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ORIGINAL

# Impact of L-Arginine Supplementation on Endometrial Thickness in Infertile Patients with Refractory Thin Endometrium: A Randomized Controlled Trial

*Impacto de la Suplementación con L-Arginina en el Grosor Endometrial en Pacientes Infértiles con Endometrio Delgado Refractario: Un Ensayo Controlado Aleatorizado*

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## Abstract

***Background:*** This randomized controlled trial aimed to evaluate the effects of L-arginine supplementation on endometrial thickness and reproductive outcomes in women with infertility associated with refractory thin endometrium.

***Methods:*** Patients with infertility (N=84) were randomly assigned to L-arginine (N=42) or control (N=42) groups. Baseline demographic parameters, infertility-related factors, and gynecologic histories were recorded. L-arginine treatment was administered, and endometrial thickness was measured using transvaginal ultrasound pre- and post-treatment. Reproductive outcomes, including embryo quality, implantation rate, pregnancy rate, and abortion rate, were assessed. Statistical analysis was performed with GraphPad Prism 10.0, with $P<0.05$ being considered as the threshold of significance.

***Results:*** A total of 84 infertile with a mean age of 35.5 ± 6.78 years were recruited. Following L-arginine treatment, a significant increase in endometrial thickness was observed (3.3 ± 0.72 mm pre-treatment vs. 7.79 ± 1.72 mm post-treatment, $P < 0.001$). The pregnancy rate was comparable between the L-arginine and control groups (21.4% vs. 28.6%, $P > 0.05$). No statistically significant differences were observed between the two groups in terms of embryo quality, endometrial preparation failure, or gestational sacs. A significantly higher proportion of cleaved embryos was found in the L-arginine group (97.6% vs. 78.6%, P=0.002), though this did not translate to improved pregnancy outcomes.

***Conclusion:*** L-arginine significantly increased endometrial thickness, but no substantial improvements in pregnancy rates or other reproductive outcomes were observed compared to the control group. Further studies are needed to determine the role of L-arginine in fertility treatment.

*Key words:* Refractory Thin Endometrium, Endometrial Thickness, L-arginine, Embryo, Infertility, Pregnancy.

## *Resumen*

***Antecedentes:*** Este ensayo controlado aleatorizado tuvo como objetivo evaluar los efectos de la suplementación con L-arginina sobre el grosor endometrial y los resultados reproductivos en mujeres con infertilidad asociada a un endometrio delgado y refractario.

***Métodos:*** Las pacientes con infertilidad (N=84) fueron asignadas aleatoriamente a los grupos de L-arginina (N=42) o control (N=42). Se registraron parámetros demográficos iniciales, factores relacionados con la infertilidad e historiales ginecológicos. Se administró tratamiento con L-arginina y se midió el grosor endometrial mediante ultrasonido transvaginal antes y después del tratamiento. Se evaluaron los resultados reproductivos, incluyendo calidad embrionaria, tasa de implantación, tasa de embarazo y tasa de aborto. El análisis estadístico se realizó con GraphPad Prism 10.0, considerando significativo un valor de $P<0.05$.

***Resultados:*** Se reclutó un total de 84 mujeres con infertilidad, con una edad promedio de 35.5 ± 6.78 años. Tras el tratamiento con L-arginina, se observó un aumento significativo en el grosor endometrial (3.3 ± 0.72 mm antes del tratamiento frente a 7.79 ± 1.72 mm después del tratamiento, $P < 0.001$). La tasa de embarazo fue comparable entre los grupos de L-arginina y control (21.4% vs. 28.6%, $P > 0.05$). No se observaron diferencias estadísticamente significativas entre los dos grupos en términos de calidad embrionaria, falla en la preparación endometrial o sacos gestacionales. Se encontró una proporción significativamente mayor de embriones segmentados en el grupo de L-arginina (97.6% vs. 78.6%, P=0.002), aunque esto no se tradujo en mejores resultados de embarazo.

***Conclusión:*** La L-arginina incrementó significativamente el grosor endometrial, pero no se observaron mejoras sustanciales en las tasas de embarazo u otros resultados reproductivos en comparación con el grupo control. Se necesitan más estudios para determinar el papel de la L-arginina en el tratamiento de la fertilidad.

*Palabras clave:* Endometrio delgado refractario, Grosor endometrial, L-arginina, Embrión; Infertilidad, Embarazo.

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## Introduction

The endometrium is a dynamic tissue that plays a crucial role in the process of embryo implantation, which is pivotal for the establishment of pregnancy[1]. Structurally, the endometrium consists of two distinct layers: the basal layer, which remains relatively unchanged throughout the menstrual cycle, and the functional layer, which undergoes periodic transformations to prepare for potential embryo implantation[2]. These changes occur during the menstrual, proliferative, and secretory phases of the cycle. In the absence of pregnancy, the functional layer is shed during menstruation, maintaining regenerative capacity[3]. For successful conception, a specific endometrial thickness is necessary, with a minimum threshold of 6 mm considered ideal for embryo implantation[4].

A thin endometrium, defined as an endometrial thickness below 6 mm, is a recognized obstacle to achieving pregnancy. Refractory thin endometrium, a condition where the endometrium remains persistently thin despite therapeutic intervention, significantly reduces the probability of conception[5], whether through natural intercourse or assisted reproductive techniques (ART) such as in vitro fertilization (IVF)[6,7]. The causes of refractory thin endometrium are multifactorial, ranging from infections and previous medical interventions to idiopathic origins. In most cases, this condition remains challenging to treat, with conventional therapies often yielding less than promising results, leading to cycle cancellations and failed implantation attempts[5].

