders globally, affect approximately 10% of women in their reproductive years (Cardoso 2020). cystectomy is a frequently used surgical technique in gynecology (Brilhante et al. 2017). The consequences of ovarian tissue damage resulting from endometrioma cystectomy might be substantial, particularly for women desiring to conceive (Vercellini et al. 2014). Endometriosis, as described by Ballard et al. (2008), is a prevalent condition where endometrial glands grow outside the uterus, leading to potential health concerns and a detrimental impact on the patient's lifestyle. The primary symptoms linked to this uterine-infiltrating ailment include intense pain, which can significantly affect specific patients, as well as the inability to conceive (Abrao, Muzii, and Marana 2013). Dysmenorrhea, dyspareunia, low back pain, tenesmus, painful bowel movements, pelvic pain that persists, and urinary dysfunction are among the common complaints (Andres 2014).

Endometriomas are ovarian cysts caused by endometriosis. These cysts account for roughly 17–44% of all cases of endometriosis. Endometriomas induce pain and infertility by inflicting harm on the adjacent ovarian tissue (Brosens et al. 2013; Fassbender et al. 2015). Drug administration alone is ineffective in treating endometrioma; hence, cystectomy surgery is necessary. However, the use of cystectomy surgery is still a topic of debate (Garcia-Velasco and Somigliana 2009; Kitajima et al. 2014; Margarida 2017). Cystectomy can result in the extraction of viable ovarian tissue, as well as a reduction in ovarian reserve (Barnhart, Dunsmoor-Su, and Coutifaris 2002; Raffi, Shaw, and Amer 2012; Yeung et al. 2011). In order to assess the decline in ovarian reserve, the levels of anti-mullerian hormone (AMH) are evaluated (Brosens et al. 2014; Gardner 2011; Keyhan et al. 2015; Obstet et al. 2016). The diameter of an endometrioma increases, leading to a drop in the value of anti-Müllerian hormone (AMH). The AMH value will also decrease if the endometrioma remains untreated surgically (Carnahan et al. 2013; Chen et al. 2014). Postponing surgery exacerbates ovarian harm (Chen et al. 2014; Gordts et al. 2015; Rosen et al. 2012). 71–83% of recurring cases detect endometriosis advancement during a second laparoscopy (Fassbender et al. 2015; Keyhan et al. 2015). Brosens et al. (2014), Kitajima et al. (2011), Obstet et al. (2016), it is necessary to remove tumor tissue in order to safeguard ovarian reserve. This should be done early, before the tumor reaches a size of 4 cm. Endometriosis causes the initiation of inflammatory responses in the abdominal cavity, which negatively impact the quality and quantity of oocytes. These effects are further intensified by the presence of endometriomas. Endometriomas serve as an indicator of the extent of endometriosis disease (Briley et al. 2016; Matsuzaki 2010). According to Gupta et al. (2008), endometriosis patches in the peritoneal area affect 99% of instances with an established endometrioma, impairing the patient's ability to become pregnant. It has been mentioned by various researchers (Brosens et al. 2004; Foti et al. 2018; Koninckx et al. 2021) in their studies.

The peritoneal fluid of patients with endometriosis has a higher concentration of macrophages. 22 A measure of inflammation, tumor necrosis factor alpha (TNF-α), is produced by macrophages. Patients with endometrioma exhibit a significant presence of TNF-α in their blood, peritoneum, and endometrioma fluid, along with other harmful chemicals. Toxic substances cause harm to the surrounding healthy tissue of the ovary, resulting in fibrosis. This, in turn, leads to a decrease in the density of follicles (Isono et al. 2019; Somigliana et al. 2012; Sugita et al. 2013). It is important to understand the function of TNF-α in regulating the damage to healthy ovarian tissue surrounding endometrioma. An investigation was conducted to examine the TNF-α receptors present on the surface of endometrioma epithelial cells. We can assess epithelial damage, which acts as a natural protective barrier, by examining the expression of specific genes involved in apoptosis. We can determine apoptosis, a form of programmed cell death, by comparing the levels of Bax expression, a pro-apoptotic factor, to the expression of Bcl-2, an anti-apoptotic factor, and by measuring caspase-3 activity, which executes cell death (Obstet et al. 2016; Sugita et al. 2013). In endometrioma patients, it is possible to prevent harm to ovarian tissue by removing the cyst and its protective capsule from the ovarian tissue as soon as possible with a cystectomy (Canis et al. 2001; Celik et al. 2012). This procedure can lead to the excision of healthy tissue surrounding the endometrioma, resulting in the depletion of follicles (Raffi et al. 2012). Granulosa cells in ovarian follicles synthesize Anti-Müllerian hormone (AMH). A reduction in AMH levels might serve as an indicator of the remaining egg supply in the ovaries, also known as the ovarian reserve (Iwase et al. 2010; Somigliana et al. 2012; Sugita et al. 2013). The European Society of Human Reproduction and Embryology, or ESHRE, suggests doing laparoscopic cystectomy for endometriomas in cysts that are equal to or larger than 3 cm. Identifying an endometrioma already damages the adjacent healthy tissue, and delaying the cystectomy will worsen the condition of the surrounding healthy tissue (Matsuzaki 2010). Nevertheless, the intensity of discomfort does not correlate with the extent of ovarian injury (Porpora et al. 1999; Shi et al. 2011). As a result, cystectomy is recommended for endometrioma patients who do not experience pain. The impact of early cystectomy on ovarian reserve before the endometrioma reaches a size of 3 or 4 cm is currently uncertain in terms of its benefits or drawbacks. Furthermore, it remains unclear how much the endometrioma has affected the healthy tissue surrounding it before its diameter grows to 3 or 4 cm, particularly in terms of ovarian tissue apoptotic factor expression (Bast 2011; Miller, Samec, and Alexander-Bryant 2021; Vercellini et al. 2018). We must decide when to perform a cystectomy.

The objective of this study is to establish the best time for a cystectomy while maintaining ovarian reserve by analyzing the damage to ovarian tissue in endometriomas with dimensions of < 4 cm and > 4 cm. The research aims to provide evidence for the benefits of early surgical intervention by comparing AMH values and the expression of apoptotic genes (p53, Bcl-2, Bax, caspase-8, caspase-9, caspase-3, and TNF-α receptor) before and after cystectomy. Because the results may improve ovarian reserve protection in endometrioma-affected women, lowering the chance of infertility, they will have important socioeconomic ramifications. Theoretical implications include a more profound understanding of the relationship between the growth of endometriomas and the harm inflicted on ovarian tissue, facilitated by apoptotic pathways. The study also seeks to innovate by identifying biomarkers that might predict the optimal timing for cystectomy, which could potentially result in more personalized and efficient treatment approaches for individuals with endometrioma.

Material and methods

Research Sample

This quasi-experimental study was conducted to evaluate the impact of laparoscopic cystectomy on serum Anti-Müllerian Hormone (AMH) levels in patients with endometrioma. The study was performed at RSIA YPK Jakarta and RSIA Bunda Jakarta from February 2018 to December 2019. A total of 44 patients were initially recruited, but only 32 subjects met the inclusion criteria and completed the study. Inclusion criteria were women aged 18 to 45 years with endometriomas ≤7 cm in diameter and ≤2 cysts, regular menstrual cycles, no hormonal treatment or contraceptive use for three months prior, and no pregnancy or history of recurrent miscarriages. Exclusion criteria included patients with malignancies, previous ovarian surgeries, smoking within six months, or endocrine disorders. AMH levels were measured before and one month after surgery. Non-probability consecutive sampling was employed to select participants. The sample size was calculated using a standard formula with α=5%, β=20%, standard deviation of AMH (s=0.25), and a clinically significant difference (Δ=15%), resulting in a minimum of 16 subjects per group, with a final sample size of 32 accounting for a 10% dropout rate. This methodological approach ensures the reliability and validity of the findings regarding the effect of cystectomy on ovarian reserve as indicated by AMH levels.

Research Variables and Operational Definition

The independent variable in this study is the size of the endometrioma, while the dependent variables include AMH levels, and the expression of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, and TNFR-1. The operational definitions for these variables are as follows: Endometrioma size is measured via two-dimensional ultrasound by a specialist, with the result recorded in centimeters (Nominal). The expressions of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, and TNFR-1 are assessed through immunohistochemistry (IHC) by comparing the epithelial cell responses to negative controls, indicating cellular damage, survival, apoptosis initiation, and TNF-α induced apoptosis, respectively (Ordinal). AMH levels, before and one month after surgery, are measured using ELISA to evaluate ovarian reserve (Ordinal). The difference in AMH levels pre- and post-operation is calculated to observe changes in ovarian reserve (Ordinal). The AMH normogram is referenced from the Yasmin Clinic 2011 data to determine normal blood levels based on age (Ordinal). These precise measurements and definitions ensure accurate evaluation of the study's outcomes related to endometrioma impact and surgical effects on ovarian function.

Observation Research Methods

The preparation phase included drafting the research proposal and obtaining ethical approval and permissions for the study at RSIA Bunda and RSIA YPK Mandiri Jakarta. The study involved experienced and trained healthcare professionals, including doctors and nurses, who managed patients with endometrioma and performed laparoscopic cystectomy procedures. The researcher, with the help of these professionals, observed and recorded relevant patient data. Laboratory staff were responsible for blood sample collection and immunohistochemistry (IHC) analysis, while a pathology expert assisted in reading and documenting the IHC results. The laboratory staff also prepared materials to measure the expression of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, and TNFR-1, and conducted AMH testing.

Subjects were consecutively selected based on inclusion and exclusion criteria. Blood samples were drawn from the antecubital vein to measure preoperative AMH levels. Endometrioma capsule tissue samples were obtained via stripping during cystectomy and immediately transported fresh to the Anatomical Pathology Laboratory for paraffin block preparation and histopathological examination. IHC staining was performed, and the expression of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, and TNFR-1 was evaluated by three doctors: a general practitioner (research assistant), the primary researcher, and an anatomical pathology expert. The staining was assessed using an Olympus CX 21 light microscope, and results were documented with a Nikon Eclipse E 200 and Indomicro camera. Each variable's immunoexpression was quantified using the histoscore (H-score) formula;

$$\text{H-score}=\sum(i\times Pi) \quad (1)$$

Where ii is the staining intensity score and PiPi is the percentage of cells with positive staining. The epithelial cells were examined at three fields of view at 400x magnification. Staining intensity was categorized as weak (1), moderate (2), or strong (3), and

percentages of positive cells were scored as 1 (< 20%), 2 (20–50%), 3 (50–80%), or 4 (> 80%). IHC expression was considered weak if the H-score was < 6 and strong if the H-score was > 6, based on the receiver operating characteristic (ROC) curve. One month post-surgery, another blood sample was taken from the antecubital vein to measure postoperative AMH levels, which are expected to increase after six months. The initial blood sample was collected just before entering the operating room.

Data Management and Analytics

The collected data were processed comprehensively. Data entry was performed using SPSS version 20, including sample characteristics and the expression levels of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, TNFR-1, and AMH values before and after surgery. Each data point was coded according to the endometrioma size groups (≤ 4 cm and > 4 cm) and entered into tables categorized accordingly. Rows represented the expression levels and AMH values, while columns denoted the endometrioma size groups.

