HUE UNIVERSITY
UNIVERSITY OF MEDICINE AND PHARMACY

LE VIET NGUYEN SA

PHENOTYPE, REPRODUCTIVE ENDOCRINOLOGY, METABOLIC PROFILE, EFFECTS OF METFORMIN AND INOSITOL ON INFERTILITY WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

SUMMARY OF MEDICAL DOCTORAL DISSERTATION

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Supervisors:

Assoc. Prof. LE MINH TAM

Prof. CAO NGOC THANH

Reviewer 1: **Prof. NGUYEN DUY ANH**

Reviewer 2: **Assoc. Prof. PHAM HUY HIEN HAO**

Reviewer 3: **Assoc. Prof. TRAN THUA NGUYEN**

The thesis will be presented in front of the jury board

At the thesis defense theater of Hue University, No 3 Le Loi Street, Hue city.

At: 8 am, 16-08-2023

The dissertation can be found at:

- Library of University of Medicine and Pharmacy-Hue University
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INTRODUCTION

1. BACKGROUND AND AIMS

Polycystic ovarian syndrome is a prevalent endocrine disorder that affects women of reproductive age. Depending on diagnostic criteria and study population, the prevalence of the syndrome ranges from 4% to 21%.

According to the available data, women with polycystic ovarian syndrome are associated with increased rates of early miscarriage, metabolic syndrome, risk of developing glucose intolerance and type 2 diabetes compared to women without. Polycystic ovarian syndrome is a frequent cause of ovulatory disorders that result in infertility. In clinical practice, ovulation treatment is common, with medical treatment being the primary indication. Multiple studies have demonstrated the positive effects of Metformin and Inositol on the menstrual cycle, pregnancy, and certain metabolic and endocrine issues in polycystic ovarian syndrome women.

Differences in phenotype and incidence of metabolic disorders among women with polycystic ovarian syndrome of different ethnic groups have been confirmed. Therefore, it is crucial to examine the clinical, subclinical characteristics and phenotypes of polycystic ovarian syndrome Vietnamese women in order to identify the most significant phenotypes and the main risk groups for treatment strategies and long-term management. In Vietnam, a number of interventional trials have been conducted to evaluate the efficacy of metformin in women with polycystic ovarian syndrome, but the sample sizes remained limited, with the primary objective being to examine the pregnancy rate or the change in parameters related to insulin resistance without a comprehensive assessment of changes in clinical, endocrine, and metabolic characteristics; or compared to other insulin sensitizers. For these reasons, I carried out the research "Phenotype, reproductive endocrinology, metabolic profile, effects of metformin and inositol in infertile women with polycystic ovary syndrome" aimed to:

1. To investigate the phenotypic, endocrine, metabolic, and ultrasound characteristics of infertile women with polycystic ovary syndrome.
2. To evaluate the effectiveness of metformin and inositol; ovulation induction in infertile patients with polycystic ovary syndrome.

2. CONTRIBUTION OF THE STUDY

Polycystic ovarian syndrome is a common endocrine disorder in women of reproductive age and is associated with numerous long-term health consequences. Research on polycystic ovary syndrome is crucial in Vietnam because of the variety of clinical manifestations, the complexity of the pathogenesis, and the impact of race on the phenotype. This research is necessary to identify the main phenotypes, clasify risk groups for long-term treatment, and develop management strategies. The findings of this study can be used in clinical practice to manage polycystic ovarian syndrome and to provide evidence for infertility treatments with the insulin-sensitive medications such as metformin and inositol as well as regimens for ovulation induction.

Scientific value: The research has revealed distinctive phenotypes and endocrine metabolism, emphasizing the elevated risk of glucose uptake disorders, metabolic disorders and dyslipidimia in women with polycystic ovarian syndrome. The study also makes conclusions about a number of infertility treatments for women with polycystic ovarian syndrome, including insulin-sensitive medicines such as metformin and inositol, and ovulation induction letrozole, giving scientific support for the use of these treatments in infertile women with polycystic ovary syndrome.

Practical value: There is evidence to support the need for endocrine and metabolic disorders screening and preventive treatment options in women with polycystic ovary syndrome who are of reproductive age in order to avoid pregnancy compilcations and long-term issues thereafter. Provide evidence for using metformin and inositol to improve weight, waist circumference and menstrual cycle in obese infertile women with polycystic ovary syndrome. Provide support for the use of letrozole as a first-line protocol for ovulation induction in infertile women with polycystic ovary syndrome.