Various strategies have been developed to address thin endometrium, including extended use of estrogen therapy[8], vitamin E supplementation[9], vaginal sildenafil citrate[10], and more recently, regenerative approaches like platelet-rich plasma (PRP) infusions[11] and granulocyte colony-stimulating factor (G-CSF)[12]. However, many of these treatments have shown limited success, particularly in patients with refractory thin endometrium, highlighting the need for more effective therapeutic interventions.

L-Arginine, a semi-essential amino acid and a precursor to nitric oxide, has been recognized for its potential to improve blood flow to various tissues, including the endometrium. Its ability to enhance uterine blood flow has made it a promising candidate for addressing issues of thin endometrium, with the aim of improving endometrial receptivity and increasing the chances of successful embryo implantation[13]. L-Arginine has been shown to have beneficial effects on fertility and pregnancy, largely due to its role in increasing nitric oxide production and reducing oxidative stress. In animal studies, L-Arginine supplementation has demonstrated its potential to alleviate fertility issues caused by oxidative stress. For instance, a study on female rats exposed to chronic intermittent hypoxia (CIH) revealed that oxidative stress in the ovaries adversely affected fertility by inducing germ cell apoptosis and disrupting ovarian function. However, when L-Arginine was given, it improved the antioxidant capacity, reduced ovarian tissue damage, and restored fertility by decreasing oxidative stress markers[14]. In human studies, L-Arginine has similarly shown promising results, particularly in improving pregnancy outcomes. A systematic review of clinical studies found that L-Arginine supplementation reduced the risk of developing preeclampsia and decreased blood pressure in pregnant women, while improving fetoplacental circulation[15]. Despite its theoretical benefits, the clinical impact of L-Arginine supplementation in patients with refractory thin endometrium has not been thoroughly investigated.

In this randomized controlled trial, we sought to evaluate the effects of L-Arginine supplementation on endometrial thickness in 84 infertile patients with refractory thin endometrium, who were randomly assigned to trial and control groups each involving 42 patients. By exploring the potential of L-Arginine as a therapeutic intervention, this study aimed to provide new insights into the management of thin endometrium-related infertility and contribute to improving reproductive outcomes for patients who have limited treatment options.

## Methods

### Study Design

This study was a single-blind randomized clinical trial designed to evaluate the impact of L-Arginine supplementation on endometrial thickness in infertile women with refractory thin endometrium. The trial was conducted on women undergoing in vitro fertilization (IVF) cycles with frozen embryo transfer (FET).

### Participants

Participants included infertile women aged 20 to 42 years with a history of thin endometrium, defined as an endometrial thickness of less than 7 mm measured by transvaginal ultrasound (5–9 MHz probe) in previous evaluation cycles. All participants were resistant to standard treatments for thin endometrium.

### Patient Eligibility Criteria

***Inclusion Criteria:***

- Infertile women aged 20–42 years
- Endometrial thickness < 7 mm (assessed via transvaginal ultrasound)
- Resistance to standard treatment for thin endometrium
- Normal hysterosalpingography, with no uterine anomalies

***Exclusion Criteria:***

- Uterine abnormalities (e.g., recurrent adhesions, submucosal fibroids, endometrial polyps)

- Severe endometriosis, adenomyosis, or congenital uterine anomalies
- Use of additional infertility treatments (e.g., growth hormone, dexamethasone, prednisolone, sildenafil, G-CSF)
- Known allergy to L-Arginine
- Declined participation

