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RESEARCH ARTICLE

Onaran et al: Vitamin D and uterine leiomyoma

Vitamin D deficiency and uterine leiomyoma in unexplained infertility

Yüksel Onaran[1*], Esra Goktas[3], Beyza Altın Öztürk[2], Serkan Kahyaoglu[1], Hatice Akkaya[1]

[1]Department of Obstetrics and Gynecology, Bilkent City Hospital, University of Health Sciences, Ankara, Türkiye;

[2]Department of Obstetrics and Gynecology, Bilkent City Hospital, Ankara, Türkiye;

[3]Department of Obstetrics and Gynecology, Aksaray University Training and Research Hospital, Aksaray, Türkiye.

*Correspondence to Yüksel Onaran: dryukselonaran@yahoo.com

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ABSTRACT

Uterine leiomyomas are the most common benign tumors of the female genital tract, and alongside hormonal and genetic factors, emerging evidence implicates vitamin D deficiency in their pathogenesis. We investigated the association between serum 25-hydroxyvitamin D [25(OH)D] and the presence of uterine leiomyomas in women with unexplained infertility. In this retrospective case–control study, 148 women aged 18–45 years presenting to the Infertility Clinic of Ankara Bilkent City Hospital between July 2019 and February 2024 were included: 74 had imaging-confirmed leiomyomas (non-submucosal; FIGO types 4–6) and 74 infertile controls had no leiomyomas. Serum 25(OH)D was measured and demographic/clinical data were analyzed with appropriate parametric and non-parametric tests; correlations used Spearman's rho, and an ANCOVA adjusted for body mass index (BMI) and season assessed group differences. Groups were comparable in age and BMI (e.g., age 35.08 ± 5.79 vs 33.30 ± 5.57 years; $p = 0.062$). Mean serum 25(OH)D was significantly lower in women with leiomyomas than in controls (41.4 ± 23.7 vs 62.0 ± 34.2 nmol/L; $p < 0.001$), and this difference remained significant after adjustment for BMI and season (ANCOVA $F = 10.7$, $p = 0.001$). Vitamin D levels did not differ by leiomyoma number (single vs multiple: 44.1 ± 21.6 vs 38.5 ± 25.83 nmol/L; $p = 0.32$) or location (intramural vs subserosal: 40.7 ± 24.9 vs 43.1 ± 21.1 nmol/L; $p = 0.69$), and were not correlated with leiomyoma size (Spearman $r = -0.04$; $p = 0.70$). Among women with unexplained infertility, uterine leiomyomas are thus associated with significantly lower serum 25(OH)D levels, independent of BMI and season, whereas vitamin D status is unrelated to leiomyoma number, size, or location. These findings support a potential role of vitamin D deficiency in leiomyoma pathogenesis and underscore the need for larger, multicenter prospective studies to clarify causality and clinical implications.

Keywords: Vitamin D, leiomyomas, infertility.

INTRODUCTION

Uterine leiomyomas are the most prevalent tumors of the female genital tract [1]. These benign monoclonal tumors originate from the smooth muscle cells and fibroblasts of the myometrium [2]. The growth and development of leiomyomas are associated with progesterone, estrogen, and related growth factors and proteins. While the precise etiology of uterine leiomyomas remains elusive, ongoing research explores their molecular biology, hormonal growth factors, and genetic underpinnings [3]. A systematic review by Stewart et al. identified several factors that increase leiomyoma incidence, including high body mass index, black ethnicity, family history, nulliparity, and reproductive age [4]. Beyond these established risk factors, some studies have proposed that low vitamin D levels contribute to leiomyoma formation [5]. The investigation into the effect of vitamin D deficiency on leiomyomas has particularly focused on African-American women [6], primarily due to their higher melanin levels, which predispose them to vitamin D deficiency [7]. A decrease in vitamin D levels has been linked to reduced expression of vitamin D receptors (VDRs) in adjacent myometrial tissue [8].