3. DISSERTATION OUTLINE

The dissertation consists of 147 pages: 2 pages of introduction of the study, 41 pages of literature review, 21 pages of subjects and methods, 39 pages of results, 41 pages of discussion, 2 pages of conclusion, 1 page of recommendations. The dissertation has 31 tables, 16 figures, 1 diagrams, 10 charts, 201 references, including 16 Vietnamese documents and 185 English documents.

Chapter 1
LITERATURE REVIEW

1.1. OVERVIEW OF POLYCYSTIC OVARIAN SYNDROME

1.2. ETIOLOGY AND PATHOPHYSIOLOGY

The role of insulin resistance in the pathogenesis of PCOS.

1.3. SYMTOMPS OF POLYCYSTIC OVARIAN SYNDROME

1.3.1. Clinical features: ovulation disorders, hyperandrogenism, obesity, insulin resistance, metabolic syndrome

1.3.2. Paraclinical features: hormonal changes: increased androgens, elevated LH, LH/FSH ratio, decreased SHBG, increased Prolactin, AMH; polycystic ovary morphology on ultrasound

1.4. DIAGNOSIS, PHENOTYPES AND GENETIC ASPECT OF PCOS

1.4.1. Diagnosis:

1.4.1.1. Diagnostic criteria for polycystic ovary syndrome

- NIH 1990
- Rotterdam criteria 2003
- AES 2006, AE/PCOS 2009.

1.4.1.2. Exclusion of other disorders

1.4.2. PCOS phenotypes

The different phenotypes in PCOS based on Rotterdam criteria:

- Type A: hyperandrogenism, chronic anovulation and polycystic ovaries
- Type B: hyperandrogenism and chronic anovulation
- Type C: hyperandrogenism and polycystic ovaries
- Type D: chronic anovulation and polycystic ovaries

1.4.3. Genetic aspect of PCOS

1.5. PCOS AND INFERTILITY

It has been showed that the effect of PCOS which may result in subfertility acts at multiple levels: central (hypothalamic-pituitary-ovarian axis) results in anovulation, intra ovarian which adversely affects the quality of oocytes which in turn affects the quality of the resulting embryos or intrauterine level results in implantation failure and recurrent pregnancy loss.

1.5.1. PCOS and chronic anovulation

1.5.2. Effect of PCOS on oocyte development

1.5.3. Effect of PCOS on embryo quality

1.5.4. Effect of PCOS on embryo implantation and recurrent implantation failure

1.6. TREATMENT OF INFERTILITY IN WOMEN WITH PCOS

1.6.1. Insulin sensitizers

1.6.1.1. *Inositols*

Functions as insulin second messenger and mediate different actions of insulin in human. Any defect in the phosphatidylinositol 3-kinase signaling pathway will eventually reduce the glucose uptake insulin-sensitve tissues and lead to insulin resistance.

1.6.1.2. *Metformin*

A member of biguanide family, Metformin works by improving the sensitivity of peripheral tissues to insulin, which results in a reduction of circulating insulin levels. Metformin inhibits hepatic gluconeogenesis and it also increases the glucose uptake by peripheral tissues and reduces fatty acid oxidation.

1.6.2. Regimens for ovulation induction in PCOS

1.6.2.1. Aromatase inhibitors

Inhibits the Aromatase – the enzyme that catalyzes the conversion of androgens to estrogens, resulting in a decrease in blood estradiol levels, which counteract the estrasdiol's negative feedback mechanism on gonadotropin secretion.

1.6.2.2 Gonadotropins

FSH stimulates growth and development of ovarian follicles in the ovary, stimulate granula cells. It is believed that FSH preparations provide the most physiological approach to the relative elevations of circulating LH/FSH in patients with PCOS.

1.6.3. Surgical treatment

1.6.4. In vitro fertilization

1.6.5. In vitro maturation

1.7. MEDIUM AND LONG-TERM COMPLICATIONS OF PCOS

1.7.1. Pregnancy complications related to PCOS

1.7.1.1. Early loss of pregnancy and preterm birth

1.7.1.2. Gestational hypertension

1.7.1.3. Gestational diabetes mellitus

1.7.1.4. Adverse pregnancy outcomes

1.7.2. Long-term complications of PCOS

- Type 2 diabetes
- Cardiovascular diseases
- Anxiety and depression disorders
- Endometrial cancer

1.8. CURRENT RESEARCH

1.8.1. In the world

1.8.1.1. Metformin therapy

- The meta-analysis by Morley et al. (2017) revealed an advantage of metformin on live birth versus placebo. Sharpe et al. concluded that metformin was more beneficial than placebo in terms of live birth, but it was associated with more frequent gastrointestinal adverse effects.

1.8.1.2. Inositol therapy

- According to Morley et al. (2017), inositol could increase ovulation rate (OR 3.57; 96% CI 1.92 - 7.45).