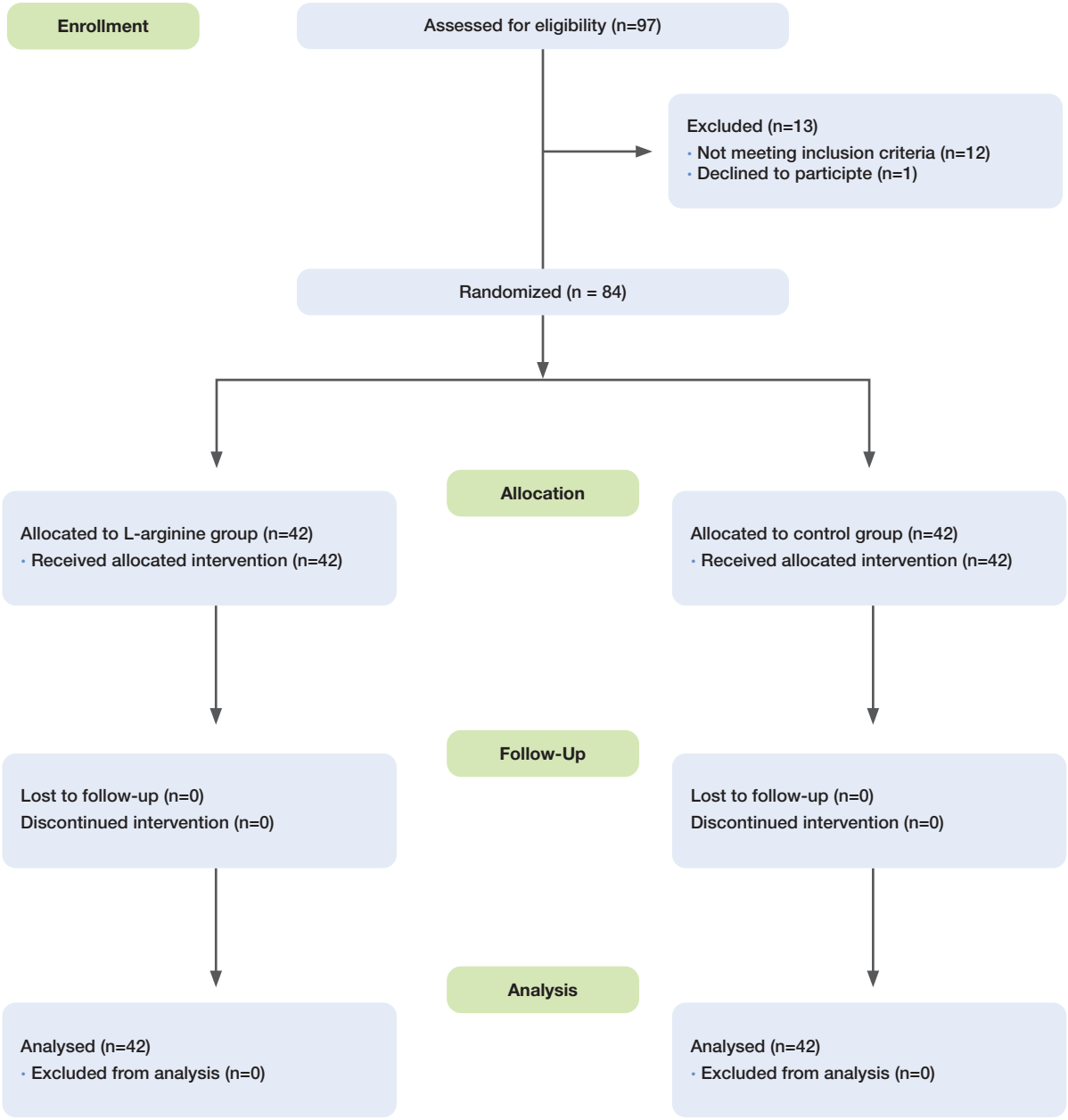
## Randomization and Blinding

Block randomization was performed using Stata 14.0 software, with blocks of four participants, for a total of 21 blocks. Participants were randomly assigned to the intervention (L-Arginine) or control group. The outcome assessor was blinded to the treatment allocation to minimize bias. The study procedure is reported in the CONSORT flowchart in **Figure 1**.

## Intervention Protocol

Patients in the intervention group were administered L-Arginine sachets (1 g every 12 hours) starting from the cycle preceding embryo transfer. L-Arginine supplementation was discontinued after embryo transfer. Estradiol valerate was initiated on cycle days 1-2 (2 mg every 8 hours for 3 days, then every 6 hours for another 3 days), along with L-Arginine, to stimulate endometrial growth. If the endometrial thickness remained below 7 mm, the estradiol dosage was increased incrementally every 3 days up to a maximum of 12 mg daily.

**Figure 1:** CONSORT flowchart of patient recruitment.



Heparin (5000 units every 12 hours) was administered starting with the estradiol treatment. When the endometrial thickness exceeded 7 mm, progesterone was administered (75 mg for 2 days, followed by 100 mg for another 2 days). On the fourth day of progesterone treatment, 2-3 grade A embryos were transferred. Estradiol valerate and vaginal progesterone (100 mg) were continued until pregnancy was confirmed. Fourteen days after embryo transfer, serum beta-HCG was checked to determine pregnancy status. Hormonal support was maintained until the 12th week of pregnancy if beta-HCG was positive.

### Sample Size Calculation

The sample size was determined using G*Power software, based on an alpha error of 5% and a power of 80%. According to a study by So et al., the expected mean increase in endometrial thickness was 9.5 ± 1.5 mm in the control group and 10.2 ± 1.8 mm in the intervention group[16]. With an estimated effect size of 0.42, the minimum sample size required was 42 participants per group, for a total of 84 participants.

### Outcome Measures

The primary outcome was the change in endometrial thickness after L-Arginine supplementation, measured by transvaginal ultrasound. Secondary outcomes included pregnancy rates (assessed via serum beta-HCG), quality of implanted embryos, failed endometrial preparation and cycle cancellation.

### Statistical Analysis

Data were analyzed using GraphPad Prism 10.0 software. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as frequencies (percentages). Independent t-tests were used to compare normally distributed continuous variables, while the Mann-Whitney U test was applied to non-normally distributed data. Categorical variables were analyzed using the Chi-square test, and Fisher's exact test was used when the expected frequency in any cell was less than 5. A p-value of 0.05 was considered statistically significant.

### Ethical Considerations

The protocol of the present clinical trials was approved by Research Ethics Committee (REC) of Tabriz University of Medical Sciences (Approval ID: IR.TBZMED.REC.1403.452). All patients were asked to provide written informed consent prior to participation in the trial. Patient credentials and identifying information were kept confidential throughout the study. The trial was registered with the Iranian Registry of Clinical Trials (No.: IRCT20130115012146N10).

## Results

### Demographic Information and Past Medical History

A total of 84 patients with a mean age of 35.5 ± 6.78 years were recruited to the trial. Demographic information of patients is reported in Table 1. The mean reported age for the spouses was 39.4 ± 6.52 years. In general, the patients were overweight with a mean body mass index (BMI) of 27.2 ± 3.95 corresponding to an average weight and height of 69.8 ± 14.5 kg and 161.0 ± 5.98 cm, respectively. Hypothyroidism was the most frequently reported comorbidity with a prevalence of 26.2%, followed by hypertension (2.4%) and diabetes mellitus (2.4%). Seropositivity for HBs antigen was reported in 2 (2.4%) patients. In terms of gynecologic history, 4 (4.8%) patients reported positive history of past myomectomy. Curettage, with the aim of abortion, was only noted in 2 (2.4%) patients, while dilatation and curettage (D&C) was reported in a single patient (1.2%). To ensure proper randomization of patients to the L-arginine (trial) and control groups, P-values were calculated for all demographic parameters (**Table I**), which indicated no statistically significant difference between the two groups in any of demographic parameters, confirming homogeneous distribution of patients in the two groups.