Uterine leiomyomas exhibit variability in their location, size, number, and symptomatology [4]. While a large proportion of leiomyomas are asymptomatic, approximately one-quarter can cause a range of severe and chronic symptoms. [4]. The most common symptom is abnormal uterine bleeding, which can lead to secondary iron deficiency anemia. Other potential symptoms include pelvic pain, bloating, constipation, and obstetric pathologies [9].

Clinically symptomatic leiomyomas are most frequently managed surgically [10]. Surgical options include hysterectomy, myomectomy, and hysteroscopic resections [11]. Leiomyomas are a common indication for hysterectomy [12]. The primary goal of optimal treatment is to preserve fertility while minimizing blood loss and tumor burden [13]. Accumulating evidence suggests that progesterone plays a pivotal role in leiomyoma development and acts as a stimulant for their growth to larger sizes [14]. This understanding has led to the development of ulipristal acetate (UPA), a selective progesterone receptor modulator (SPRM), as a significant pharmacological treatment for leiomyomas [15]. Due to their efficacy, UPA agents were used as first-line therapy to prepare leiomyomas for surgery. However, utilization of UPA has recently been abandoned due to hepatotoxicity. However, UPA

is an expensive substance, and research is ongoing regarding the risk of liver failure with long-term use [16, 17].

Given the diverse origins of leiomyomas, an effective prophylaxis method has not yet been established [18]. Numerous studies have been conducted to develop prophylactic strategies, but a definitive method remains elusive [18]. Therefore, vitamin D prophylaxis, given its significant role in the biological development of leiomyomas, may offer a potential solution [19].

Vitamin D refers to a group of steroid compounds that are fat-soluble and exert potent effects within the human body, with receptors (VDRs) found in various organs, including the myometrium and leiomyomas [20]. Diet, dietary supplements, and sunlight are the primary sources of vitamin D in humans. Vitamin D is synthesized in the skin from 7-dehydrocholesterol. The liver then converts this molecule to 25-hydroxyvitamin D [25(OH)D], which is subsequently converted by the kidneys to 1,25-dihydroxyvitamin D [1,25(OH)D]. Optimal serum vitamin D level is defined as 25(OH)D concentration of 50 nmol/L (20ng/mL) [21]. Vitamin D plays a role in regulating the cell cycle and cell differentiation, and it also possesses anti-angiogenic activity [22]. Vitamin D deficiency is emerging as a significant risk factor in the development of leiomyomas [23]. Many ideas have been proposed regarding the use of vitamin D in the prophylaxis and treatment of leiomyomas [24], but clinical studies in this area are still insufficient.

Abnormal serum levels of vitamin D have been detected in various gynecological and obstetric pathologies, such as polycystic ovary syndrome, infertility, and preterm birth [25]. Unexplained infertility is defined as the inability to achieve pregnancy after 12 months of regular unprotected intercourse, despite normal results in standard infertility evaluations including ovulation assessment tubal patency and semen analysis. Although no clear abnormality is identified in routine investigations several factors such as oocyte and sperm quality issues, fertilization failure, endometrial receptivity defects, immunological factors, genetic and epigenetic abnormalities and subtle hormonal imbalances may contribute to unexplained infertility [26]. Currently, low serum vitamin D levels are considered a potential risk for the development of leiomyomas [19]. In recent years, the idea that vitamin D plays a role in the pathogenesis of leiomyomas has gained prominence [27]. Recent studies