Univariate analysis was conducted to describe the characteristics of the variables in frequency distribution form. For numerical data, mean and standard deviation were used for normally distributed data, while median and range were applied for non-normally distributed data. The Kolmogorov-Smirnov test was used to assess the normality of numerical data distribution. Bivariate analysis examined the relationships between the expression levels of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, TNFR-1, and endometrioma size. It also explored the relationship between the change in AMH values before and after surgery and endometrioma size, as well as changes in preoperative AMH values against age-adjusted AMH normograms. Independent t-tests were used for normally distributed data and Mann-Whitney tests for non-normally distributed data. Bivariate analysis also assessed the relationship between preoperative and postoperative AMH values within each endometrioma size group, using paired t-tests for normally distributed data and Wilcoxon-signed rank tests for non-normally distributed data. Correlation tests determined the relationships between the expression levels of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, TNFR-1, and AMH values before and after surgery. Multivariate analysis was performed to ascertain the direction and strength of the relationships between the expression levels of p53, Bcl-2, Bax, caspase-3, caspase-8, caspase-9, TNFR-1, and postoperative AMH values. Linear regression was applied to significant variables ($p < 0.2$) assuming normal, linear, and homogeneous data distributions. A significance level of $\alpha = 0.05$ was used, with p-values < 0.05 considered statistically significant.

The study was conducted after receiving ethical approval from the Ethics Committee of the Faculty of Medicine, University of Indonesia. Blood samples were taken from patients before and after surgery, and tissue samples were obtained during the removal of the endometrioma capsule and accompanying healthy tissue. All samples and blood materials were recorded and numbered systematically. All procedures were carried out with the knowledge and consent of the patients or their families, documented in the medical records.

Results

Table 1. shows that in the paired t test there is no significant difference in the decrease in AMH values in endometriomas with a diameter of ≤ 4 cm. In endometriomas with a diameter of > 4 cm, analysis with a paired t test found a significant difference indicating that AMH values were associated with surgery.

Comparison of Delta AMH in the Endometrioma Group

In Table 2. It can be seen that the difference in the decrease in AMH (delta AMH) before cystectomy with normogram values in groups ≤ 4 cm and > 4 cm (delta AMH 2) unpaired t test does not show significant differences. In both endometrioma groups, there was also no significant difference in the decrease in AMH before and after cystectomy (delta AMH 1).

Comparison of Delta AMH in Age Groups

To determine the difference in AMH difference before and after surgery (delta AMH 1) in endometriomas ≤ 4 cm based on age, an unpaired t test was conducted and the results showed no significant difference. The difference between preoperative AMH and normal values in endometriomas ≤ 4 cm (delta AMH 2) was also not significantly different in both age groups (Table 3.).

In Table 4. Statistical tests were conducted on the endometrioma > 4 cm group in the age group (< 30 years and ≥ 30 years) on the difference between AMH before and after surgery (delta AMH 1), as well as the difference between AMH before surgery and normal values (delta AMH2).

Statistical test Table 4., there was no significant difference in the decrease in AMH endometrioma > 4 cm (delta AMH 1) in both age groups. However, delta AMH 2 of the endometrioma > 4 cm group found a significant difference (p 0.028) in the age group.

Comparison of TNF-α Receptor p53, Bcl-2 and Bax Expression

In Table 5, with IHK examination, there was an increase in TNFR-1 expression and an increase in diameter > 4 cm, but not significant. Statistical tests performed on both endometrioma diameter groups did not find significant differences in the expression of p53 and Bcl-2. A significant increase in Bax expression (p 0.010) in the endometrioma > 4 cm group. Increased Bax expression illustrates heavier tissue damage in ovarian tissue with endometrioma diameter > 4 cm.

In Table 6., immunohistochemical examination showed an increase in the expression of Caspase-8, Caspase-9, Caspase-3. Statistical tests performed on both endometrioma diameter groups found no significant differences in overall gene expression.

Correlation of Apoptosis Factor Gene Expression and Endometrioma Diameter

In Table 7, the correlation test of Caspase-3 and Caspase-9 found a strong positive relationship (r 0.780 and r 0.653) and significant (p = 0.005 and p = 0.001, respectively) in both endometrioma groups (≤ 4 cm and > 4 cm), illustrating apoptosis takes place through the instrinsic pathway and apoptosis is strongly associated with both endometrioma groups. Moderate positive association of Caspase-9 and p53 (r 0.594) and significant (p = 0.054) in endometriomas ≤ 4 cm. Moderate positive association of Bax and p53 (0.508) was not significant (p = 0.111) in endometrioma ≤ 4 cm.

In Table 7, the TNFR-1 and Caspase-3 correlation test found a very strong positive relationship (r 0.859) significant (p = 0.001) in endometrioma diameter ≤ 4 cm and moderate positive (r 0.425) significant (p = 0.055) in endometrioma diameter > 4 cm. The TNFR-1 and Caspase-9 correlation test found a strong positive relationship (r 0.747) significant (p = 0.008) in the endometrioma ≤ 4 cm group and a moderate positive correlation (r 0.495) significant (p = 0.023) in the endometrioma diameter > 4 cm. Increased expression of TNFR-1 and p53, positively related moderate (r 0.595) significant (p = 0.053) in endometrioma ≤ 4 cm and weakly positively related not significant (r 0.375 and p = 0.094) in endometrioma > 4 cm.

Comparison of AMH, Apoptosis Factor Gene Expression and Endometrioma Diameter

In Table 8., a comparison was made of AMH before and after cystectomy in both endometrioma groups. In AMH before cystectomy, there was no significant decrease in AMH in both endometrioma groups (≤ 4 cm and > 4 cm) although AMH was found to be lower in the endometrioma group > 4 cm. In Table 4.9, there was an increase in the expression of apoptotic factor genes in endometriomas > 4 cm except p53. A significant difference was found in the expression of Bax (p = 0.010).

Comparison of AMH, Apoptosis Factor Gene Expression and Age Groups

In Table 9, AMH before and after cystectomy was higher in the age group < 30 years compared to age ≥ 30 years but no significant difference was found. Comparison of apoptotic factor gene expression in both age groups (≥ 30 years and < 30 years) found no significant difference. Bax was found to be higher in the < 30 year old group but not significant.

Expression of Bax, Caspase-9 and Diameter Increase

In this study, it was found that the increase in diameter was followed by an increase in gene expression, especially Bax. Other parameters in the 3 cm cyst and 4-5 cm diameter cyst groups were also followed by an increase in gene expression except in the 6 cm diameter group where there was a downward trend.

There was an increasing trend in Bax expression values with increasing cyst diameter, but not in the expression of other genes in the study. In other gene expressions, the increase occurred up to diameter 4-5 and decreased at diameter 6.

Percentage of Positive Values of Apoptosis Factor Gene Expression

In Figure 3, it appears that in this study all factors examined showed positive values or positive.

CPI reactions with the lowest exposure presented by Caspase-8 at 25% and the highest was Bax 97%.

Figure 3. Percentage of Positive Values of Apoptosis Factor Expression

In Table 10, the number of cysts and age were found to be weakly negative and not significant, as well as postoperative, there was a weak negative relationship not significant in more than one cyst and age after surgery.

Table 10., Correlation between the number of cysts and age

In Figure 4A, there is a decrease in AMH values from normal values with increasing age of endometrioma patients who have more than one cyst and AMH values lower than normal before surgery.

Relationship between AMH-post Surgery and Age

In Figure 4B., this study found a decrease in AMH as the number of cysts increase;

In Figure 4C. it is found that the age of patients with endometrioma and more than one cyst will have a lower decrease in AMH value after surgery. The decrease in AMH value in cysts that are more than one is much lower.

In Table 11, the correlation test found a positive association of decreased AMH values before surgery with apoptotic factor gene expression. Increased p53 expression was positively associated very strongly (r 0.895) and significantly (p < 0.001) with delta AMH-2 in the endometrioma ≤ 4 cm group. Increased expression of TNFR-1 had a moderate positive relationship and was not significant with delta AMH-2 in the endometrioma ≤ 4 cm group. A moderate and significant positive relationship was found for Bax expression, in the endometrioma > 4 cm group.

In multivariate analysis Table 12. it was found that the expression of apoptotic factors was significantly positively and strongly associated with a decrease in baseline AMH levels (before surgery) in the endometrioma ≤ 4 cm group (R2 = 0.918) and significantly positively and strongly associated with the > 4 cm group.

Relationship between Apoptosis Factor and Delta AMH-1 Gene Expression

In Table 13, the correlation test of AMH difference after before surgery in the endometrioma group > 4 cm, TNFR-1 expression was found to have a strong negative relationship (r -0.687) significant (p = 0.001) and a moderate negative relationship (-0.506) significant (p = 0.019) Bax expression.

In Table 13. the endometrioma ≤ 4 cm group, TNFR-1 expression had a moderate negative relationship and was not significant. A strong negative relationship (r-0.654) significant (p = 0.029) was found in Caspase-9 expression, Caspase-3 expression (r -0.705 p = 0.010) and p53 (r -0.738 p = 0.010). The expression of these

apoptotic factors illustrated a strong association with decreased AMH after surgery, except for Bcl-2.

In Table 14, there was a very strong relationship (R2 0.957) of apoptosis factor gene expression to AMH values after surgery in the endometrioma ≤ 4 cm group. There was a moderate positive association (R2 0.405) in the endometrioma > 4 cm group in the decrease of AMH after surgery.

In this study, the gene expression of apoptotic factors was examined by means of IHK to see the role of pro-inflammatory TNF-α inducing cell apoptosis in ovarian tissue damage. TNFR-1 expression was found to be 47% strong intensity, Caspase-8 strong intensity 25%, p53 expression strong intensity 66% and Bax expression strong intensity increased 97%. This increase in Bax expression was found in both groups of endometriomas ≤ 4 cm and > 4 cm.

Bcl-2 expression (Figure 5A) was found in 48% of strong positive reactions and 97% % of cases were found in strong intensity Bax expression (Figure 5B). The normal ratio of cells in defending themselves from exposure is high anti-apoptotic (Bcl-2) and low pro apoptotic (Bax). Increased expression of Bax followed by high expression of Caspase-9 (85%) (Figure 5C) proves mitochondrial damage which shows a strong positive reaction and high expression of Caspase-3 (Figure 5D) (56%) as the executor of cell death indicates apoptosis occurs.

Figure 5. Blue arrows indicate positive CPI values, black arrows indicate negative CPI values.

Discussion

Laparoscopic Cystectomy for Endometrioma

This study divided the diameter of endometriomas into two groups, namely ≤ 4 cm and > 4 cm. This grouping refers to the HIFERI policy on the management of endometriosis pain with endometriomas. Currently, surgery is performed on large diameters. In accordance with Chen et al. (2014) Henes and Engler (2018) because AMH values decrease after 5 cm and 7 cm endometrioma diameter. In contrast to them, this study showed that the AMH value of endometriomas ≤ 4 cm had decreased before surgery. This proves that exposure to toxic substances damages healthy tissue since small diameters before reaching 4 cm (Rumph et al. 2020). In accordance with Feng, Gao, and Peng (2019), Kitajima et al. (2014), Matsuzaki (2010), reported high 8-hydroxydeoxyguanosine as an indicator of DNA damage due to oxidative stress and the formation of fibrosis in the tissue around endometriomas as an indication of chronic inflammatory processes. It is necessary to perform small diameter surgery to avoid extensive fibrosis formation...

In endometriomas ≤ 4 cm and > 4 cm, AMH values had decreased before surgery and worsened significantly after surgery in endometriomas > 4 cm. However, it was not significant in ≤ 4 cm. Endometriomas decrease ovarian reserve and the risk increases with increasing diameter. AMH values falling from normal preoperatively are in line with (Davies et al. 2019; Uncu et al. 2013). Delaying surgery is avoided because surgery of small endometriomas (≤ 4 cm) does not significantly reduce AMH values. Also to prevent worsening of ovarian reserve due to time, as well as when cystectomy of endometriomas > 4 cm.