**Table I:** Demographic information of patients.

| Demographic | | Trial | | | P-value |
|---|---|---|---|---|---|
| | | L-Arginine (N=42) | Control (N=42) | Total (N=84) | |
| **Age (yr)** | Patient (Female) | 35.4 ± 6.36 | 35.5 ± 7.26 | 35.5 ± 6.78 | 0.949 |
| | Spouse (Male) | 38.8 ± 6.06 | 40.0 ± 6.96 | 39.4 ± 6.52 | 0.396 |
| **Anthropometrics** | Weight (kg) | 70.7 ± 15.3 | 69.0 ± 13.8 | 69.8 ± 14.5 | 0.592 |
| | Height (cm) | 162.0 ± 6.58 | 161.0 ± 5.39 | 161.0 ± 5.98 | 0.638 |
| | BMI (kg/m$^2$) | 27.6 ± 4.21 | 26.9 ± 3.67 | 27.2 ± 3.95 | 0.395 |
| **Comorbidity** | HTN | 2 (4.8%) | 0 (0%) | 2 (2.4%) | 0.494 |
| | DM | 0 (0%) | 2 (4.8%) | 2 (2.4%) | 0.494 |
| | Hypothyroidism | 12 (28.6%) | 10 (23.8%) | 22 (26.2%) | 0.620 |
| | Hyperprolactinemia | 1 (2.4%) | 0 (0%) | 1 (1.2%) | 1.000 |
| | Skin Cancer | 0 (0%) | 1 (2.4%) | 1 (1.2%) | 1.000 |
| | Depression | 0 (0%) | 1 (2.4%) | 1 (1.2%) | 1.000 |
| | HBs Ag$^+$ | 2 (4.8%) | 0 (0%) | 2 (2.4%) | 0.494 |
| **Gynecologic History** | Curettage (Abortion) | 2 (4.8%) | 0 (0%) | 2 (2.4%) | 0.494 |
| | D&C | 1 (2.4%) | 0 (0%) | 1 (1.2%) | 1.000 |
| | Myomectomy | 3 (7.1%) | 1 (2.4%) | 4 (4.8%) | 0.616 |

**BMI:** body mass index; **D&C:** dilation and curettage; **DM:** diabetes mellitus; **HBs Ag$^+$:** HBs antigen seropositivity; **HTN:** hypertension

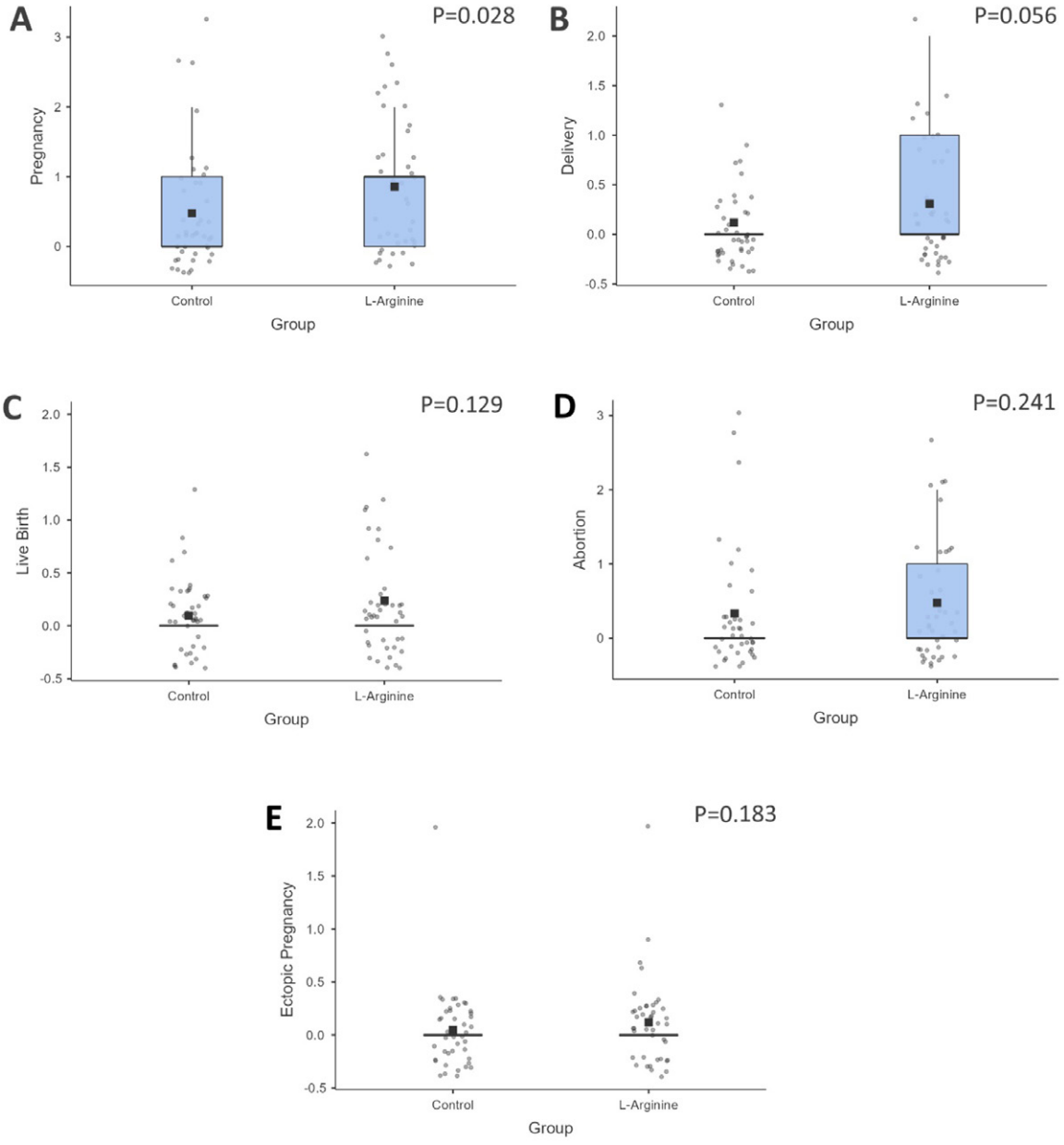
The obstetric history of patients from both groups, with over-dispersed distribution, is reported in **table II** and visualized in **figure 2**.