have shown a negative correlation between serum vitamin D levels and leiomyomas [28]. Low serum vitamin D levels in women have been associated with leiomyomas, irrespective of ethnic origin [28]. It has been observed that patients with adequate serum vitamin D levels are less likely to develop leiomyomas compared to those with low serum vitamin D levels. Additionally, exposure to sunlight has been found to decrease the risk of leiomyoma formation [28]. A correlation study between vitamin D and leiomyomas demonstrated that patients with symptomatic leiomyomas had lower vitamin D levels [29]. A study conducted in Turkey similarly found lower serum vitamin D levels in patients with leiomyomas but no correlation between low vitamin D levels and the volume, location, or number of leiomyomas [30]. The first study to better elucidate the effect of vitamin D on leiomyoma growth was published by Blauer et al. [31]. This study demonstrated a relationship between 1,25(OH)D levels and the growth of leiomyoma cells [31]. The inhibition of growth was correlated with vitamin D concentrations; increasing vitamin D concentrations inhibited leiomyoma growth [31]. Subsequently, another study by Sharan et al. observed that 1,25(OH)D inhibited the proliferation of leiomyoma cells *in vitro* [32].

According to a study by Al-Hendy et al., 1,25(OH)D exhibits potent antiprogestogenic and antiestrogenic effects [32]. In this study, they observed an inverse correlation between estrogen and progesterone receptors and vitamin D receptor (VDR) expression. The same study also observed that active vitamin D treatment significantly reduced progesterone and estrogen receptor levels [32]. Studies by Halder et al. have demonstrated a significant reduction in leiomyoma growth under the influence of vitamin D [33,34]. The authors concluded that 1,25(OH)D decreases transforming growth factor beta 3 (TGF-beta3)-related gene expression, and that 1,25(OH)D treatment slows the growth of uterine leiomyomas and inhibits the proliferation of leiomyoma cells [33,34].

MATERIALS AND METHODS

This retrospective case-control research encompassed patients who sought treatment at the Infertility Clinic of Ankara Bilkent City Hospital for infertility from July 2019 to February 2024, had a confirmed diagnosis of leiomyoma through any imaging technique (Patients were evaluated using transvaginal ultrasonography with GE Voluson E10 and assessed for the absence of tubal pathologies using

hysterosalpingography (HSG)) and had vitamin D levels recorded in our hospital's patient database. None of the included leiomyomas were submucosal in nature; specifically, no cases of FIGO type 0, 1, 2, or 3 myomas were present, whereas types 4, 5, and 6 were observed. All patients had undergone office hysteroscopy as part of their infertility evaluation. Semen analyses were within normal reference ranges, and patients with abnormal findings were excluded. Women with hypogonadotropic hypogonadism, pituitary insufficiency, hyperprolactinemia, polycystic ovary syndrome (PCOS), or premature ovarian insufficiency (POI) characterized by low ovarian reserve were also excluded from the study. Our control group consisted of infertile individuals without leiomyomas, and no other uterine or tubal pathology confirmed with ultrasonography and hysterosalpingography, who also had vitamin D levels documented in our hospital's patient record system.

A total of 148 individuals experiencing unexplained infertility were included in this research. Two groups were established: 74 individuals with unexplained infertility having leiomyomas, and 74 individuals with unexplained infertility without leiomyomas. The control group, which comprised patients with unexplained infertility lacking leiomyomas, was selected through sequential admission at the polyclinic until we reached 74 patients with leiomyomas in the study group (Figure 2).

Participants aged 18 to 45 years, diagnosed with primary infertility, and confirmed to have leiomyoma by any imaging modality were included in the study. Those taking Vitamin D supplements and/or hormonal therapies, including oral contraceptives, as well as individuals with chronic systemic diseases, were excluded.

The biochemical analysis segment of the study was conducted at the Central Biochemistry Laboratory of Ankara Bilkent City Hospital. Venous blood samples were obtained from both the patient group and healthy control participants using gel serum tubes. Venous blood samples were collected from all participants under standardized conditions to ensure consistency and minimize pre-analytical variability. All samples were drawn in the morning hours at the same certified blood collection center of Ankara Bilkent City Hospital, and all participants were instructed to fast overnight (at least 8–12 hours) prior to sampling. This protocol was applied uniformly across both the case and control groups to eliminate potential confounding factors related to circadian variation or postprandial biochemical fluctuations, particularly

relevant for vitamin D and metabolic parameters. Following blood collection, samples were permitted to clot for 20 minutes before being centrifuged at 1300xg for 10 minutes. The total 25(OH)D vitamin levels were quantified using an immunoassay method on an Atellica IM analyzer (Siemens Healthineers, Mannheim, Germany).