- Pundir et al. (2017) found that Inositol was associated with enhanced ovulation, menstrual cycle regulation, and decreased serum androgen, total and free Testosterone and DHEA concentrations versus placebo.

1.8.1.3. Ovulation induction with Letrozole

- Franik et al. (2018) concluded from a meta-analysis of 42 clinical trials that the live birth rate and pregnancy rate were higher in the letrozole ovulatory group than in the Clomiphene Citrate group.

1.8.1.2. In Vietnam

1.8.2.1. Defining PCOS phenotypes and metabolic profiles

- Cao Ngoc Thanh (2019) conducted a large sample study to report that the Vietnamese infertile PCOS population was slim, less hirsutism, anovulate, and had elevated LH and AMH concentrations. Phenotype D was the common phenotype. 12.5% of them were affected by metabolic syndrome. According to Nguyen Thi Gia Khanh (2021), the prevalence of

metabolic syndrome among women with PCOS was 28.6%.

1.8.2.2. Use of insulin-sensitizing agents

- Ho Manh Tuong (2004) reported a natural pregnancy rate of 16.2% after 3 months treatment with metformin 1000mg/day, but ovulation rate was not mentioned.

- Vu Van Tam (2009) conducted a clinical trial with metformin in over 100 PCOS women with insulin resistance. He concluded that metformin alone and in combination with CC decreased LH, insulin, the HOMA-IR index, and increased the QUICKI index, significantly.

- No clinical trials have compared the clinical, endocrine, and metabolic effects of metformin with those of other insulin sensitizers.

Chapter 2
SUBJECTS AND METHODS

2.1. RESEARCH SUBJECTS

2.1.1. Selection criteria

Infertile women diagnosed with PCOS visited The Center for Reproductive Endocrinology & Infertility, Hue University of Medicine and Pharmacy hospital for examination and treatment from May 2018 through August 2022, meeting the selection criteria:

- infertility as defined by the WHO
- diagnosis of PCOS based on the 2003 Rotterdam consensus
- patent or normal fallopian tubes diagnosed by hysterosalpingography
- normal husband's semen analysis
- agree to participate in the research

2.1.2. Exclusion criteria

- congenital adrenal hyperplasia, Cushing's syndrome
- under 18 and over 40 years of age
- hyperthyroidism, hyperprolactinemia
- history of oral contraceptive use within three months
- type 1 and type 2 diabetes
- history of ovarian surgery, presence of ovarian disease (ovarian cyst, tumor, or endometrioma)

2.2. RESEARCH METHODS

2.2.1. Study design: a cross-sectional design and a randomized clinical trial

2.2.2. Sample size: 171 infertile women with PCOS, satisfying the estimated sample size.

2.2.3. Study instruments

- Protocols, infertility medical record documentation
- Bench scale, Omiron automatic blood pressure monitor (HEM-7117-AP).
- Diagnostic ultrasound system ALOKA Prosound SSD-3500 with 7 MHz vagnial probe, Hitachi, Japan.
- Roche/Cobas C assay analyzer (Module Cobas 6000/8000), Roche Diagnostics, Indianapolis, USA.
- Microscope, sperm counting chambers, slides and cover slips
- Centrifuge, CO_2 incubator
- Sil-silect 90%, Sil-silect 45% (Fertipro, Belgium), Ferticult Flushing (Fertipro, Belgium)
- 1 ml syringe, IUI smooze long catheter (Gynetics, Belgium)
- Speculum, Kelly.

2.2.4. Procedure

Objective 1:

2.2.4.1. To investigate the phenotypic, endocrine, metabolic, and ultrasound characteristics of infertile women with PCOS

- Interview general characteristics: age, occupation, geography
- Interview medical histories:.
- Perform baseline clinical evaluation included height, weight, BMI, waist and hip circumferences, blood pressure and evaluation of hirsutism, acne, alopecia, and acanthosis nigricans.
- Perform transvaginal ultrasound examination: measure the sizes of the ovaries in three dimensions and define polycystic ovarian morphology; calculate the ovarian volume; measure the uterus in three dimensions: length, depth and width; measure endometrial thickness on day 2-4 of cycle.
- Serum testing included anti-mullerian hormone (AMH), day 3 estradiol, follicle stimulating hormone (FSH), and luteinizing hormone

(LH), testosterone, prolactin, fasting lipid profile, fasting blood glucose, two-hour glucose tolerance testing (GTT), and HbA1C.

- Determine the PCOS phenotypes.
- Identify MetS and IR.