While the number of past pregnancies was higher in the L-arginine group (0.86, [0.56-1.15]), compared with the controls (0.48, [0.21-0.74]), to a significant extent (P=0.028), no statistically significant difference was observed between the two groups in terms of deliveries, live births, abortions and ectopic pregnancies (P > 0.05).

**Table II:** Obstetric history of patients in L-arginine and control groups. (Data are reported as mean with 95% confidence intervals due to over-dispersed distribution).

| Obstetric History | Trial | | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | L-Arginine (N=42) | | Control (N=42) | | Total (N=84) | | |
| | Mean | 95% CI | Mean | 95% CI | Mean | 95% CI | |
| **Pregnancy** | 0.86 | [0.56-1.15] | 0.48 | [0.21-0.74] | 0.66 | [0.46-0.86] | 0.028 |
| **Delivery** | 0.31 | [0.15-0.47] | 0.12 | [0.02-0.22] | 0.21 | [0.12-0.31] | 0.056 |
| **Live Birth** | 0.24 | [0.09-0.39] | 0.09 | [0.003-0.19] | 0.17 | [0.08-0.25] | 0.129 |
| **Abortion** | 0.48 | [0.23-0.72] | 0.33 | [0.10-0.57] | 0.41 | [0.24-0.57] | 0.241 |
| **Ectopic Pregnancy** | 0.12 | [-0.004-0.24] | 0.05 | [-0.05-0.11] | 0.08 | [0.006-0.160] | 0.183 |

**Figure 2:** Reproductive history of patients in L-arginine and control groups, including the number of **A)** pregnancies, **B)** deliveries, **C)** live births, **D)** abortions and **F)** ectopic pregnancies.



## Infertility Type and Causal Factors

**Table III** summarizes the findings regarding infertility and related contributing factors in the L-arginine and control groups, as well as the entire trial. As reported, the mean duration of infertility among all patients was 7.30 ± 4.90 years, with patients in the L-arginine group being infertile for a significantly longer period compared with those in the control group (8.79 ± 5.34 vs. 5.82 ± 3.96, P=0.005). Conversely, no statistically significant difference was noted between the two group with regard to other infertility-related factors, indicating homogeneous distribution of contributing factors in the two groups. Patients with primary infertility constituted the majority of the population (79.8%), leaving a total prevalence of 20.2% for secondary infertility. Few patients (2, 2.4%) had unexplained infertility, while no patients were found to have combined infertility. The majority of patients reported regular menstrual cycles (79.8%). Polycystic ovary (PCO) and decreased ovarian reserve (DOR) were fairly common with prevalence rates of 32.1% and 35.7%, respectively, while tubal factor and hypomenorrhea were reported to a considerably lesser extent, with prevalence rates falling below 10%. Male factor was comparably frequent among patients, with an overall prevalence of 36.9%.

**Table III:** Prevalence of infertility types and causal factors among patients.

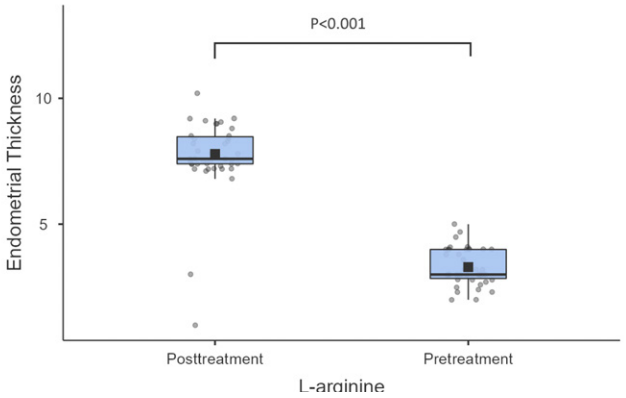
| Factor | | Trial | | | P-value |
|---|---|---|---|---|---|
| | | L-Arginine (N=42) | Control (N=42) | Total (N=84) | |
| Duration of Infertility (yr) | | 8.79 ± 5.34 | 5.82 ± 3.96 | 7.30 ± 4.90 | 0.005 |
| Infertility | Primary<br>Secondary | 33 (78.6%)<br>9 (21.4%) | 34 (81.0%)<br>8 (19.0%) | 67 (79.8%)<br>17 (20.2%) | 0.786 |
| Unexplained Infertility | | 1 (2.4%) | 1 (2.4%) | 2 (2.4%) | 1.000 |
| Combined Infertility | | 0 (0%) | 0 (0%) | 0 (0%) | 1.000 |
| Menstrual Cycle | Regular<br>Irregular | 33 (78.6%)<br>9 (21.4%) | 34 (81.0%)<br>8 (19.0%) | 67 (79.8%)<br>17 (20.2%) | 0.786 |
| PCO | | 14 (33.3%) | 13 (31.0%) | 27 (32.1%) | 0.815 |
| DOR | | 15 (35.7%) | 15 (35.7%) | 30 (35.7%) | 1.000 |
| Tubal Factor | | 3 (7.1%) | 3 (7.1%) | 6 (7.1%) | 1.000 |
| Hypomenorrhea | | 2 (4.8%) | 0 (0%) | 2 (2.4%) | 0.152 |
| Male Factor | | 16 (38.1%) | 15 (35.7%) | 31 (36.9%) | 0.821 |