Statistical evaluations were carried out using the SPSS for Windows version 21.0 (SPSS Inc. IL, USA) software package. A 95% confidence interval was established, with $p<0.05$ regarded as a statistically significant differentiation between the groups. The assumption of normal distribution was assessed using the Shapiro-Wilk test. Data are expressed as means ± SD for continuous variables. To evaluate the differences of variables across groups, the independent sample t-test and Mann-Whitney U-test were applied. Correlations among the parameters were determined using the Spearman correlation test. The statistical program available on the website of the statistical department of the University of British Columbia was employed to compute the sample size and statistical power for our study (http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html).

According to this calculation, including 74 patients in each study group was determined to be adequate to achieve 80% statistical power at a significance level of $p<0.05$.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Ankara Bilkent City Hospital (TABED 2-24-85, 20.03.2024).

RESULTS

A total of 148 patients with unexplained infertility were included in the study. Two groups were formed: 74 cases with unexplained infertility and leiomyomas, and 74 cases with unexplained infertility without leiomyomas. The control group, consisting of unexplained infertility patients without leiomyomas, was selected by sequential polyclinic admission until the number of 74 leiomyoma patients in the study group was reached. The age distribution for the leiomyoma group was 22-42 years, while for the control group it was 23-38 years. Both groups consisted of primary infertile and nulligravid patients. The mean age of the study group and

control group was 35.08±5.79 years and 33.3±5.57 years, respectively, indicating similar age distributions between the two groups (p=0.062). (Table 1) No significant differences were found in sociodemographic characteristics when comparing the groups. The mean serum 25(OH)D value in the study group was 41.4±23.7 nmol/L, while in the control group it was 62.0±34.2 nmol/L. A statistically significant difference was found when these values were compared (p<0.001) (Figure 1).

In the leiomyoma group, 38 (51.4%) cases had solitary leiomyomas, while 36 (48.6%) had multiple leiomyomas. When examining the relationship between solitary or multiple leiomyomas and vitamin D levels, no significant difference was observed, with the solitary group having an average vitamin D level of 44.1±21.6 nmol/L and the multiple leiomyoma group having 38.5±25.83 nmol/L (p=0.32). Regarding leiomyoma location, most cases in our study were intramural (70.3%, 52 cases) or subserosal (29.7%, 22 cases) with an intramural component. When investigating the relationship between location and vitamin D levels, no significant difference was observed between the intramural group having an average vitamin D level of 40.7±24.9 and the subserosal leiomyoma group having 43.1±21.1 nmol/L (p=0.69).

The relationship between leiomyoma size and vitamin D levels was examined using the Spearman correlation test (r=−0.04, p=0.7), indicating no statistically significant correlation. Similarly, no statistically significant relationship was observed between vitamin D levels and other factors such as leiomyoma number, location, or size (p>0.05). Variance Inflation Factor (VIF) values ranged between 1.00 and 1.25, indicating no significant multicollinearity among the predictors. The group difference in vitamin D levels persisted after adjustment for potential confounders, including BMI and season.

An ANCOVA was conducted to compare serum vitamin D levels between women with and without myoma, adjusting for BMI and season. After controlling for these covariates, the difference in vitamin D levels between the groups remained statistically significant (ANCOVA, $F = 10.7$, $p = 0.001$). Adjusted vitamin D levels have been included in Table 1.

The reference intervals of vitamin D status used in the laboratory of Bilkent City Hospital are indicated as follows: deficiency (<50 nmol/L), insufficiency (50–75 nmol/L), and normal (75–250 nmol/L) (Table 3). Median serum levels were lower in the leiomyoma group compared to controls, consistent with a higher prevalence of deficiency and insufficiency.