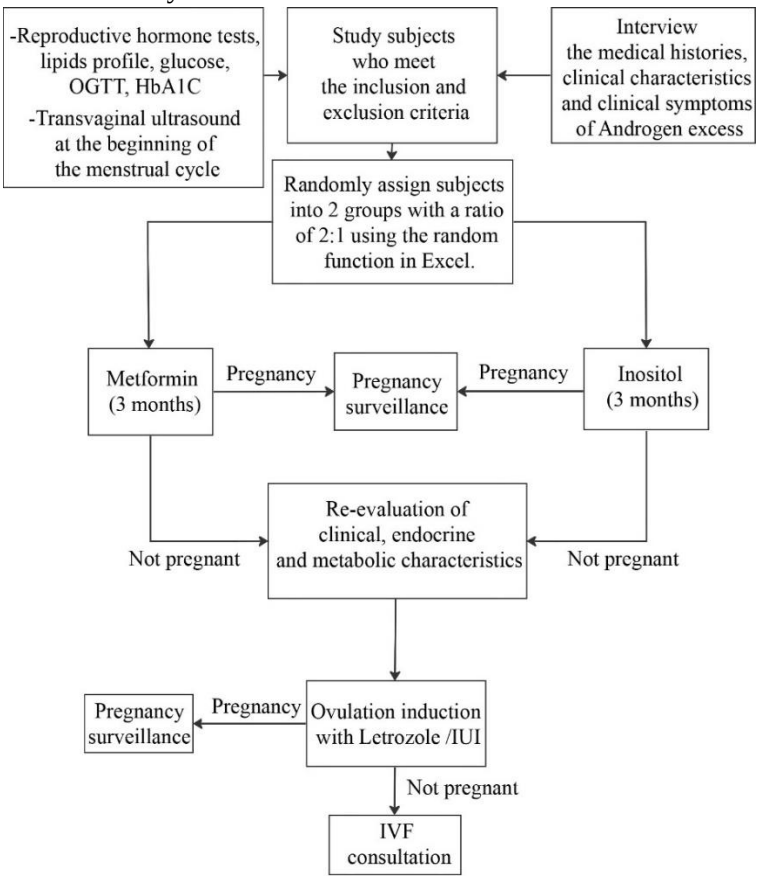

Diagram 2.1. Study diagram

Objective 2:

2.2.4.2. To evaluate the effiectiveness of Metformin and Inositol; ovulation induction in infertile patients with polycystic ovary syndrome.

- Randomly assign subjects to the Metformin intervention group (Met group) and the Inositol intervention group (Ino group) in a ratio of

2:1 using the random function in Excel.

+ Met group: Glucophage dose 850mg x 2 tablets/day, take 1 tablet with the breakfast and 1 tablet with the evening meal for 3 months.

+ Ino group: Inositol 500mg x 4 tablets / day, take 2 tablets after breakfast and 2 tablets after dinner 15-30 minutes for 3 months.

- Monitor intervention results after three months: adverse effects (if any); menstrual cycle characteristics; re-examination of clinical features; re-testing of blood tests; spontaneous pregnancy; pregnancy surveillance.

- If the patient is not conceive, use letrozole alone or in combination with FSH for ovulation induction

- Monitor follicular development and administer Ovitrelle 250 mcg when there is at least one follicle measuring 17 mm or more.

- Cancel the cycle if there are more than three follicles of 14mm or greater in size or if there are no developing follicles on day 28 of the menstrual cycle.

- Perform IUI 34-40h after hCG administration.

- Pregnancy surveillance.

2.2.5. Criteria of research variables

2.3. DATA ANALYSIS

2.3.1. Data collection

2.3.2. Data analysis methods

- The normal distribution of the results was checked by the Kolmogorov-Smirnov test.

- Compare the difference between two means using an unpaired t-test or a Mann-Whitney U test.

- Use a paired t-test or Wilcoxon test to examine the difference between the values before and after treatment.

- Compare the difference between the two ratios utilizing the 2-tailed $\chi 2$ test; Fisher's exact test for 2x2 tables containing 20% of cells with expected frequency 5.

- Use the McNerma test with a significance level of = 0.05 to examine the difference between the rates before and after treatment.

- Analyze a diagnostic test using the ROC curve.

- Analysis of univariate and multivariable logistic binary regression among dependent variables: MetS, IR, pregnancy after treatment, response to ovulation induction.

2.4. RESEARCH ETHICS

The study was approved by the Biomedical Research Ethics Committee, Hue University of Medicine and Pharmacy, Hue University, no H2018/432.