**DOR:** decreased ovarian reserve; **PCO:** polycystic ovary.

## Effect of L-arginine Treatment on Endometrial Thickness

To determine the effect of L-arginine treatment on endometrial thickness, patients were evaluated using transvaginal ultrasound prior to and after receiving the scheduled dose of L-arginine. Overall, the mean thickness of endometrium was 5.54 ± 2.61 mm. As shown in **figure 3**, treatment with L-arginine was found to significantly increase endometrial thickness from a mean pretreatment thickness of 3.3 ± 0.72 mm to a mean posttreatment thickness of 7.79 ± 1.72 mm (P < 0.001).

**Figure 3:** Comparison of pretreatment and posttreatment endometrial thickness (mm) in patients receiving L-arginine.




## Reproductive Outcomes in L-arginine and Control Groups

To determine the effect of L-arginine on reproductive outcomes in patients with infertility, several reproductive outcomes were monitored and reported in **table IV**.The mean number of implanted embryos was 2.32 ± 0.62, with patients in the control group showing slightly higher numbers, which were deemed as statistically insignificant. The majority of implanted embryos were in the cleavage stage (88.1%), with the L-arginine group showing a markedly high frequency of cleaved embryos (97.6%) compared with the control group, among which only 78.6% of embryos were in the cleavage state. Conversely, embryos implanted to control patients also comprised other stages including cleavage-to-morula (9.5%) and morula (11.9%). This discrepancy between the two groups with regard to the embryo type was deemed as statistically significant (P=0.002). Of the 84 pregnancy tests, a total of 21 returned positive results, without a significant difference between the two groups (21.4% vs. 28.6%). Post-implantation abortion was rare and only reported in one patient from the L-arginine group, without any meaningful difference. Ectopic pregnancy was not observed in any patients following embryo implantation.

Reproductive outcomes with over-dispersed distribution are reported in **table V** and visualized in **figure 4**.

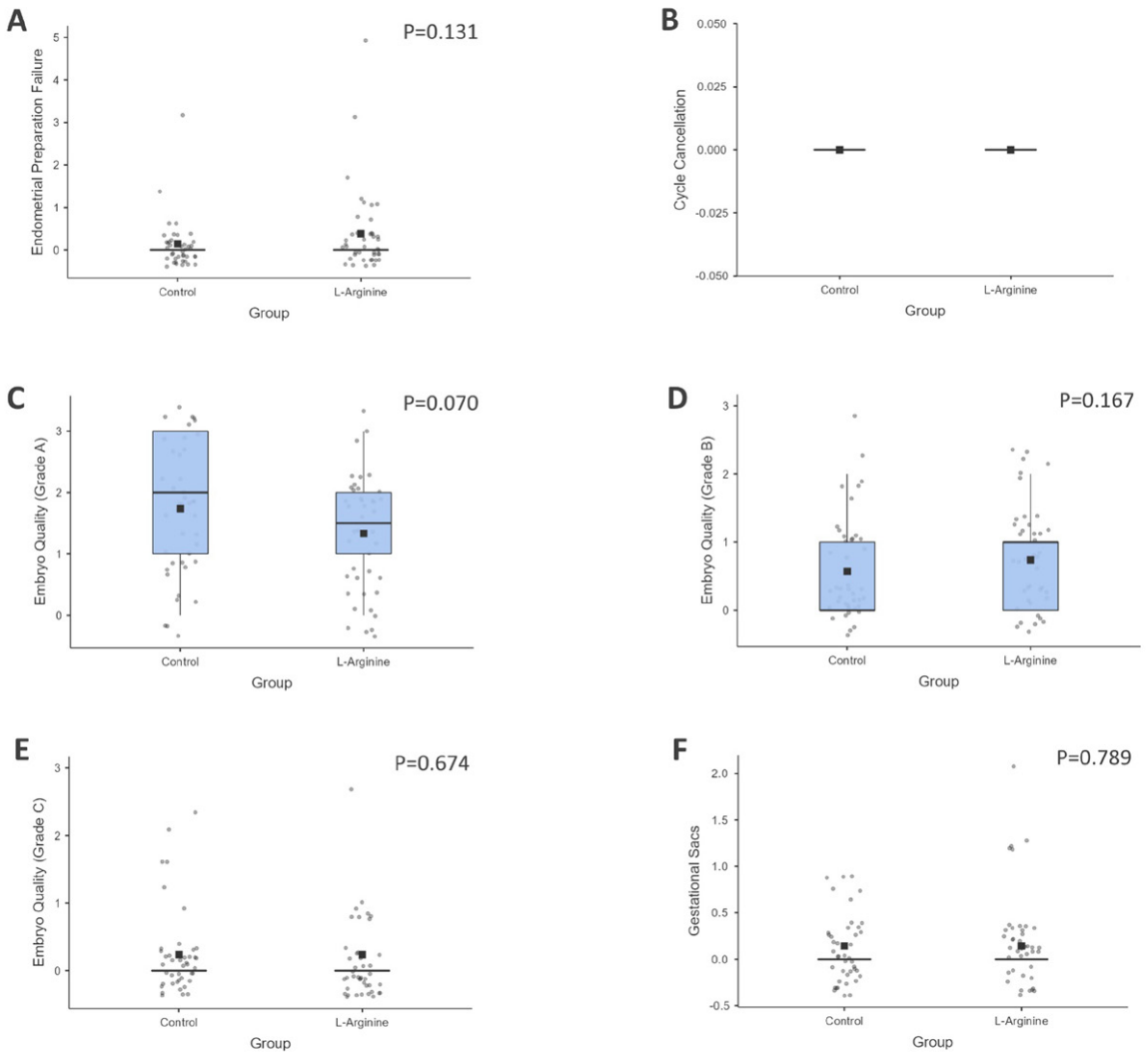
**Table IV:** Reproductive outcomes of treatment in the L-arginine and control groups.