In this study, AMH values after and before surgery for endometriomas ≤ 4 cm did not differ significantly. In line with Brosens et al. (2014), early preventive measures are needed to maintain fertility. Endometrioma surgery ≤ 4 cm is recommended, because small endometriomas without surgery have reduced AMH values. According to Dr. Kasapoglu131, the rate of AMH decline was greater in endometriomas (26.4%) than in normal ovaries (7.4%). The rate of decline based on age in normal ovaries was 0.2 ng/mL/year130. In line with Gordts (2017), loss of ovarian reserve in endometriomas < 4 cm coincides with fibrosis in the ovarian cortex. Therefore, early detection and removal of endometrioma toxic substances is necessary to minimize the risk of ovarian tissue damage with endometrioma surgery before it is > 4 cm in diameter.

Endometrioma Diameter and Age

When compared to unilateral endometriomas, large, bilateral endometriomas considerably lower AMH values. AMH readings were much lower in this study due to the age of the individuals and several endometriomas. Bilateral AMH readings were lower than unilateral AMH values both before and one month after surgery. According to research by Busacca and Vignali (2009), Hwu et al. (2011), Kitajima et al. (2011), the postoperative AMH value drop will depend on the cyst's width and bilaterality. Prior to surgery, the basal AMH value was low, which is a factor that exacerbates the fall in AMH value and should be taken seriously. In order to prevent harm early on and prevent the basal AMH value from worsening the postoperative AMH value, surgery should be performed before the diameter reaches 4 cm. 62% of the participants in this study had AMH readings that were below normal and that dramatically dropped following surgery for endometriomas larger than 4 cm. This shows the possibility of a significant drop in AMH values in the event that surgery is postponed. Large diameter surgery carries a higher risk of increased ovarian reserve depletion and decreased basal AMH levels. According to Chiu et al. (2022), there is a significant correlation between large endometriomas and the high incidence of recurrence following surgery. Surgery at small diameters is recommended because to the potential for greater loss of healthy tissue in larger diameters and the significance of the basal AMH value prior to surgery (Alammari, Lightfoot, and Hur 2017). In this study, 77% of the participants were older than 30, and 40% of the subjects had AMH values less than 1.4 ng/mL. If the embryo transfer is done at a young age, Iwase et al. (2010), Shi et al. (2011) states that these low AMH values are strongly linked to the failure

Table 1. AMH Values Before and After Cystectomy in Endometriomas ≤ 4 cm and

Endometrioma	**Sebelum**	**Sesudah**	p
≤ 4 cm	2,393 (± 1,619)	1,752 (± 1,955)	0,083
> 4 cm	1,656 (± 0,945)	1,276 (± 0,911)	**0,023**

Table 2. Association of Delta AMH 1 and Delta AMH 2 with Endometrioma ≤ 4 cm versus > 4 cm

Delta AMH	**≤ 4 cm (n = 11)**	**> 4 cm (n = 21)**	p
Delta AMH 1	-1,029 (± 0,710)	-0,599 (±0,854)	0,164
Delta AMH 2	-0,971 (±1,951)	-0,985 (±1,484)	0,982

Table 3. Delta AMH in Endometrioma ≤ 4 cm by Age Group

Delta AMH	**≥ 30 tahun (n = 7)**	**< 30 Tahun (n = 4)**	p
Delta AMH 1	-0,878 (± 0,618)	-1,292 (± 0,878)	0,380
Delta AMH 2	-0,512 (± 1,642)	-1,772 (± 2,438)	0,328

Table 4. Delta AMH in Endometrioma > 4 cm by Age Group

Delta AMH	**≥ 30 tahun (n = 13)**	**< 30 Tahun (n = 8)**	*p*
Delta AMH 1	-0,512 (± 0,761)	-0,738 (± 1,027)	0,570
Delta AMH 2	-0,441 (± 0,950)	-1,868 (± 1,817)	**0,028**

Table 5. Apoptosis Factor Gene Expression in Endometriomas < 4 cm and ≥ 4 cm

Ekspresi Gen	**≤ 4 cm (n = 11)**	**> 4 cm (n = 21)**	p
TNFR-1, Rerata (SB)	5,414 (± 3,350)	5,855 (± 3,387)	0,728
Ekspresi p53, Rerata (SB)	6,831 (± 3,304)	6,127 (± 2,270)	0,483
Bcl-2, Rerata (SB)	5,107 (± 2,328)	5,981 (± 3,691)	0,420
Bax , Rerata (SB)	11,340 (± 4,125)	14,981 (± 3,222)	**0,010**

Table 6. Expression of Caspase-8, Caspase-9, Caspase-3 in Endometriomas ≤ 4 cm and > 4 cm

Ekspresi Gen	**≤ 4 cm (n = 11)**	**> 4 cm (n = 21)**	p
Caspase-3, Rerata (SB)	5,841 (± 4,096)	7,367 (± 4,675)	0,369
Caspase-8, Rerata (SB)	3,969 (± 2,269)	4,436 (± 4,624)	0,756
Caspase-9, Rerata (SB)	8,353 (± 3,948)	11,245 (± 4,109)	0,065

Table 7. Correlation of Apoptosis Factor Gene Expression on Endometrioma Diameter

Gene Expression	≤ 4 cm (n = 11)		> 4 cm (n = 21)	
	r	*p*	r	*p*
Caspase-3 – Caspase-9	0,780	0,005	0,653	0,001
Caspase 3 – Bax	0,607	0,048	0,380	0,089
Caspase 9 – Bax	0,630	0,038	0,443	0,045
Caspase-9 – p53	0,594	0,054	0,078	0,736
TNFR-1 – Caspase-3	0,859	0,001	0,425	0,055
TNFR-1 – Caspase-8	0,467	0,147	0,231	0,313
TNFR-1 – Caspase-9	0,747	0,008	0,495	0,023
TNFR-1 – p53	0,595	0,053	0,375	0,094
TNFR-1 – Bax	0,586	0,056	0,456	0,038
p53 – Bax	0,508	0,111	0,172	0,455
Bcl-2 – Bax	0,487	0,137	0,407	0,067
Caspase 8– Bax	0,160	0,638	0,116	0,617

Table 8. AMH and Apoptosis Factor Gene Expression in Endometrioma Diameter ≤ 4 cm and > 4 cm

AMH dan Ekspresi Gen	**≤ 4 cm (n = 11)**	**> 4 cm (n = 21)**	p
AMH			
Sebelum	2,392 (± 1,619)	1,656 (± 0,945)	0,113
Sesudah	1,753 (± 1,955)	1,276 (± 0,911)	0,352
Expression			
Expression p53, Rerata (SB)	6,831 (± 3,304)	6,127 (± 2,270)	0,483
Bcl-2, Rerata (SB)	5,107 (± 2,328)	5,981 (± 3,691)	0,420
Bax, Average (SB)	11,340 (± 4,125)	14,981 (± 3,222)	**0,010**
Caspase-3, Rerata (SB)	5,841 (± 4,096)	7,367 (± 4,675)	0,369
Caspase-8, Rerata (SB)	3,969 (± 2,269)	4,436 (± 4,624)	0,756
Caspase-9, Rerata (SB)	8,353 (± 3,948)	11,245 (± 4,109)	0,065
TNFR-1, Rerata (SB)	5,414 (± 3,350)	5,855 (± 3,387)	0,728

Table 9. AMH and Apoptosis Factor Gene Expression by Age Group ≥ 30 Years and < 30 Years

AMH dan Ekspresi Gen	**≥ 30 tahun (n = 20)**	**< 30 Tahun (n = 12)**	p
AMH			
Before	1,747 (± 1,180)	2,180 (± 1,353)	0,349
After	1,350 (± 1,515)	1,590 (± 1,063)	0,635
Expression			
Ekspresi p53, Rerata (SB)	6,596 (± 2,403)	5,991 (± 3,065)	0,539
BCL-2, Rerata (SB)	6,340 (± 3,194)	4,581 (± 3,248)	0,144
Bax , Rerata (SB)	13,489 (± 4,207)	14,130 (± 3,494)	0,661
Caspase-3, Rerata (SB)	7,00 (± 3,870)	6,575 (± 5,526)	0,798
Caspase-8, Rerata (SB)	4,806 (± 4,772)	3,392 (± 1,731)	0,333
Caspase-9, Rerata (SB)	10,256 (± 4,146)	10,243 (± 4,546)	0,993
TNFR-1, Rerata (SB)	6,045 (±3,260)	5,133 (± 3,501)	0,461

Table 10. Correlation between the number of cysts and age

Kista	**r**	p
Unilateral (pre)	-0,052	0,832
Bilateral (pre)	-0,319	0,289
Unilateral (post)	-0,104	0,672
Bilateral (post)	-0,286	0,343

Table 11. Association of Apoptosis Factor Gene Expression with Delta AMH-2 Based on Endometrioma

Gene Expression	≤ 4 cm (n = 11)		> 4 cm (n = 21)	
	r	p	r	p
TNFR-1 – Delta AMH-2	0,569	0,068	0,437	0,048
p53 – Delta AMH-2	0,895	< 0,001	0,181	0,432
Bax – Delta AMH-2	0,455	0,160	0,533	0,013
Caspase-3– Delta AMH-2	0,539	0,087	0,198	0,389
Caspase-8– Delta AMH-2	-0,279	0,406	0,159	0,490
Caspase-9– Delta AMH-2	0,532	0,092	0,170	0,461
BCL-2 – Delta AMH-2	0,648	0,031	0,005	0,984

Table 12. Correlation of Apoptosis Factor Gene Expression to AMH-1 Based on Endometrioma Size

Gene Expression	**≤ 4 cm (R^2 = 0,918)**		**> 4 cm (R^2 = 0,648)**	
	Koefisien	*p*	**Koefisien**	*p*
TNFR --1	1,390	0,035	-0,620	0,016
p53	-0,989	0,024	0,182	0,386
Bax	0,371	0,127	-0,280	0,191
Caspase-3	-1,069	0,033	0,156	0,544
Caspase-8	-0,581	0,094	-0,090	0,629
Caspase-9	-403	0,180	-0,234	0,368
BCL-2	-	-	0,197	0,416

Table 13. Association of Apoptosis Factor Gene Expression with Delta AMH-1 by Endometrioma Size

Ekspresi	**≤ 4 cm (n = 11)**		**> 4 cm (n = 21)**	
	r	*p*	r	*p*
TNFR-1 - Delta AMH	-0,472	0,143	-0,687	0,001
p53 - Delta AMH	-0,738	0,010	-0,014	0,953
Bax - Delta AMH	-0,313	0,349	-0,506	0,019
Caspase-3 - Delta AMH	-0,705	0,010	-0,239	0,296
Caspase-8 - Delta AMH	0,099	0,773	-0,146	0,528
Caspase-9 - Delta AMH	-0,654	0,029	-0,484	0,026
BCL-2 - Delta AMH	-0,227	0,502	-0,027	0,906

Table 14. Multivariate Analysis of Apoptosis Factor Gene Expression on AMH-2 in the Endometrioma Group

Ekspresi	**≤ 4 cm (R^2 = 0,957)**		**> 4 cm (R^2 = 0,405)**	
	Koefisien	*p*	**Koefisien**	*p*
TNFR-1	0,491	0,286	-	-
p53	0,594	< 0,001	-	-
Bax	-0,114	0,555	0,295	0,184
Caspase-3	-0,318	0,340	0,521	0,031
Caspase-8	-0,319	0,249	-	-
Caspase-9	0,055	0,808	-	-
BCL-2	0,387	0,074	-0,301	0,165