Chapter 3
RESEARCH RESULTS

3.1. PHENOTYPES, REPRODUCTIVE ENDOCRINOLOGY AND METABOLIC PROFILE ON INFERTILE WOMEN WITH PCOS

3.1.3. Clinical features of infertile women with PCOS

Table 3.3. Clinical features of infertile women with PCOS

Characteristic	Total (n = 171)	Metformin (n = 113)	Inositol (n = 58)	p
Irregular menses	153 (89.5%)	105 (92.9%)	48 (82.8%)	**0.040**
BMI	21.10 ± 2.43	21.30 ± 2.32	20.69 ±2.60	0.058
Waist circumference (cm)	75.78 ± 8.27	76.06 ± 8.13	75.22 ± 8.57	0.377*
WHR	0.83 ± 0.06	0.83 ± 0.06	0.83 ± 0.06	0.547
mFG	1.79 ± 2.79	1.40 ± 2.15	2.55 ± 3.64	0.068*
Hirsustism	64 (37.4%)	38 (33.6%)	26 (44.8%)	0.152
Acne	19 (11.1%)	10 (8.8%)	9 (15.5%)	0.189
Alopecia	8 (4.7%)	5 (4.4%)	3 (5.2%)	1.000**
Acanthosis nigricans	1 (0.6%)	1 (0.9%)	0 (0.0%)	1.000**

* Mann-Whitney U test ** Fisher's Exact test

Comment: PCOS infertile women had irregular menstrual cycles, a low BMI, less hirsutism, and few hyperandrogenism symptoms.

3.1.4. Reproductive endocrine features of infertile women with PCOS

Table 3.4. Reproductive endocrine features of infertile women with PCOS

Parameters	Total (n = 171)	Metformin (n = 113)	Inositol (n = 58)	p
FSH (IU/L)	6.05 ± 1.32	6.00 ± 1.41	6.16 ± 1.13	0.448
LH (IU/L)	10.02 ± 5.94	9.69 ± 5.73	10.67 ± 6.34	0.352*

LH/FSH	1.67 ± 0.95	1.62 ± 0.89	1.76 ± 1.05	0.765*
E2 (pg/ml)	40.45 ± 20.88	40.13 ± 22.26	41.07 ± 18.07	0.299*
Testosterone (ng/mL)	0.287 ± 0.136	0.295 ± 0.139	0.270 ± 0.127	0.324*
AMH(ng/mL)	7.42 ± 3.70	7.37 ± 3.43	7.52 ± 4.21	0.921*

*Mann-Whitney U test

Comment: Infertile women with PCOS had high LH and AMH levels but low total testosterone levels.

3.1.5. Ultrasound characteristics of infertile women with PCOS

Table 3.5. Ultrasound characteristics of infertile women with PCOS

Characteristic	**Total (n = 171)**	**Metformin (n = 113)**	**Inositol (n = 58)**	**p**
Left ovary volume (ml)	8.42 ± 3.56	8.16 ± 3.60	8.93 ± 3.46	0.146*
Right ovary volume (ml)	9.41 ± 3.87	9.22 ± 3.58	9.79 ± 4.39	0.788*
Length of uterus (mm)	47.45 ± 5.98	46.92 ± 6.21	48.50 ± 5.39	0.102
DAP (mm)	36.24 ± 5.64	35.88 ± 5.87	36.95 ± 5.16	0.094*
PCOM n (%)				
One side	26 (15.2%)	20 (17.7%)	6 (10.3%)	0.447**
Two sides	142 (83.0%)	91 (80.5%)	51 (87.9%)	
None	3 (1.8%)	2 (1.8%)	1 (1.7%)	

* Mann-Whitney U test ** Fisher's Exact test

Comment: The mean volumen of the ovaries of infertile women with PCOS is less tan 10ml.

3.1.10. Metabolic profiles of infertile women with PCOS

Table 3.10. Metabolic profiles of infertile women with PCOS

Parameters	**Total (n = 171)**	**Metformin (n = 113)**	**Inositol (n = 58)**	**P**
Lipid profile (mmol/L)				
Cholesterol	4.48 ± 0.80	4.46 ± 0.85	4.50 ± 0.71	0.778
Triglycerid	1.32 ± 1.02	1.37 ± 1.24	1.22 ± 0.84	0.682*
LDL-Cho	3.03 ± 0.72	3.00 ± 0.75	3.08 ± 0.67	0.486
HDL-Cho	1.31 ± 0.31	1.30 ± 0.31	1.34 ± 0.31	0.479
Glucose profile (mmol/L)				

G0	5.16 ± 0.42	5.20 ± 0.41	5.08 ± 0.43	0.094*
G2	6.54 ± 1.45	6.58 ± 1.44	6.46 ± 1.48	0.610
HbA1c (%)	5.11 ± 0.37	5.18 ± 0.38	4.99 ± 0.32	**0.001***
	n (%)	**n (%)**	**n (%)**	**P**
Impaired glucose tolerance	10 (5.8%)	7 (6.2%)	3 (5.2%)	1.000**
MetS	21 (12.3%)	13 (11.5%)	8 (13.8%)	0.666
Dyslipidemia	75 (43.9%)	51 (45.1%)	24 (41.4%)	0.640

*Mann-Whitney U test ** Fisher's Exact test

Comment: There were 5.8% of women with impaired glucose tolerance, 12.3%with MetS, and 43.9% with at least one aberrant blood lipid parameter.