| Outcome | | Trial | | | P-value |
|---|---|---|---|---|---|
| | | L-Arginine (N=42) | Control (N=42) | Total (N=84) | |
| **Implanted Embryos** | | 2.31 ± 0.56 | 2.33 ± 0.69 | 2.32 ± 0.62 | 0.862 |
| **Implanted Embryo Stage** | Cleavage | 41 (97.6%) | 33 (78.6%) | 74 (88.1%) | 0.002 |
| | Blastocyte | 1 (2.4%) | 0 (0%) | 1 (1.2%) | |
| | Cleavage-to-Morula | 0 (0%) | 4 (9.5%) | 4 (4.8%) | |
| | Morula | 0 (0%) | 5 (11.9%) | 5 (6.0%) | |
| **Pregnancy Test** | Positive | 9 (21.4%) | 12 (28.6%) | 21 (25.0%) | 0.450 |
| | Negative | 33 (78.6%) | 30 (71.4%) | 63 (75%) | |
| **Post-Implantation Abortion** | | 1 (2.4%) | 0 (0%) | 1 (1.2%) | 0.314 |
| **Post-Implantation Ectopic Pregnancy** | | 0 (0%) | 0 (0%) | 0 (0%) | – |

**Table V:** Reproductive outcomes of treatment in the L-arginine and control groups. (Data are reported as mean with 95% confidence intervals due to over-dispersed distribution).

| Outcome | | Trial | | | | | | P-value |
|---|---|---|---|---|---|---|---|---|
| | | L-Arginine (N=42) | | Control (N=42) | | Total (N=84) | | |
| | | Mean | 95% CI | Mean | 95% CI | Mean | 95% CI | |
| **Endometrial Preparation Failure** | | 0.38 | [0.08-0.68] | 0.14 | [-0.02-0.30] | 0.26 | [0.09-0.43] | 0.131 |
| **Cycle Cancellation** | | 0 | – | 0 | – | 0 | – | – |
| **Embryo Quality** | Grade A | 1.33 | [1.04-1.62] | 1.74 | [1.41-2.06] | 1.54 | [1.32-1.75] | 0.070 |
| | Grade B | 0.74 | [0.52-0.96] | 0.57 | [0.32-0.82] | 0.65 | [0.49-0.82] | 0.167 |
| | Grade C | 0.12 | [-0.004-0.24] | 0.24 | [0.11-0.37] | 0.08 | [0.006-0.160] | 0.674 |
| **Gestational Sacs** | | 0.14 | [0.01-0.27] | 0.14 | [0.03-0.25] | 0.14 | [0.06-0.22] | 0.789 |

**Figure 4:** Reproductive outcomes of treatment in L-arginine and control groups, including **A)** endometrial preparation failure, **B)** cycle cancellation, **C)** embryo quality – grade A, **D)** embryo quality – Grade B, **E)** embryo quality – grade C and **F)** gestational sacs.



As can be seen, patients had generally low rates of endometrial preparation failure (0.26, [0.09-0.43]), without any noticeable difference between the two groups. Cycle cancellation was not reported in any of the patients from either group. Embryo quality (grades A to C) was largely comparable between the two groups; however, the control group was found to have a slightly higher mean count of grade A embryos (1.74, [1.41-2.06]) compared to the L-arginine group (1.33, [1.04-1.62]), although this difference was not statistically significant (P=0.070), indicating that L-arginine treatment did not have any meaningful effect on embryo quality. Lastly, gestational sacs were few overall (0.14, [0.06-0.22]) and highly similar between the two groups (P=0.789), though, one patient from the L-arginine group had 2 gestational sacs as visualized in the box plot. Collectively, treatment with L-arginine was not accompanied by meaningful differences between the two groups with regard to endometrial preparation failure, embryo quality and gestational sacs.

## Discussion

In this study, we investigated the effects of L-arginine treatment on endometrial thickness and reproductive outcomes in a cohort of 84 women with infertility of different either male or female origin. Patients were randomly assigned to receive either L-arginine + ART protocol or ART protocol alone, and the baseline characteristics, including demographic and infertility-related parameters, were comparable between the two groups. Significant findings included an increase in endometrial thickness following L-arginine treatment ($P < 0.001$), by approximately 2.36-fold, and a notable difference in the stage of implanted embryos, with the L-arginine group showing a higher prevalence of cleavage-stage embryos (P=0.002). However, no statistically significant differences were observed in other reproductive outcomes, including pregnancy rates, embryo quality, or gestational sacs, suggesting that while L-arginine may enhance endometrial thickness, it does not substantially improve overall reproductive success.