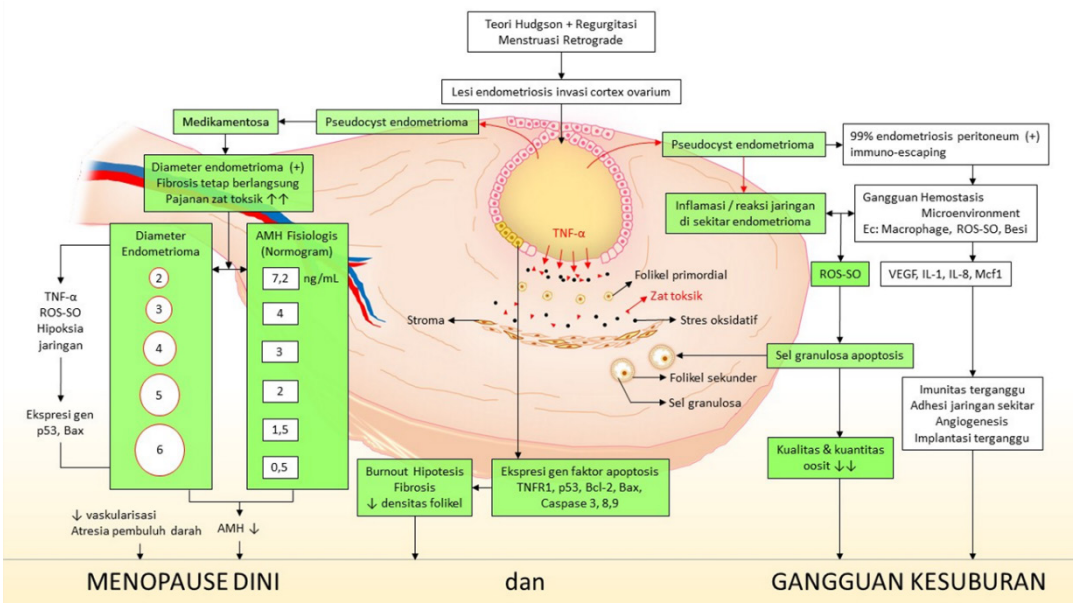

Figure 1. Theoretical Framework of Ovarian Reserve Damage in Endometrioma

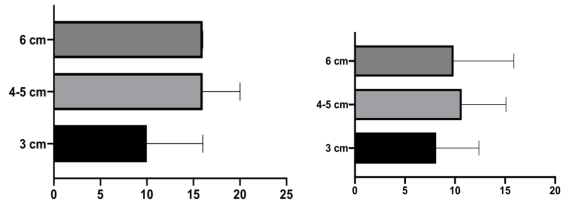

Figure 2. Bax Expression and Endometrioma Diameter Increase.

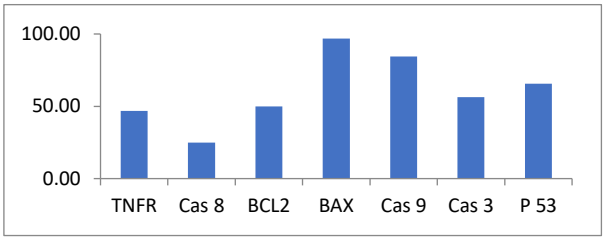

Figure 3. Percentage of Positive Values of Apoptosis Factor Expression.

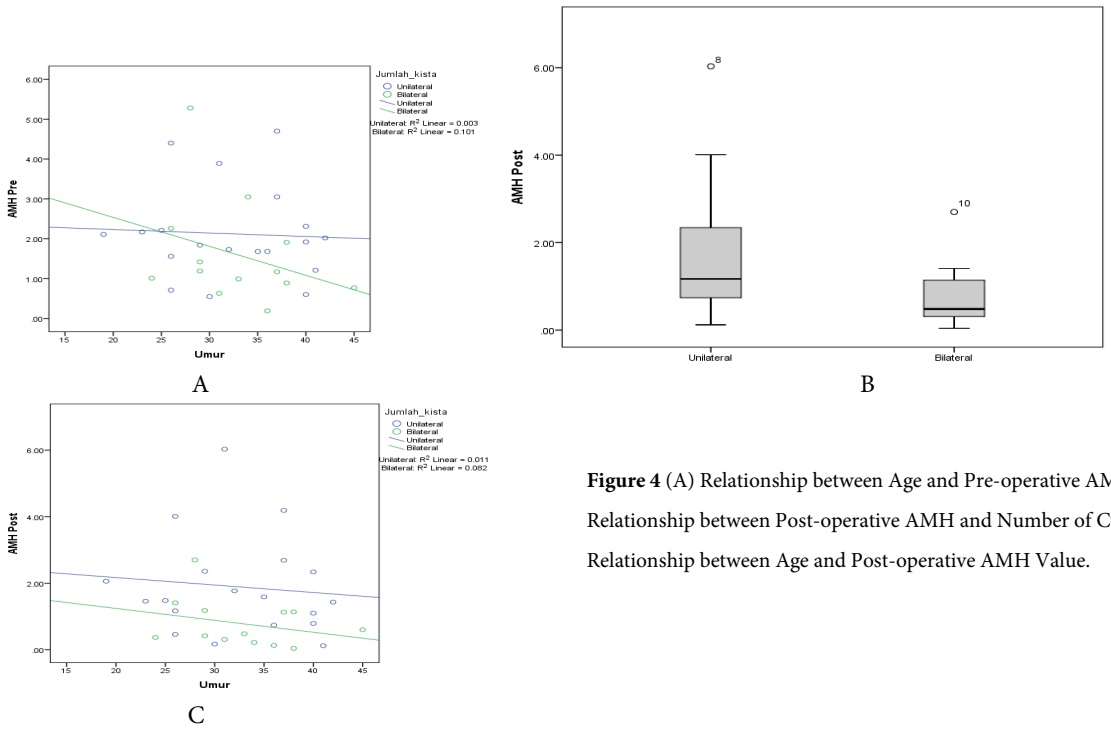

Figure 4 (A) Relationship between Age and Pre-operative AMH. (B) Relationship between Post-operative AMH and Number of Cysts. (C) Relationship between Age and Post-operative AMH Value.

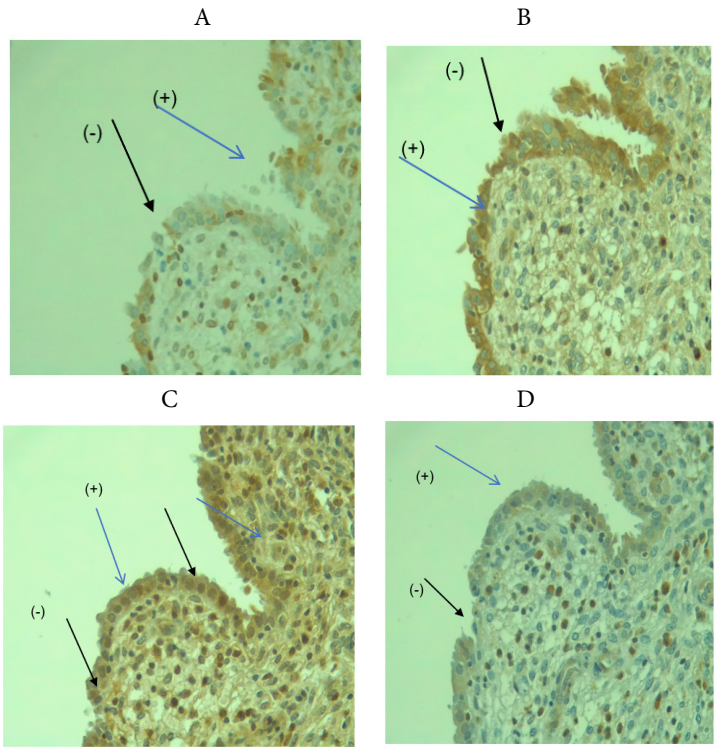

Figure 5 (A) Expression of Bcl-2; Cytoplasm of Moderately Positive Epithelium by Immunohistochemistry. **(B) Bax** Expression; Strong Positive Epithelial Cytoplasm by Immunohistochemistry. (C) Caspase-9 Expression; Cytoplasm of Strongly Positive Epithelial Cells by Immunohistochemical Reaction. (D) Caspase-3 Expression; Strong Positive Epithelial Cell Cytoplasm by Immunohistochemical Reaction

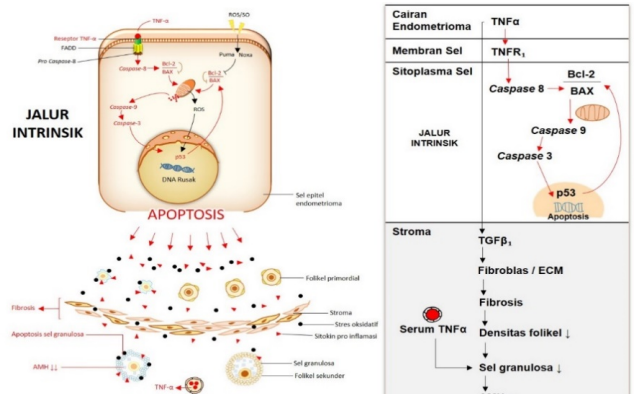

Figure 6. Proposed Mechanism of Ovarian Tissue Damage by TNF-α

of the embryo transfer process. An estimated 17–44% of endometriosis patients also have endometriomas, and 20–40% of them will use assisted reproductive technologies. AMH readings will decline with age, and Briley et al. (2016), Seifer, Baker, and Leader (2011), state that age is a significant factor in aggravating the loss of ovarian reserve. It is critical to identify endometriomas as soon as possible to preserve ovarian reserve. 25% of the individuals had an AMH value of less than 1 ng/mL when they were brought to the hospital; this indicated a poor prognosis for a successful induction of ovulation and a lowered likelihood of becoming pregnant. The group with the highest severity was composed of 6% of patients who arrived at the hospital with an AMH result below 0.5 µg/mL, which is the cutoff point for early menopause. The proportion of patients with low fertility rose following cystectomy. In that order, 31% (< 0.5 ng/mL), 44% (< 1 ng/mL), and 56% (< 1.4 ng/mL) were found. This is in line with the findings of Celik et al. (2012), who have indicated that patients with basal or preoperative AMH concentrations will have worse reproductive outcomes. Therefore, surgery should be done while AMH readings are still good in order to prevent more serious harm (Lew 2019; Sugita et al. 2013; Zikargae 2018).

The 30-year restriction used in this study is based on Isono et al. (2019), who stated that severe endometriosis was managed before the age of 30 in order to conceive. Atkins' research indicates that primordial follicles are also strongly impacted by age.49 AMH readings become basal values prior to surgery as people age, according to Briley et al. (2016), Seifer, Baker, and Leader (2011). Age is therefore a factor in this investigation. The deterioration of healthy ovarian tissue is related to age. Age-related increases in ovarian tissue damage are marked by elevated Bax expression at 30 years of age and greater attempts by the body to repair the damage by markedly raising Bcl-2 at 30 years of age. This study also indicated that age has an impact on AMH values. Before surgery, there was a statistically significant difference in the low AMH values between the age 30 and endometrioma > 4 cm groups. According to Isono et al. (2019), it is advised that attempts be made to achieve conception before the age of thirty. The findings of this study suggest that endometrioma surgery should be performed before to the age of thirty years and before the endometrioma diameter reaches four centimeters (Gordts et al. 2015).