3.1.13. Predictors of metabolic syndrome in infertile women with PCOS

Table 3.12. Univariate and multivariate logistic regression analysis of predictors of MetS in infertile women with PCOS

Factors		**MetS (n = 21)/ Total (n = 171)**			
		OR (95% CI)	**p**	**OR adjusted (95% CI)**	**P**
BMI (kg/m^2)	< 18.5	NA	NA	NA	NA
	18.5 -< 23	1	-	1	-
	23 -< 24.9	3.65 (1.08 – 12.26)	0.037	0.68 (0.05 – 10.12)	0.780
	≥ 25	13.61 (3.76 – 49.22)	< 0.001	4.16 (0.20 – 84.93)	0.354
Waist circumference (cm)	< 80	1	-	1	-
	≥ 80	39.64 (8.74 – 179.85)	< 0.001	103.56 (6.34 – 1690.37)	0.001
Testosterone (ng/mL)	> 0.70	NA	NA	-	-
	≤ 0.70			-	-
Triglycerid (mmol/L)	< 1.7	1	-	1	-
	≥ 1.7	100.11 (20.95 – 478.39)	< 0.001	570.48 (27.03– 12040.28)	< 0.001

Comment: Multivariate logistic regression analysis showed that waist circumference and TG are two predictors of MetS in infertile women with PCOS.

3.2. EFFECTIVENESS OF SOME INTERVENTIONS IN INFERTILE WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

3.2.1. Changes in clinical features following metformin therapy

Table 3.19. Changes in clinical characteristics in infertile PCOS patients treated with metformin for three months

Factors	Total (n = 88)		Overweight/Obese (n = 17)		Non Overweight/ Obese (n = 71)	
	Δ (T3-T0)	p	Δ (T3-T0)	p	Δ (T3-T0)	P
Regular cycle	42.1%	**<0.001****	41.2%	**0.016****	42.2%	**<0.001****
Weight (kg)	- 0.54 ± 1.64	**0.002***	- 2.06 ± 1.47	**0.001***	-0.18 ± 1.46	0.228*
BMI (kg/m^2)	- 0.22 ± 0.67	**0.002***	- 0.84 ± 0.60	**0.001***	-0.08 ± 0.60	0.250*
WC (cm)	- 0.24 ± 2.25	0.066*	- 1.12 ± 2.98	**0.041***	- 0.03 ± 2.01	0.457*
HC (cm)	- 0.32 ± 2.09	0.095*	- 0.88 ± 1.93	0.075*	- 0.18 ± 2.12	0.384*
mFG	- 0.10 ± 0.48	**0.038***	- 0.06 ± 0.24	0.317*	-0.11 ± 0.52	0.063*
Hirsutism	-1.1%	1.000**	0%	1.000**	-1.4%	1.000**
Acne	- 2.3%	0.625**	5.9%	1.000**	- 4.2%	0.250**
Alopecia	1.1%	1.000**	0%	NA	1.4%	1.000**

*Wilcoxon Signed Ranks Test **McNemar test

Comment: The treatment with metformin improved menstrual regularity. weight. BMI. and mFG score. The effect was more prominent in the obese cohort.

3.2.2. Changes in endocrine and metabolic characteristics following metformin therapy

Table 3.20. Changes in endocrine and metabolic characteristics in infertile patients with PCOS after three months of metformin

Parameters	Total (n = 88)		Overweight/Obese (n = 17)		Non Overweight/ Obese (n = 71)	
	Δ (T3-T0)	p	Δ (T3-T0)	P	Δ (T3-T0)	p
FSH (IU/L)	- 0.10 ± 1.40	0.453*	-0.01 ± 1.06	0.982	- 0.12 ± 1.48	0.494
LH (IU/L)	- 1.21 ± 4.94	**0.031***	- 0.89 ± 4.60	0.436	- 1.28 ± 5.05	**0.019***
Testosterone (ng/mL)	- 0.020 ± 0.100	**0.011***	- 0.027 ± 0.105	0.266*	-0.017 ± 0.096	**0.017***
Prolactin (IU/L)	2.39 ± 146.21	0.758*	-17.68 ± 181.39	0.906*	7.20 ± 137.58	0.651*