The significant increase in endometrial thickness observed in the trial following L-arginine supplementation is supported by a body of evidence suggesting that L-arginine exerts positive effects on endometrial function through various molecular and cellular mechanisms. Notably, the role of L-arginine as a precursor for nitric oxide (NO), a potent vasodilator, is likely pivotal to these effects. Increased NO levels contribute to improved blood flow and angiogenesis within the endometrium, which are crucial for endometrial receptivity and embryo implantation[13,17]. The ability of L-arginine to ameliorate oxidative stress, as demonstrated by Kalehoei et al. in 2023, further supports its potential in enhancing the uterine environment. In their study, L-arginine administration normalized oxidative status by restoring total antioxidant capacity and reducing NO and total oxidative stress levels, hence, improving oocyte quality and subsequent embryonic development. These antioxidative properties may also be involved in promoting endometrial thickness by reducing oxidative damage and promoting tissue regeneration[18].

Moreover, the regulation of water transport in the endometrium, as reported by Zhu et al. in 2021, highlights another mechanism by which L-arginine may enhance endometrial thickness. Their study found that L-arginine supplementation increased the expression of aquaporins (AQPs), proteins facilitating water transport, in the endometrial and placental tissues of pregnant gilts. This effect could augment the hydration of the endometrial stroma, hence, facilitating its expansion and improving its capacity to support embryo implantation[19]. Additionally, the modulation of steroid hormone receptor expression, as demonstrated by Gao et al. in 2019, provided further insights into the molecular pathways affected by L-arginine. L-arginine was found to increase the expression of estrogen receptor α (ERα) and progesterone receptor (PGR) in the ovine endometrium, which are highly important for endometrial proliferation and differentiation during the menstrual cycle[20]. The upregulation of these receptors was speculated to contribute to the enhanced endometrial response observed in the trial, as estrogen and progesterone are key regulators of endometrial growth and receptivity.

The influence of L-arginine on angiogenesis is another important factor that may contribute to increased endometrial thickness. In 2018, Gao et al. reported that L-arginine supplementation restored microvessel density (MVD) and decreased angiogenic growth factors, such as vascular endothelial growth factor A (VEGFA) and vascular endothelial growth factor receptor 2 (VEGFR2), in nutrient-restricted sheep, suggesting a role in maintaining vascular homeostasis in the endometrium[21]. Enhanced angiogenesis is thought to sustain sufficient blood supply to the endometrium, promoting tissue expansion and creating an optimal environment for embryo implantation[22,23]. Furthermore, the ability of L-arginine to decrease oxidative stress and improve endothelial function may further enhance endometrial receptivity by maintaining the integrity of the endometrial vasculature[21].

The findings of Greene et al.[6], reported in 2013, also suggested that L-arginine might exert direct effects on endometrial cells by promoting cell proliferation and inhibiting apoptosis. Their study observed that L-arginine increased cell proliferation and reduced mitochondrial-mediated apoptosis in human endometrial cells, effects that were mediated through NO and polyamine biosynthesis[24]. This dual action of promoting cell growth while protecting against apoptosis could explain the significant increase in endometrial thickness observed in the current trial. The reduced apoptosis may also

improve the overall quality and function of the endometrial tissue, further contributing to its receptivity and capacity to support implantation[25].

Beyond the regulatory mechanisms facilitating the potentially beneficial effects of L-arginine supplementation in patients with infertility, the clinical applicability of this approach is equally important, as well. Clinical trials investigating the use of L-arginine in patients seeking pregnancy have shown promising results, particularly in improving endometrial thickness and uterine blood flow, which are critical factors for successful implantation. For instance, Pilania et al. (2021) reported increased endometrial thickness in patients with previously thin endometrium, suggesting a positive role for L-arginine in enhancing uterine conditions during fertility treatments[26]. Similarly, So et al. (2020) found that L-arginine supplementation significantly improved pregnancy rates in women undergoing assisted reproductive technology (ART) for male-factor infertility, indicating its potential benefit in this subgroup[16]. Nevertheless, we did not observe significantly higher rates of positive pregnancy tests in our L-arginine group compared with the controls. Moreover, studies by Takasaki et al. (2010) and Battaglia et al. (1999) demonstrated that L-arginine could improve uterine and ovarian blood flow, leading to better ovarian response and higher pregnancy rates in poor responders[27,28]. These findings, though with different patient populations and study designs, consistently highlight the beneficial effects of L-arginine in fertility treatments, particularly in cases involving thin endometrium or poor ovarian response, reinforcing its potential as an adjunct therapy in ART programs.

### Strengths and Limitations

This study's strengths include proper randomization, as demonstrated by the comparable demographic and gynecologic histories between the L-arginine and control groups ($P > 0.05$), reducing potential confounding factors. The focus on a specific population —patients with refractory thin endometrium— adds clinical relevance, while the inclusion of a control group allows for a clear comparison of outcomes. However, the trial's small sample size and single-center design limit its generalizability, with potential geographic and ethnic homogeneity reducing external validity. Additionally, variability in IVF protocols across different settings, not accounted for in the study, may influence the broader applicability of the results.

## Conclusion

Taken together, the results of this trial and the supporting evidence from previous studies suggest that L-arginine supplementation enhances endometrial thickness through a combination of increased blood flow, improved angiogenesis, modulation of steroid hormone receptors, enhanced water transport, and reduced oxidative stress and apoptosis. These mechanisms highlight the multifaceted role of L-arginine in improving endometrial receptivity and its potential as a therapeutic agent in assisted reproductive technologies, particularly for patients with refractory thin endometrium. However, further research is warranted to fully elucidate the precise molecular pathways involved and optimize L-arginine dosing and timing in clinical settings.

### Conflict of Interest

The authors declare no conflicts of interest.

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