Apoptosis Factor and AMH Gene Expression

The study observed a correlation between the growth of endometrioma and the upregulation of genes associated with apoptosis. This was demonstrated by the increased expression of TNF-α receptors, Caspase-8, Bcl-2, Bax, Caspase-9, and Caspase-3, which are known to play a role in cell death. Apoptosis of the endometrioma epithelium is enhanced, leading to the removal of natural barriers and causing more harm to the ovarian reserves, particularly the primordial follicles. According to Gupta's findings, it has been observed that granulosa cells, which are responsible for producing AMH, undergo apoptosis. The study observed a rise in apoptosis, which was subsequently accompanied by a decrease in AMH levels from their initial normal values before to surgery. There was no significant difference in the expression of apoptotic factor genes between the two endometrioma groups, one with a size of 4 cm or less and the other with a size greater than 4 cm. This demonstrates that the exposure to hazardous compounds occurs over a range of dimensions, starting from small (2.5 cm) and extending to larger (6 cm) sizes. This study demonstrates that the existence of endometrioma has caused early harm to healthy tissue and is linked to preoperative exposure to toxic substances. This study additionally demonstrated that pro-inflammatory cytokines contribute to the destruction of ovarian tissue.

The expression of caspase increased as the diameter of the endometrioma increased. The enlargement of endometrioma diameter to more than 4 cm is indicative of heightened Caspase-9 expression, which signifies mitochondrial impairment. This process commences with an upregulation of Bax expression, leading to the creation of apoptosomes that trigger the conversion of procaspase-9 into active Caspase-9. Elevated levels of Caspase-9 induce the activation of Caspase-3, leading to the initiation of programmed cell death. This diagram depicts the progressive deterioration of ovarian tissue as the size of the endometrioma increases. According to Sanchez et al. (2014), Gene expression alters the physical barrier separating cyst fluid from healthy ovarian tissue, allowing reactive oxygen species (ROS) to induce fibrosis by crossing the cyst wall. This study found that TNF-α stimulated the expression of genes associated with programmed cell death and was associated with harm to nearby healthy tissue. Brosens et al. (2004), Chiu et al. (2022), provided evidence that smooth muscle metaplasia, fibrosis of the cortical layer, and the extent of the endometrioma are associated with a decrease in AMH levels. Cystectomy is necessary to halt the enlargement of the cyst's diameter and minimize exposure to harmful substances.

Activation of TNF-α receptors by pro-inflammatory cytokines, which in turn initiated the process of programmed cell death in the epithelial cells of both endometrioma groups (≤ 4 cm and > 4 cm). This aligns with the findings of Vetvicka,11 who demonstrated the involvement of pro-inflammatory cytokines in triggering the apoptotic cascade. The IHK's response to TNFR-1 is feeble, which aligns with the findings of Salmeri's research.The number is 68. An escalation in the severity of endometriosis leads to a reduction in the level of TNF-α binding with its receptor (TNFR-1). Endometriosis is a persistent inflammatory condition. During the chronic inflammatory process, the M1 phenotype transitions into the M2 phenotype. M2 is a type of immune cell called a macrophage that plays a role in tissue regeneration and the development of fibrosis. As a result, the presence of TNF-α, a pro-inflammatory

molecule, is reduced. Hence, it is imperative to halt the inflammatory process that triggers damage to ovarian tissue and the development of permanent fibrosis once the diagnosis is established.

Gene expression is the process by which organisms modify gene transcription in order to respond to environmental factors such as hormones, heavy metals, and toxins. This work demonstrates that cells undergo a process of adaptation in response to the risk of mortality, characterized by an upregulation of Bcl-2 expression, albeit with low intensity. Similarly, studies carried out by (Dufournet et al. 2006; Korkmaz et al. 2013; Pejovic et al. 2020). revealed a low level of Bcl-2 expression in 25% of the analyzed cases. The Bcl-2/Bax ratio serves as an indicator of the cell's capacity to withstand exposure to harmful chemicals. The endometrioma epithelium exhibited a significant increase in Bax expression in comparison to Bcl-2 expression. Elevated levels of pro-apoptotic expression are indicative of the intensity of toxicant exposure, leading to cellular demise. Eliminating exposure to hazardous substances is a viable strategy to enhance the flexibility of the natural barrier in the ovaries.

The cell membrane contains TNF-α receptors, which are referred to as death receptors (DRs) or death signals. The correlation analysis between TNFR-1 expression and apoptosis occurrence revealed a robust positive association, indicating that the pro-inflammatory cytokine TNF-α initiates the apoptotic pathway in the epithelium of endometriomas. Apoptosis occurs via the intrinsic mechanism, namely including upregulation of Bax, Caspase-9, and Caspase-3. (Illustration of the proposed mechanism can be found in Figure 6) This contrasts with the findings of Haupt et al. (2003), Obstet et al. (2016), Salmeri et al. (2015), propose that the active TNF-α receptor induces apoptosis via the extrinsic pathway. Specifically, the active death receptor activates Caspase-8 and Caspase-3. Contrarily, this work aligns with Bredesen's findings that TNF-α effectively attaches to death receptors and triggers apoptosis both directly via caspases and indirectly through mitochondria.

The study found a drop in the balancing ratio of Bcl-2/Bax. The levels of pro-apoptotic Bax were higher in both endometrioma groups (≤ 4 cm and > 4 cm) and showed a significant difference. Elevated Bax expression leads to mitochondrial impairment, which serves as a vital energy source for cells.Prolonged exposure to harmful substances will result in cellular apoptosis and irreversible cellular harm. 121 Activation of death receptors triggers the production of Bax and p53, which are involved in preserving the integrity of the genome (the guardian of the genome).One hundred eighteen If cell damage cannot be healed, the presence of activated Bax alters the proportion of Bcl-2 to Bax (Depalo et al. 2009; Haupt et al. 2003). The investigation revealed a notable increase (66%) in p53 expression, indicating the presence of significant damage. Consistent with (Fauvet et al. 2005; Haupt et al. 2003). Dufournet et al. (2006) Fauvet et al. (2005), observed that the expression of p53 was elevated in borderline cysts, specifically endometriomas and ovarian cancers, compared to benign ovarian cysts. Ensuring the cessation of contact with harmful substances is of utmost importance after endometrioma is diagnosed. According to Lincenstein, mitochondrial damage is both permanent and progressively worsens from a mild to severe state.One hundred seventeen The study demonstrates a significant upregulation of Bax and p53, which are indicators of apoptosis, indicating the severity of exposure. The upregulation of p53 in this study is consistent with the findings of (Chen et al. 2014; Fauvet et al. 2005). In contrast to Nezhat's research, which found negative p53 staining in 30 cases of ovarian endometriosis cysts, this study observed positive Bcl-2 expression that aligns with its own findings. Higher levels of p53 expression serve as a sign of advancing cases that necessitate immediate intervention, hence it is advisable to refrain from postponing endometrioma surgery. All instances showed a high level of Bax expression (97%), suggesting a reduced Bcl-2/Bax ratio. Cell death occurs as a result of mitochondrial dysfunction caused by damage to the mitochondrial membrane (Bateman, Blanco, and Sheffi 2017; Heca et al. 2018). Apoptosis is observed by the upregulation of Caspase-3, which acts as the executor of cell death. This diverges from(Depalo et al. (2009), Giacomini et al. (2017), states that endometrial lesions sustain their survival by evading apoptosis or enhancing the Bcl-2/Bax ratio. Nevertheless, this distinction aligns with the viewpoint of Nisolle and Donnez (2019), who argues that endometrioma, as a cyst caused by endometriosis in the ovary, is a distinct entity from endometriosis lesions in the peritoneal cavity and deep infiltrating endometriosis located in the rectum. Therefore, medicine alone is not a successful treatment for endometrioma, and surgery is the preferred initial option.

Apoptotic factor gene expression (Bax, Caspase-9, Caspase-3) was greater in endometriomas larger than 4 cm compared to those that were 4 cm or less. There was a notable disparity in the expression of Bax, with a p-value of 0.01. The levels of Caspase-9 and Caspase-3 were elevated in endometriomas larger than 4 cm, however the difference was not statistically significant. A correlation test was conducted to examine the expression of apoptotic factor genes in the endometrioma group with a size more than 4 cm. The results showed a significant and strong positive association between Caspase-3 and Caspase-9. The expression of TNFR-1 showed a modest and statistically significant positive connection with Caspase-9 ($r = 0.495$) and Caspase-3 ($r = 0.425$). This demonstrates how the intrinsic pathway's apoptotic process is triggered by proinflammatory cytokines (TNF-α) in both endometrioma groups. This study demonstrates that gene expression reveals serious damage when the diameter exceeds 4 cm. Therefore, to prevent the widespread deterioration of the ovarian natural barrier,

it is advisable to undergo surgery prior to the ovarian diameter reaching 4 cm.

This study observed a rise in the mortality of the epithelial cells in the endomyoma wall. According to Muzii et al. (2007) out of 40% of the cyst surface lacks epithelium. The absence of a natural barrier facilitates the susceptibility of surrounding healthy tissue to damage from exposure to poisonous substances released by endometrioma. The injury is identified by a reduction in the AMH value prior to the surgical procedure. These findings align with the research conducted by (Garcia-Velasco and Somigliana 2009; Giacomini et al. 2017). They indicate that endometriomas decrease the anti-Müllerian hormone (AMH) levels in both blood and follicular fluid. Endometriomas have a detrimental impact on the surrounding tissue as a result of disruptions in iron metabolism and the occurrence of oxidative stress. Kitajima et al. (2011), in their study, observed a higher occurrence of primary follicle degeneration in the ovarian cortex of endometriomas, along with increased caspase-3 expression and reduced diameter of primordial follicles, as compared to ovaries without cysts. This study examines how the absence of the normal protective barrier in the ovaries affects the harm caused to the surrounding healthy tissue by the endometrioma. Consequently, it is imperative to perform surgery promptly upon diagnosing endometrioma in order to prevent the spread of harmful compounds from the endometrioma and prevent more harm.

Consistent with the findings of Khan et al. (2013), Kitajima et al. (2014), Matsuzaki (2010), our study demonstrates that the absence of endometrioma epithelium raises the risk of further harm to the ovarian reserve from additional hazardous substances associated with endometrioma. Measures must be implemented to safeguard the quantity and quality of oocytes, particularly the primordial follicles, as well as the ovarian reserve. Consistent with the findings of Coccia et al. (2014), Nisolle and Donnez (2019), the mesothelium that covers the ovary might fold inward, causing the primordial follicles to be displaced towards the rear of the cyst. The study found that the lack of epithelium allowed hazardous chemicals to directly access the primordial follicles. The reduction in the quantity of primordial follicles will impact the AMH level. Prior to surgery, it is crucial to assess the basal anti-Müllerian hormone (AMH) levels in order to prevent any potential ovarian dysfunction. Early surgical intervention is necessary in order to safeguard fertility and prevent harm to the adjacent healthy tissue surrounding the endometrioma. This study revealed that the expression of apoptotic factor genes influences the ability of free radicals to traverse the natural barrier. The upregulation of TNF-α receptors marks the initiation of the apoptotic pathway, leading to ovarian tissue damage and decreased AMH levels prior to surgery in both groups with endometrioma. According to Sanchez's research, the AMH value decreases as a sign of ovarian reserve loss caused by fibrosis surrounding the endometrioma. ROS, or reactive oxygen species, penetrates the cyst wall and contributes to the development of ovarian fibrosis, resulting in a decrease in follicular density. Miller et al. (2021), it has been observed that reactive oxygen species (ROS) can permeate past the cyst membrane barrier and cause harm to the surrounding healthy tissue around the endometrioma. It is crucial to inhibit the activation of death receptors by pro-inflammatory cytokines to prevent extensive damage to the ovaries (Akhtar Muhammad 2021; Foti et al. 2018).