Chol (mmol/L)	- 0.06 ± 0.64	0.345	-0.09 ± 0.75	0.638	-0.06 ± 0.62	0.432
TG (mmol/L)	0.04 ± 1.16	**0.040**[*]	-0.11 ± 2.20	0.332[*]	0.03 ± 072	0.097[*]
LDL-Cho (mmol/L)	- 0.09 ± 0.56	0.159	- 0.08 ± 0.54	0.544	-0.09 ± 0.57	0.208
HDL-Cho (mmol/L)	0.02 ± 0.17	0.493[*]	0.04 ± 0.16	0.381[*]	0.01 ± 0.17	0.740[*]
G0 (mmol/L)	0.04 ± 0.53	0.878[*]	- 0.08 ± 0.57	0.191[*]	0.06 ± 0.52	0.501[*]
G2 (mmol/L)	0.27 ± 1.31	0.059[*]	0.78 ± 1.05	**0.008**	0.15 ± 1.34	0.344
HbA1c (%)	- 0.04 ± 0.29	0.451[*]	- 0.09 ± 0.38	0.346	-0.02 ± 0.27	0.756[*]

*Wilcoxon Signed Ranks Test

Comment: Metformin significantly decreased basal LH and Testosterone levels, particularly in the non-obese group.

3.2.4. Changes in clinical features following inositol therapy

Table 3.22. Changes in clinical characteristics in infertile PCOS patients treated with inositol for three months

Factors	**Total (n = 44)**		**Overweight/Obese (n = 11)**		**Non overweight/obese (n = 33)**	
	Δ (T3-T0)	**p**	**Δ (T3-T0)**	**p**	**Δ (T3-T0)**	**p**
Regular cycle	18.2%	**0.008**[**]	16.7%	0.500[**]	18.4%	**0.031**[**]
Weight (kg)	- 0.50 ± 1.63	0.089	- 2.09 ± 0.83	**<0.001**	0.03 ± 1.48	0.761[*]
BMI (kg/m^2)	- 0.11 ± 0.93	0.102[*]	- 0.85 ± 0.30	**<0.001**	0.02 ± 0.60	0.885
WC (cm)	-0.20 ± 2.60	0.367[*]	- 1.82 ± 1.47	**0.002**	0.33 ± 2.67	0.580[*]
HC (cm)	- 0.82 ± 2.85	**0.016**	- 1.82 ± 2.63	**0.045**	- 0.48 ± 2.87	0.340
mFG	-0.05 ± 0.21	0.157[*]	0.00	1.000[*]	- 0.06 ± 0.24	0.157[*]
Hirsutism	0%	1.000[**]	0%	1.000[**]	0%	1.000[**]
Acanthosis nigricans	2.3%	1.000[**]	0%	NA[**]	2.6%	1.000[**]

*Wilcoxon Signed Ranks Test **McNemar test

Comment: Inositol treatment increased the frequency of regular menstruation; inositol improved significantly BMI. waist and hips circumference in the overweight and obese group.

3.2.5. Changes in endocrine and metabolic characteristics following inositol therapy

Table 3.23. Changes in endocrine and metabolic characteristics in infertile patients with PCOS after three months of inositol

Parameters	Total (n = 44)		Overweight/Obese (n = 11)		Non overweight/obese (n = 33)	
	Δ (T3-T0)	p	Δ (T3-T0)	P	Δ (T3-T0)	p
FSH (IU/L)	- 0.18 ± 0.82	0.148	0.19 ± 1.26	0.626	- 0.30 ± 0.59	**0.005**
LH (IU/L)	- 0.84 ± 4.48	0.104*	0.79 ± 4.89	0.604	- 1.39 ± 4.28	**0.047***
Testosterone (ng/mL)	- 0.032 ± 0.098	**0.033**	-0.015 ± 0.100	0.618	- 0.038 ± 0.098	**0.033**
Prolactin (IU/L)	-19.40 ± 170.55	0.455	-151.86 ± 278.67	0.101	24.75 ± 82.32	0.094
Chol (mmol/L)	0.05 ± 0.90	0.735	0.42 ± 1.29	0.286*	-0.08 ± 0.71	0.534
TG (mmol/L)	0.04 ± 0.54	0.674*	0.08 ± 0.90	0.764	0.03 ± 0.71	0.681*
LDL-Cho (mmol/L)	0.01 ± 0.90	0.918	0.31 ± 1.27	0.437	- 0.08 ± 0.74	0.516
HDL-Cho (mmol/L)	- 0.02 ± 0.25	0.647	0.03 ± 0.29	0.713	- 0.03 ± 0.24	0.420
G0 (mmol/L)	- 0.07 ± 0.35	0.194	- 0.19 ± 0.34	0.095	- 0.03 ± 0.34	0.633
G2 (mmol/L)	- 0.27 ± 1.30	0.171	- 0.26 ± 1.44	0.560	- 0.27 ± 1.27	0.221
HbA1c (%)	0.03 ± 0.41	0.579	0.07 ± 0.50	0.667	0.02 ± 0.38	0.726