DISCUSSION

Vitamin D receptors (VDRs) are ubiquitously distributed throughout the human body, not limited to organs primarily associated with calcium metabolism, but identified in a wide range of tissues and organs. Numerous studies have demonstrated vitamin D's involvement in a multitude of biological processes within the body [35]. Didriksen et al. suggested that genetic polymorphisms in vitamin D-related enzymes can significantly influence serum 25(OH)D levels [36]. Lower levels of Vitamin D, in turn, have been proposed as a risk factor for the formation and growth of leiomyomas [28]. A study by Halder et al. reported that leiomyomas contain lower levels of VDRs compared to normal myometrial tissue [19]. An inverse relationship has been observed between VDR levels and upregulated estrogen and progesterone receptors in leiomyomas. This suggests that vitamin D may act as an antagonist to sex steroid hormones in leiomyoma tissue [33,37].

In our study, we found a statistically significant difference in serum 25(OH)D levels between our leiomyoma patient group and the control group. However, when comparing our study group cases with solitary leiomyomas versus multiple leiomyomas, we found no significant difference in serum 25(OH)D levels. This indicates that serum 25(OH)D levels are unrelated to the location, size, and number of leiomyomas. A 2018 study conducted in Turkey reported similar findings with our study, while in our study, we also demonstrated the association between vitamin D deficiency and infertility in women with leiomyomas [30]. One of the strongest aspects of our study was the screening of the patient group for leiomyomas by an experienced radiologist. Limitations of our study included the unknown onset of the disease and the inability to assess vitamin D levels before and after leiomyoma development.

In summary, our study concludes that vitamin D levels are lower in patients with leiomyomas, and lower levels of Vitamin D contribute to the development of

leiomyoma. To definitively confirm this conclusion, further large-scale, multi-center studies with greater patient numbers are warranted. Besides, further studies investigating the therapeutic role of vitamin D administration to patients with uterine leiomyomas are needed in the future.

CONCLUSION

This study demonstrated that serum 25(OH) vitamin D levels are significantly lower in patients with leiomyomas compared to those without, among individuals evaluated for primary or secondary infertility. However, no significant association was found between vitamin D levels and the location, number, or size of the leiomyoma. These findings suggest that vitamin D deficiency may play a role as a risk factor in leiomyoma development. Given the study's limitations, further large-scale, multicenter, and prospective research is needed to clarify this relationship. To conclude, while our findings suggest a potential link between vitamin D status and leiomyomas, further studies are required to elucidate the role of vitamin D in the reproductive system. Should the role of vitamin D deficiency in leiomyoma pathogenesis be confirmed, monitoring and correcting vitamin D levels could offer a promising new strategy in leiomyoma management.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Demographic data of patients

Variables	Infertility with leiomyoma (*n*=74)	Infertility without leiomyoma (*n*=74)	*p* value*
Age (year)	35.08±5.79	33.3±5.57	0.062
Weight (kg)	70.5±13	72.6±12.3	0.326
Height (cm)	164±6.1	164±6.0	0.77
BMI (kg/m²)	25.93±4.56	26.75±4.57	0.363
25(OH) Vit D (nmol/L)	41.4±23.7	62.0±34.2	<0.001
Adjusted 25(OH) Vit D level (nmol/L)	41.9±3.33	61.4±3.32	0.000

Note: Values are expressed as mean ± standard deviation. *Comparison between groups was performed using the Mann–Whitney U-test. Abbreviations: BMI: Body mass index; 25(OH) Vit D: 25-hydroxyvitamin D.