According to a correlation test, there was a substantial positive link between TNFR-1 expression and increased expression of Bax, caspase-9, and caspase-3 with moderate, strong, and very strong results in both endometrioma groups (≤ 4 cm and > 4 cm). This demonstrates that apoptosis takes place via the intrinsic mechanism. The activity of the intrinsic pathway was enhanced by upregulation of Bax, leading to increased production of caspase-9 and caspase-3, which exhibited a highly significant positive correlation. Both sets of endometriomas had a significant correlation between the expression of apoptotic factor genes, ranging from mild to strong. Unlike Vetvicka's study, TNF-α triggers apoptosis through the extrinsic mechanism by activating receptors. This work demonstrates that exposure to the pro-inflammatory cytokine TNF-α activates the TNFR-1 receptor in the epithelium of endometrioma, leading to apoptosis via the intrinsic pathway. The presence of natural barriers to apoptosis will regulate the toxic compounds in endometriomas, particularly the pro-inflammatory cytokine TNF-α, which stimulates the development of fibrosis and decreases follicular density. (Illustration of the proposed mechanism can be found in Figure 6.

Early Detection and Treatment of Endometrioma is Important

A significant positive connection was seen between AMH levels and the expression of apoptotic factor genes in both types of endometriomas, those with a size of 4 cm or less and those larger than 4 cm. A multivariate correlation test was conducted to examine the relationship between the expression of apoptotic factor genes and the decrease in AMH values before surgery. The results revealed a highly significant positive connection (R2 0.918). A robust and statistically significant inverse correlation was seen in the expression of TNFR-1 in the group with endometriomas larger than 4 cm. According to Obstet et al. (2016), TNF-α is a pro-inflammatory cytokine that interacts to its receptor TNFR-1, leading to the initiation of apoptosis. The detrimental effects of the endometrioma facilitate the passage of other hazardous substances beyond the natural barrier, leading to destruction of the surrounding healthy tissue. This leads to the development of fibrosis and a decrease in follicular density, resulting in lower AMH readings prior to surgery. Therefore, it is crucial and paramount to prevent any additional harm (Baker et al. 2018; Lew 2019; Somigliana et al. 2012).

This study demonstrates a decline in anti-Müllerian hormone (AMH) levels prior to surgery. In the correlation test conducted on endometrioma measuring 4 cm or less, there was a robust and statistically significant positive association between p53 expression and a decrease in AMH value prior to surgery. Similarly, there is a favorable correlation between the expression of Bcl-2 and a substantial and statistically significant reduction in AMH levels prior to surgery in endometriomas that are 4 cm or less. This suggests that there is an ongoing attempt to compensate cells in order to adjust to the encountered exposure. In contrast to larger diameters (> 4 cm), there is no correlation between Bcl-2 expression and the decrease in AMH readings prior to surgery (Parameswaran and Patial 2010). Endometrioma larger than 4 cm is linked to a notable reduction in AMH levels, which is correlated with a substantial and meaningful rise in TNFR-1 and Bax expression. This suggests that the presence of harmful compounds that stimulate TNF-α receptors leads to damage in ovarian tissue, particularly when the diameter exceeds 4 cm.

This study observed a significant upregulation of Bax expression, which exhibited a positive correlation with the cyst diameter. Greater levels of Bax and caspase-9 expression were observed in endometriomas larger than 4 cm, indicating more extensive damage. The expression of Bax showed a statistically significant difference ($p = 0.01$) between both groups of endometrioma. This demonstrates that as the diameter of an endometrioma increases, there is a corresponding increase in damage to the ovarian tissue, as observed through changes in gene expression. This study demonstrates that the damage to endometriomas begins with the lowest diameter (2.5 cm) and progresses to the largest diameter (6.5 cm). This damage is associated with the pro-inflammatory cytokine TNF-α and is influenced (Molvig 1988; Parsa et al. 2018; Sipak-Szmigiel et al. 2017). Therefore, it is crucial to discover endometriomas early.

Failure to promptly address the presence of endometrioma from the outset will exacerbate the likelihood of diminishing the patient's ovarian reserve. This study revealed a significant difference in the lowering of the AMH value following cystectomy in the group with endometrioma larger than 4 cm. It is crucial to prevent ovarian function failure, particularly during reproductive years, as it would prevent the possibility of using assisted reproductive technologies in cases where the AMH value is extremely low. This study revealed that 31% of the total participants experienced a decline in their AMH readings below 0.5 ng/mL after cystectomy. This decrease serves as a threshold, indicating that patients with endometrioma may be at risk of early menopause.

The main finding of this study is that surgery for endometriomas measuring < 4 cm does not have a significant impact on anti-Müllerian hormone (AMH) levels, but surgery for endometriomas measuring > 4 cm considerably reduces AMH levels. The size of ovarian cysts directly correlates with the extent of damage to ovarian tissue, as evidenced by the upregulation of genes associated with programmed cell death. Bax expression, in particular, continues to rise as cyst width increases. The expression upregulation begins with the activation of TNF receptor-α, which subsequently triggers the intrinsic pathway, leading to an increase in the expression of Bax and caspase-9. This ultimately results in the death of epithelial cells. The presence of caspase-3 indicates apoptosis in the epithelium of endometrioma, resulting in the loss of the ovary's natural protective barrier. This process also affects the exposure of the ovary to harmful substances, which can cause damage to the healthy tissue. The presence of tissue damage in the tiny-diameter cyst, measuring 2.5 cm, and the decline in AMH values prior to surgery are apparent. The drop in anti-Müllerian hormone (AMH) levels was a result of injury to the ovarian tissue. Investigators found a positive correlation between this decrease and the expression of genes related to programmed cell death (apoptosis). Endometrioma decreases fertility, and the presence of cyst fluid jeopardizes the progression of ovarian tissue damage. To save the ovarian reserve, it is advisable to extract the fluid from endometrioma cysts before they reach a diameter of 4 cm, particularly in patients who are 30 years of age or younger.

According to this study, 50% of endometriomas have surface epithelium that has undergone apoptosis due to the pro-inflammatory cytokine TNF-α. However, given the high expression of p53 (66%), 97% of endometriomas have Bax, and 56% of them have caspase-3, it is impossible to rule out the possibility that other endometrioma-causing toxins are also responsible for seriously harming epithelial cell mitochondria. ROS, generated by the Fenton reaction, can permeate the cyst wall in endometrioma fluid (Scutiero et al. 2017).

Conclusion

Based on the findings, laparoscopic cystectomy for endometriomas smaller than 4 cm does not significantly reduce AMH levels, whereas for endometriomas larger than 4 cm, there is a significant decline in AMH. The increase in endometrioma diameter is associated with elevated Bax expression, indicating ovarian tissue damage. Laparoscopic cystectomy of endometriomas larger than 4 cm in women over 30 years significantly decreases AMH and poses a risk of ovarian failure, with 31% of cases experiencing early menopause. The expression of apoptosis-related genes shows a very strong positive correlation with AMH reduction in endometriomas smaller than 4 cm and a strong correlation in larger endometriomas. These results suggest that immediate surgical intervention for endometriomas is crucial, particularly before the diameter reaches 4 cm, to preserve ovarian function and prevent long-term reproductive complications.

Author contributions

T.A.H. was the primary researcher and was responsible for the study design, data collection, data analysis, and manuscript preparation. Dr. W. H., Dr. S. S., and Dr. P. R. provided supervision, critical revisions, and guidance throughout the research process. All authors contributed to the interpretation of the data and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Abrao, Mauricio S., Ludovico Muzii, and Riccardo Marana. 2013. "International Journal of Gynecology and Obstetrics Anatomical Causes of Female Infertility and Their Management." International Journal of Gynecology and Obstetrics 123:S18–24. doi: 10.1016/j.ijgo.2013.09.008.

Akhtar Muhammad, Kong Yusheng. 2021. Performance , and Profitability.

Alammari, Roa, Michelle Lightfoot, and Hye-Chun Hur. 2017. "Impact of Cystectomy on Ovarian Reserve: Review of the Literature." Journal of Minimally Invasive Gynecology 24(2):247–57. doi: https://doi.org/10.1016/j.jmig.2016.12.010.

Andres, Marina de Paula. 2014. "Endometriosis Is an Important Cause of Pelvic Pain in Adolescence." 60(6):560–64.

Baker, Valerie L., Clarisa Gracia, Michael J. Glassner, Vicki L. Schnell, Kevin Doody, Charles C. Coddington, Sanghyuk S. Shin, Lorna A. Marshall, Michael M. Alper, Arlene J. Morales, Mary Ellen Pavone, Millie A. Behera, Edward A. Zbella, Bruce S. Shapiro, Joely A. Straseski, and Dennis L. Broyles. 2018. "Multicenter Evaluation of the Access AMH Antimüllerian Hormone Assay for the Prediction of Antral Follicle Count and Poor Ovarian Response to Controlled Ovarian Stimulation." Fertility and Sterility 110(3):506-513.e3. doi: https://doi.org/10.1016/j.fertnstert.2018.03.031.

Ballard, K. D., H. E. Seaman, C. S. De Vries, and J. T. Wright. 2008. "Can Symptomatology Help in the Diagnosis of Endometriosis? Findings from a National Case-Control Study - Part 1." BJOG: An International Journal of Obstetrics and Gynaecology 115(11):1382–91. doi: 10.1111/j.1471-0528.2008.01878.x.

Barnhart, Kurt, Rebecca Dunsmoor-Su, and Christos Coutifaris. 2002. "Effect of Endometriosis on in Vitro Fertilization." Fertility and Sterility 77(6):1148–55. doi: https://doi.org/10.1016/S0015-0282(02)03112-6.

Bast, R. C. 2011. "Molecular Approaches to Personalizing Management of Ovarian Cancer." Annals of Oncology 22:viii5–15. doi: https://doi.org/10.1093/annonc/mdr516.

Bateman, Alexis H., Edgar E. Blanco, and Yossi Sheffi. 2017. "Disclosing and Reporting Environmental Sustainability of Supply Chains." Springer Series in Supply Chain Management 4:119–44. doi: 10.1007/978-3-319-29791-0_6.

Benagiano, Giuseppe, Felice Petraglia, Stephan Gordts, and Ivo Brosens. 2016. "A New Approach to the Management of Ovarian Endometrioma to Prevent Tissue Damage and Recurrence." Reproductive BioMedicine Online 32(6):556–62. doi: 10.1016/j.rbmo.2016.03.001.

Briley, Shawn M., Susmita Jasti, Jennifer M. McCracken, Jessica E. Hornick, Barbara Fegley, Michele T. Pritchard, and Francesca E. Duncan. 2016. "Reproductive Age-Associated Fibrosis in the Stroma of the Mammalian Ovary." Reproduction (Cambridge, England) 152(3):245–60. doi: 10.1530/REP-16-0129.

Brilhante, Aline Veras Morais, Kathiane Lustosa Augusto, Manuela Cavalcante Portela, Luiz Carlos Gabriele Sucupira, Luiz Adriano Freitas Oliveira, Ana Juariana Magalhães Veríssimo Pouchaim, Lívia Rocha Mesquita Nóbrega, Thaís Fontes de Magalhães, and Leonardo Robson Pinheiro Sobreira. 2017. "Endometriosis and Ovarian Cancer: An Integrative Review (Endometriosis and Ovarian Cancer)." Asian Pacific Journal of Cancer Prevention : APJCP 18(1):11–16. doi: 10.22034/APJCP.2017.18.1.11.