Table 2. Multivariable regression analysis of factors associated with vitamin D levels

Predictor	Std. Error	Beta (standardized coefficient)	t	Sig. (*p* value)	95% confidence interval (lower, upper)
Age	0.471	-0.191	-1.66	0.102	[-1.721, 0.158]
BMI	0.587	-0.314	-27.75	0.007	[-2.799, -0.457]
Leiomyoma count (single / multiple)	5.850	-0.161	-1.3	0.198	[-19.2, 4.66]
Leiomyoma size	0.11	-0.015	-0.127	0.89	[-0.236, 0.208]
Leiomyoma site	6.16	-0.006	-0.047	0.96	[-12.58, 12.03]
Season effect	2.123	-0.197	-1.28	0.201	[-6.9, 1.469]

Abbreviation: BMI: Body mass index.

Table 3. Vitamin D level categorization based on provided reference levels

Category	nmol/L	ng/mL
Deficient	0-50	0-20
Insufficient	50 – 75	20 – 30
Normal	75-250	30-100

Note: The reference intervals of vitamin D status used in the laboratory of Bilkent City Hospital are as follows: deficiency (<50 nmol/L), insufficiency (50–75 nmol/L), and normal (75–250 nmol/L).

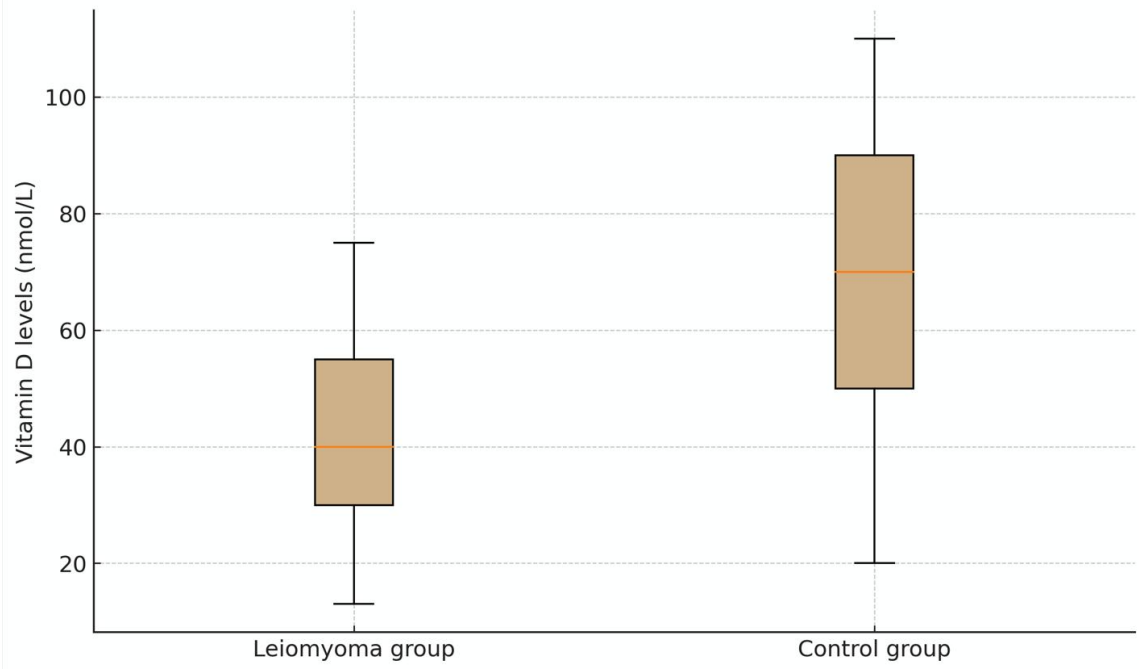

Figure 1. Distribution of serum 25-hydroxyvitamin D [25(OH)D] levels in the leiomyoma and control groups. The box plot depicts the median, interquartile range (IQR), and overall range of 25(OH)D concentrations (nmol/L) for each group. Mean ± SD values were 41.4 ± 23.7 nmol/L vs 62.0 ± 34.2 nmol/L, respectively; the between-group difference was statistically significant ($p < 0.001$).