Brosens, I., P. Puttemans, Sy Gordts, R. Campo, S Gordts, and G. Benagiano. 2013. "Early Stage Management of Ovarian Endometrioma to Prevent Infertility." FVV In OBGyn 5(4):309–14.

Brosens, Ivo, Stephan Gordts, Patrick Puttemans, and Giuseppe Benagiano. 2014. "Pathophysiology Proposed as the Basis for Modern Management of the Ovarian Endometrioma." Reproductive BioMedicine Online 28(2):232–38. doi: 10.1016/j.rbmo.2013.09.024.

Brosens, Ivo, Patrick Puttemans, Rudi Campo, Stephan Gordts, and Karen Kinkel. 2004. "Diagnosis of Endometriosis: Pelvic Endoscopy and Imaging Techniques." Best Practice & Research Clinical Obstetrics & Gynaecology 18(2):285–303. doi: https://doi.org/10.1016/j.bpobgyn.2004.03.002.

Busacca, Mauro, and Michele Vignali. 2009. "Endometrioma Excision and Ovarian Reserve: A Dangerous Relation." Journal of Minimally Invasive Gynecology 16(2):142–48. doi: https://doi.org/10.1016/j.jmig.2008.12.013.

Canis, M., J. L. Pouly, S. Tamburro, G. Mage, A. Wattiez, and M. A. Bruhat. 2001. "Ovarian Response during IVF – Embryo Transfer Cycles after Laparoscopic Ovarian Cystectomy for Endometriotic Cysts of > 3 Cm in Diameter." 16(12):2583–86.

Cardoso, Jéssica Vilarinho. 2020. "Epidemiological Profile of Women with Endometriosis : A Retrospective Descriptive Study." Revista Brasileira de Saúde Materno Infantil 20(4):1057–67.

Carnahan, Molly, Jennifer Fedor, Ashok Agarwal, and Sajal Gupta. 2013. "Ovarian Endometrioma: Guidelines for Selection of Cases for Surgical Treatment or Expectant Management." Expert Review of Obstetrics and Gynecology 8(1):29–55. doi: 10.1586/eog.12.75.

Celik, Hale Goksever, Erbil Dogan, Emre Okyay, Cagnur Ulukus, Bahadir Saatli, Sezer Uysal, and Meral Koyuncuoglu. 2012. "Effect of Laparoscopic Excision of

Endometriomas on Ovarian Reserve: Serial Changes in the Serum Antimüllerian Hormone Levels." Fertility and Sterility 97(6):1472–78. doi: https://doi.org/10.1016/j.fertnstert.2012.03.027.

Chen, Yuqing, Huihui Pei, Yajie Chang, Minghui Chen, Haihe Wang, Hongzhe Xie, and Shuzhong Yao. 2014. "The Impact of Endometrioma and Laparoscopic Cystectomy on Ovarian Reserve and the Exploration of Related Factors Assessed by Serum Anti-Mullerian Hormone : A Prospective Cohort Study." 1–8. doi: 10.1186/s13048-014-0108-0.

Chiu, Chui-Ching, Teh-Fu Hsu, Ling-Yu Jiang, I. San Chan, Ying-Chu Shih, Yen-Hou Chang, Peng-Hui Wang, and Yi-Jen Chen. 2022. "Maintenance Therapy for Preventing Endometrioma Recurrence after Endometriosis Resection Surgery – A Systematic Review and Network Meta-Analysis." Journal of Minimally Invasive Gynecology 29(5):602–12. doi: https://doi.org/10.1016/j.jmig.2021.11.024.

Coccia, Maria Elisabetta, Francesca Rizzello, Stefano Barone, Sara Pinelli, Erika Rapalini, Cristiana Parri, Domenico Caracciolo, Savvas Papageorgiou, Gianpaolo Cima, and Loredana Gandini. 2014. "Is There a Critical Endometrioma Size Associated with Reduced Ovarian Responsiveness in Assisted Reproduction Techniques?" Reproductive Biomedicine Online 29(2):259–66. doi: 10.1016/j.rbmo.2014.04.019.

Davies, Melanie J., Caroline A. Kristunas, Abualbishr Alshreef, Simon Dixon, Helen Eborall, Agnieszka Glab, Lisa Huddlestone, Nicky Hudson, Kamlesh Khunti, Graham Martin, Alison Northern, Mike Patterson, Rebecca Pritchard, Sally Schreder, Bernie Stribling, Jessica Turner, and Laura J. Gray. 2019. "The Impact of an Intervention to Increase Uptake to Structured Self-Management Education for People with Type 2 Diabetes Mellitus in Primary Care (the Embedding Package), Compared to Usual Care, on Glycaemic Control: Study Protocol for a Mixed Methods Stu." BMC Family Practice 20(1):152. doi: 10.1186/s12875-019-1038-0.

Depalo, Raffaella, Aldo Cavallini, Filomenamila Lorusso, Emma Bassi, Ilaria Totaro, and Andrea Marzullo. 2009. "Article Apoptosis in Normal Ovaries of Women with and without Endometriosis." Reproductive BioMedicine Online 19(6):808–15. doi: 10.1016/j.rbmo.2009.09.024.

Dufournet, Charlotte, Catherine Uzan, Raffaèle Fauvet, Annie Cortez, Jean-Pierre Siffroi, and Emile Daraï. 2006. "Expression of Apoptosis-Related Proteins in Peritoneal, Ovarian and Colorectal Endometriosis." Journal of Reproductive Immunology 70(1):151–62. doi: https://doi.org/10.1016/j.jri.2005.11.003.

Fassbender, Amelie, Richard O. Burney, Dorien F. O, Thomas D'Hooghe, and Linda Giudice. 2015. "Update on Biomarkers for the Detection of Endometriosis-Protein and Contraction." BioMed Research International 2015.

Fauvet, Raffaèle, Charlotte Dufournet, Christophe Poncelet, Catherine Uzan, Danièle Hugol, and Emile Daraï. 2005. "Expression of Pro-Apoptotic (P53, P21, Bax, Bak and Fas) and Anti-Apoptotic (Bcl-2 and Bcl-x) Proteins in Serous versus Mucinous Borderline Ovarian Tumours." Journal of Surgical Oncology 92(4):337–43. doi: https://doi.org/10.1002/jso.20424.

Feng, Guohua, Jiti Gao, and Bin Peng. 2019. "An Integrated Panel Data Approach to Modelling Economic Growth."

Foti, Pietro Valerio, Renato Farina, Stefano Palmucci, Ilenia Anna, Agata Vizzini, Norma Libertini, Maria Coronella, Saveria Spadola, Rosario Caltabiano, Marco Iraci, Antonio Basile, Pietro Milone, Antonio Cianci, and Giovanni Carlo Ettorre. 2018. "Endometriosis : Clinical Features , MR Imaging Findings and Pathologic Correlation." 149–72.

Garcia-Velasco, Juan A., and Edgardo Somigliana. 2009. "Management of Endometriomas in Women Requiring IVF: To Touch or Not to Touch." Human Reproduction (Oxford, England) 24(3):496–501. doi: 10.1093/humrep/den398.

Gardner, David K. 2011. Human Assisted Reproductive Technology.

Giacomini, Elisa, Ana M. Sanchez, Veronica Sarais, Soha Al Beitawi, Massimo Candiani, and Paola Viganò. 2017. "Characteristics of Follicular Fluid in Ovaries with Endometriomas." European Journal of Obstetrics, Gynecology, and Reproductive Biology 209:34–38. doi: 10.1016/j.ejogrb.2016.01.032.

Gordts, Stephan, Philippe Koninckx, and Ivo Brosens. 2017. "Pathogenesis of Deep Endometriosis." Fertility and Sterility 108(6):872-885.e1. doi: https://doi.org/10.1016/j.fertnstert.2017.08.036.

Gordts, Stephan, Patrick Puttemans, Sylvie Gordts, and Ivo Brosens. 2015. "Ovarian Endometrioma in the Adolescent: A Plea for Early-Stage Diagnosis and Full Surgical Treatment." Gynecological Surgery 12(1):21–30. doi: 10.1007/s10397-014-0877-x.

Gupta, Sajal, Jeffrey M. Goldberg, Nabil Aziz, Eric Goldberg, Natalie Krajcir, and Ashok Agarwal. 2008. "Pathogenic Mechanisms in Endometriosis-Associated Infertility." Fertility and Sterility 90(2):247–57. doi: https://doi.org/10.1016/j.fertnstert.2008.02.093.

Haupt, Susan, Michael Berger, Zehavit Goldberg, and Ygal Haupt. 2003. "Apoptosis – the P53 Network." doi: 10.1242/jcs.00739.

Heca, Cancer Cell Line, Meryem İ. Karagül, Derya Yetkin, Gülsen Bayrak, and Didem Çelikcan. 2018. "P53 , Bcl2 and Bax Expression and Apoptosis in Perifosine and Vitamin D-Treated Endometrial." 25:3–7. doi: 10.3390/proceedings2251564.

Henes, Melanie, and Engler. 2018. "Ovarian Cyst Removal Influences Ovarian Reserve Dependent on Histology, Size and Type of Operation." Women's Health 14:1745506518778992. doi: 10.1177/1745506518778992.

Hwu, Yuh-ming, Frank Shao-ying Wu, Sheng-hsiang Li, Fang-ju Sun, Ming-huei Lin, and Robert Kuo-kuang Lee. 2011. "The Impact of Endometrioma and Laparoscopic Cystectomy on Serum Anti-Müllerian Hormone Levels." Reproductive Biology and Endocrinology 9(1):80. doi: 10.1186/1477-7827-9-80.

Isono, Wataru, Osamu Wada-Hiraike, Nana Akino, Hiromi Terao, Miyuki Harada, Tetsuya Hirata, Yasushi Hirota, Kaori Koga, Tomoyuki Fujii, and Yutaka Osuga. 2019. "The Efficacy of Non-Assisted Reproductive Technology Treatment Might Be Limited in Infertile Patients with Advanced Endometriosis in Their 30s." Journal of Obstetrics and Gynaecology Research 45(2):368–75. doi: 10.1111/jog.13826.

Iwase, Akira, Wakana Hirokawa, Maki Goto, Sachiko Takikawa, Yoshinari Nagatomo, Tatsuo Nakahara, Shuichi Manabe, and Fumitaka Kikkawa. 2010. "Serum Anti-Müllerian Hormone Level Is a Useful Marker for Evaluating the Impact of Laparoscopic Cystectomy on Ovarian Reserve." Fertility and Sterility 94(7):2846–49. doi: https://doi.org/10.1016/j.fertnstert.2010.06.010.

Keyhan, Sanaz, Claude Hughes, Thomas Price, and Suheil Muasher. 2015. "An Update on Surgical versus Expectant Management of Ovarian Endometriomas in Infertile Women." 2015. doi: 10.1155/2015/204792.

Khan, Khaleque Newaz, Michio Kitajima, Akira Fujishita, Koichi Hiraki, Ayumi Matsumoto, Masahiro Nakashima, and Hideaki Masuzaki. 2013. "Pelvic Pain in Women with Ovarian Endometrioma Is Mostly Associated with Coexisting Peritoneal Lesions." Human Reproduction 28(1):109–18. doi: 10.1093/humrep/des364.

Kitajima, Michio, Sylvie Defrère, Marie-Madeleine Dolmans, Sebastien Colette, Jean Squifflet, Anne Van Langendonckt, and Jacques Donnez. 2011. "Endometriomas as a Possible Cause of Reduced Ovarian Reserve in Women with Endometriosis." Fertility and Sterility 96(3):685–91. doi: https://doi.org/10.1016/j.fertnstert.2011.06.064.