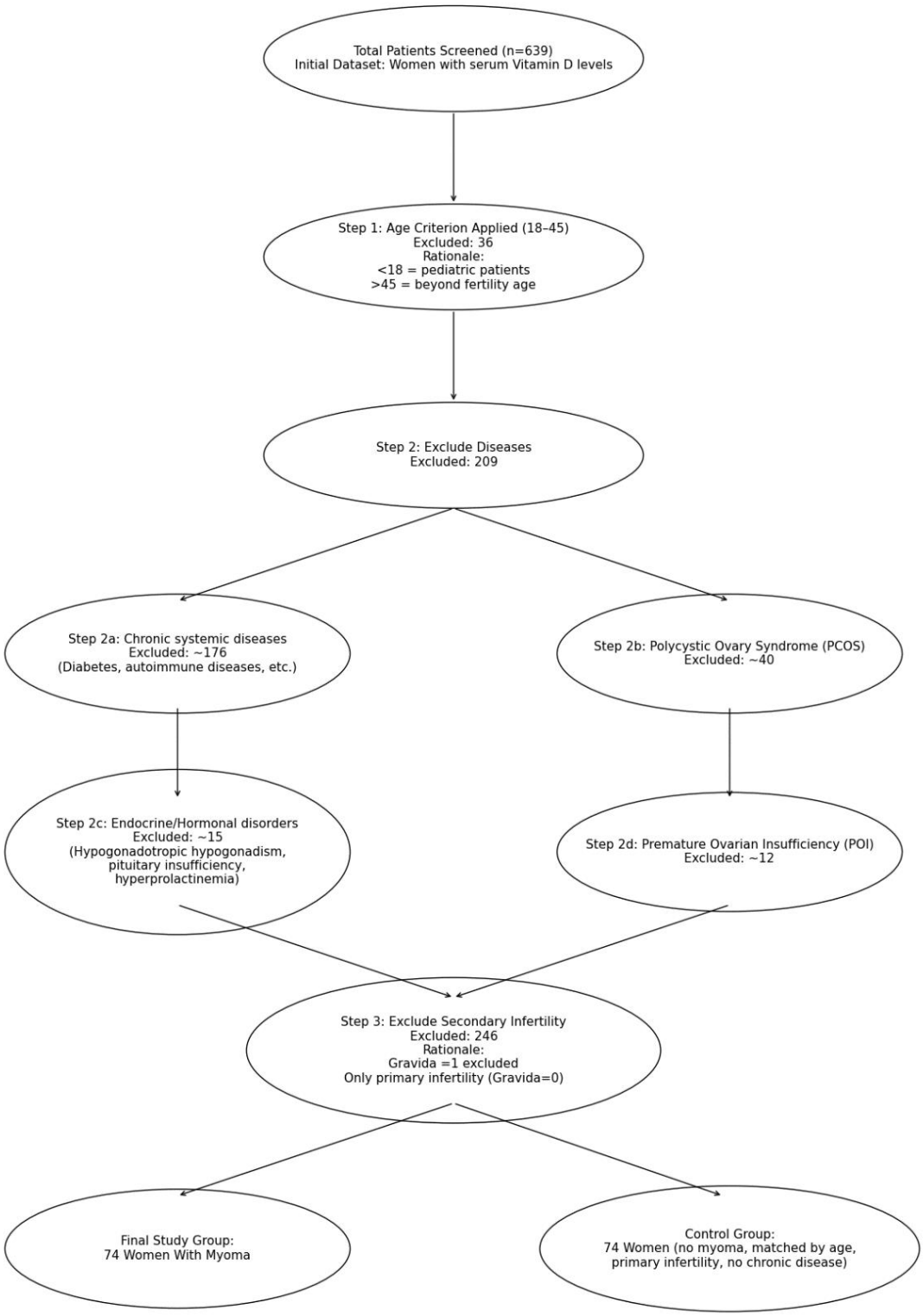

Figure 2. Flow diagram of patient selection