Kitajima, Michio, D. Ph, Marie-madeleine Dolmans, D. Ph, Olivier Donnez, and D. Ph. 2014. "Enhanced Follicular Recruitment and Atresia in Cortex Derived from Ovaries with Endometriomas." Fertility and Sterility 101(4):1031–37. doi: 10.1016/j.fertnstert.2013.12.049.

Koninckx, Philippe R., Rodrigo Fernandes, Anastasia Ussia, Larissa Schindler, Arnaud Wattiez, Shaima Al-Suwaidi, Bedayah Amro, Basma Al-Maamari, Zeinab Hakim, and Muna Tahlak. 2021. "Pathogenesis Based Diagnosis and Treatment of Endometriosis." Frontiers in Endocrinology 12(November):1–13. doi: 10.3389/fendo.2021.745548.

Korkmaz, D., E. Bastu, O. Dural, C. Yasa, E. Yavuz, and F. Buyru. 2013. "Apoptosis through Regulation of Bcl-2, Bax and Mcl-1 Expressions in Endometriotic Cyst Lesions and the Endometrium of Women with Moderate to Severe Endometriosis." Journal of Obstetrics and Gynaecology 33(7):725–28. doi: 10.3109/01443615.2013.824416.

Lew, Raelia. 2019. "Natural History of Ovarian Function Including Assessment of Ovarian Reserve and Premature Ovarian Failure." Best Practice and Research: Clinical Obstetrics and Gynaecology 55:2–13. doi: 10.1016/j.bpobgyn.2018.05.005.

Margarida. 2017. "Endometrioma Em Contexto de Infertilidade." Angewandte Chemie International Edition, 6(11), 951–952. 5–24.

Matsuzaki, Sachiko. 2010. "Oxidative Stress Status in Normal Ovarian Cortex Surrounding Ovarian Endometriosis." Fertility and Sterility 93(7):2431–32. doi: 10.1016/j.fertnstert.2009.08.068.

Miller, Emily M., Timothy M. Samec, and Angela A. Alexander-Bryant. 2021. "Nanoparticle Delivery Systems to Combat Drug Resistance in Ovarian Cancer." Nanomedicine: Nanotechnology, Biology and Medicine 31:102309. doi: https://doi.org/10.1016/j.nano.2020.102309.

Molvig. 1988. "Endotoxin-Stimulated Human Monocyte Secretion of Interleukin 1, Tumour Necrosis Factor Alpha, and Prostaglandin E2 Shows Stable Interindividual Differences." Scandinavian Journal of Immunology 27(6):705–16. doi: https://doi.org/10.1111/j.1365-3083.1988.tb02404.x.

Muzii, Ludovico, Antonella Bianchi, Filippo Bellati, Emanuela Cristi, Milena Pernice, Marzio A. Zullo, Roberto Angioli, and Pierluigi Benedetti Panici. 2007. "Histologic Analysis of Endometriomas: What the Surgeon Needs to Know." Fertility and Sterility 87(2):362–66. doi: 10.1016/j.fertnstert.2006.06.055.

Nisolle, Michelle, and Jacques Donnez. 2019. "Reprint of: Peritoneal Endometriosis, Ovarian Endometriosis, and Adenomyotic Nodules of the Rectovaginal Septum Are Three Different Entities." Fertility and Sterility 112(4):e125–36. doi: 10.1016/j.fertnstert.2019.08.081.

Obstet, Arch Gynecol, Francesca Maria Salmeri, Vaclav Vetvicka, and Antonio Simone. 2016. "Regulation of Apoptotic Pathways during Endometriosis : From the Molecular Basis to the Future Perspectives." doi: 10.1007/s00404-016-4195-6.

Parameswaran, Narayanan, and Sonika Patial. 2010. "Tumor Necrosis Factor-α Signaling in Macrophages." Critical Reviews in Eukaryotic Gene Expression 20(2):87–103. doi: 10.1615/critreveukargeneexpr.v20.i2.10.

Parsa, Sepideh, Ian Roper, Michael Muller-Camen, and Eva Szigetvari. 2018. "Have Labour Practices and Human Rights Disclosures Enhanced Corporate Accountability? The Case of the GRI Framework." Accounting Forum 42(1):47–64. doi: 10.1016/j.accfor.2018.01.001.

Pejovic, Tanja, Sarah Thisted, Michael White, and Farr R. Nezhat. 2020. "Endometriosis and Endometriosis-Associated Ovarian Cancer (EAOC) BT - Hormonal Pathology of the Uterus." Pp. 73–87 in, edited by L. Deligdisch-Schor and A. Mareş Miceli. Cham: Springer International Publishing.

Porpora, M. G., P. R. Koninckx, J. Piazze, M. Natili, S. Colagrande, and E. V Cosmi. 1999. "Correlation between Endometriosis and Pelvic Pain." The Journal of the American Association of Gynecologic Laparoscopists 6(4):429–34. doi: https://doi.org/10.1016/S1074-3804(99)80006-1.

Raffi, F., R. W. Shaw, and S. A. Amer. 2012. "National Survey of the Current Management of Endometriomas in Women Undergoing Assisted Reproductive Treatment." Human Reproduction 27(9):2712–19. doi: 10.1093/humrep/des195.

Rosen, Mitchell P., Erica Johnstone, Charles E. McCulloch, Sonya M. Schuh-Huerta, Barbara Sternfeld, Renee A. Reijo-Pera, and Marcelle I. Cedars. 2012. "A Characterization of the Relationship of Ovarian Reserve Markers with Age." Fertility and Sterility 97(1):238–43. doi: https://doi.org/10.1016/j.fertnstert.2011.10.031.

Rumph, Jelonia T., Victoria R. Stephens, Anthony E. Archibong, Kevin G. Osteen, and Kaylon L. Bruner-Tran. 2020. "Environmental Endocrine Disruptors and Endometriosis." Advances in Anatomy, Embryology, and Cell Biology 232:57–78. doi: 10.1007/978-3-030-51856-1_4.

Salmeri, Francesca M., Vincenza Sofo, Antonio S. Lagana, Onofrio Triolo, Emanuele Sturlese, Giovanni Retto, Alfonsa Pizzo, Angela D. Ascola, and Salvatore Campo. 2015. "Behavior of Tumor Necrosis Factor- α and Tumor Necrosis Factor Receptor 1 / Tumor Necrosis Factor Receptor 2 System in Mononuclear Cells Recovered From Peritoneal Fluid of Women With Endometriosis at Different Stages." Reproductive Sciences 22(2):165–72. doi: 10.1177/1933719114536472.

Sanchez, A. M., P. Viganò, E. Somigliana, P. Panina-Bordignon, P. Vercellini, and M. Candiani. 2014. "The Distinguishing Cellular and Molecular Features of the Endometriotic Ovarian Cyst: From Pathophysiology to the Potential Endometrioma-Mediated Damage to the Ovary." Human Reproduction Update 20(2):217–30. doi: 10.1093/humupd/dmt053.

Scutiero, Gennaro, Piergiorgio Iannone, Giulia Bernardi, Gloria Bonaccorsi, Savino Spadaro, Carlo Alberto Volta, Pantaleo Greco, and Luigi Nappi. 2017.

"Oxidative Stress and Endometriosis: A Systematic Review of the Literature" edited by V. M. Victor. Oxidative Medicine and Cellular Longevity 2017:7265238. doi: 10.1155/2017/7265238.

Seifer, David B., Valerie L. Baker, and Benjamin Leader. 2011. "Age-Specific Serum Anti-Müllerian Hormone Values for 17,120 Women Presenting to Fertility Centers within the United States." Fertility and Sterility 95(2):747–50. doi: https://doi.org/10.1016/j.fertnstert.2010.10.011.

Shi, Jinghua, Jinhua Leng, Quancai Cui, and Jinghe Lang. 2011. "Follicle Loss after Laparoscopic Treatment of Ovarian Endometriotic Cysts." International Journal of Gynecology & Obstetrics 115(3):277–81. doi: https://doi.org/10.1016/j.ijgo.2011.07.026.

Sipak-Szmigiel, Olimpia, Piotr Włodarski, Elżbieta Ronin-Walknowska, Andrzej Niedzielski, Beata Karakiewicz, Sylwia Słuczanowska-Głąbowska, Maria Laszczyńska, and Witold Malinowski. 2017. "Serum and Peritoneal Fluid Concentrations of Soluble Human Leukocyte Antigen, Tumor Necrosis Factor Alpha and Interleukin 10 in Patients with Selected Ovarian Pathologies." Journal of Ovarian Research 10(1):1–14. doi: 10.1186/s13048-017-0320-9.

Somigliana, Edgardo, Nicola Berlanda, Laura Benaglia, Paola Viganò, Paolo Vercellini, and Luigi Fedele. 2012. "Surgical Excision of Endometriomas and Ovarian Reserve: A Systematic Review on Serum Antimüllerian Hormone Level Modifications." Fertility and Sterility 98(6):1531–38. doi: https://doi.org/10.1016/j.fertnstert.2012.08.009.

Sugita, Atsuko, Akira Iwase, Maki Goto, Tatsuo Nakahara, Tomoko Nakamura, Mika Kondo, Satoko Osuka, Masahiko Mori, Ai Saito, and Fumitaka Kikkawa. 2013. "One-Year Follow-up of Serum Antimüllerian Hormone Levels in Patients with Cystectomy: Are Different Sequential Changes Due to Different Mechanisms Causing Damage to the Ovarian Reserve?" Fertility and Sterility 100(2):516-522.e3. doi: https://doi.org/10.1016/j.fertnstert.2013.03.032.

Uncu, Gurkan, Isil Kasapoglu, Kemal Ozerkan, Ayse Seyhan, Arzu Oral Yilmaztepe, and Baris Ata. 2013. "Prospective Assessment of the Impact of Endometriomas and Their Removal on Ovarian Reserve and Determinants of the Rate of Decline in Ovarian Reserve†." Human Reproduction 28(8):2140–45. doi: 10.1093/humrep/det123.

Vercellini, Paolo, Paola Viganò, Laura Buggio, Sofia Makieva, Giovanna Scarfone, Fulvia Milena Cribiù, Fabio Parazzini, and Edgardo Somigliana. 2018. "Perimenopausal Management of Ovarian Endometriosis and Associated Cancer Risk: When Is Medical or Surgical Treatment Indicated?" Best Practice & Research Clinical Obstetrics & Gynaecology 51:151–68. doi: https://doi.org/10.1016/j.bpobgyn.2018.01.017.

Vercellini, Paolo, Paola Viganò, Edgardo Somigliana, and Luigi Fedele. 2014. "Endometriosis : Pathogenesis and Treatment." 10(May). doi: 10.1038/nrendo.2013.255.

WHO. 2023. "Endometriosis." WHO. Retrieved (https://www.who.int/news-room/fact-sheets/detail/endometriosis).

Yeung, Patrick, Ken Sinervo, Wendy Winer, and Robert B. Albee. 2011. "Complete Laparoscopic Excision of Endometriosis in Teenagers: Is Postoperative Hormonal Suppression Necessary?" Fertility and Sterility 95(6):1909-1912.e1. doi: https://doi.org/10.1016/j.fertnstert.2011.02.037.

Zikargae, Mekonnen Hailemariam. 2018. "Analysis of Environmental Communication and Its Implication for Sustainable Development in Ethiopia." Science of the Total Environment 634:1593–1600. doi: 10.1016/j.scitotenv.2018.04.050.