*Wilcoxon Signed Ranks Test

Comment: Inositol treatment significantly decreased free Testosterone levels.

3.2.8. Comparison of metformin and inositol adverse events, tolerability, and spontaneous pregnancy rates

Table 3.26. Adverse effects, tolerability and spontaneous pregnancy rates with metformin and inositol

Factors	Metformin (n = 106)	Inostiol (n = 53)	p
Side effects	31 (29.2%)	5 (9.4%)	**0.005**
Digestive disorders	23	1	-
Tiredness	5	3	
Others	0	1	
Many side effects	3	0	
Tolerance	105 (99.1%)	53 (100%)	1.000*

Factors	Metformin (n = 105)	Inostiol (n = 53)	P
Pregnancy	22 (21.0%)	10 (18.9%)	0.758
Clinical pregnancy	20 (19.0%)	9 (17.0%)	0.751
Ongoing pregnancy	19 (17.9%)	9 (17.0%)	0.883
Miscarriage	4 (3.8%)	1 (1.9%)	0.665*
Live birth	18 (17.1%)	9 (17.0%)	0.980

* Fisher's Exact test

Comment: In the two treatment groups, there was no statistically significant difference in the rates of pregnancy, miscarriage, or live births. Threefold more adverse events occurred in the metformin group.

3.2.9. Characteristics and outcomes of IUI cycles following ovulation induction in infertile women with polycystic ovary syndrome

Table 3.27. Characteristics and outcomes of IUI cycles

Parameters	Total (*n* = 83)	Letrozole (n =47)	Letrozole +FSH (n = 36)	P
Completed cycles	75 (90.4%)	46 (97.9%)	29 (80.6%)	**0.019***
Duration of stimulation	15.84 ± 3.91	14.33 ± 3.03	18.17 ± 4.00	< **0.001****
Endometrial thickness (mm)	7.66 ± 2.20	7.02 ± 2.14	8.69 ± 1.92	**0.001**
No of follicles	1.43 ± 1.19	1.23 ± 0.60	1.69 ± 1.65	0.407
0	5 (6.0%)	1 (2.1%)	4 (11.1%)	0.062*
1	57 (68.7%)	37 (78.7%)	20 (55.6%)	
2	11 (13.3%)	6 (12.8%)	5 (13.9%)	
3	7 (8.4%)	3 (6.4%)	4 (11.1%)	
> 3	3 (3.6%)	0 (0.0%)	3 (8.3%)	
Pregnancy/cycle	22 (26.5%)	13 (27.7%)	9 (25.0%)	0.786
Miscarriage/cycle	3 (3.6%)	1 (3.2%)	2(7.1%)	0.599*
Ongoing pregnancy/ cycle	17 (20.5%)	12 (25.5%)	5 (13.9%)	0.193

Comment: Comparison between LET and LET + FSH cycles showed that the LET alone cycle had a higher completion rate, shorter number of days of stimulation, thinner endometrial thickness ($p<0.05$) but the pregnancy rate difference was not significant.

Chapter 4
DISCUSSION

4.2. PHENOTYPES, REPRODUCTIVE ENDOCRINOLOGY AND METABOLIC PROFILE ON INFERTILE WOMEN WITH PCOS

4.2.3. Clinical features of infertile women with PCOS

Weight and BMI: The average weight and BMI of the women in our study were lower than those of other Caucasian women, but comparable to studies conducted in Vietnam.

The clinical manifestations of hyperandrogenism: were less prevalent in infertile women with PCOS in Vietnam with a low mean mFG score (1.79 ± 2.79) and a low proportion of women with clinical hyperandrogen symptoms. This result was comparable to the findings of Cao Ngoc Thanh et al. (2019). Studies conducted by foreign authors indicated that the mFG scores of Caucasian women were significantly higher than those of Vietnamese women. The low incidence of hirsutism in East Asian women of Chinese, Korean, Thai, and Japanese populations may be attributable to weak alpha reductase activity in the hair follicles.

The 2018 International evidence-based guidelines for the assessment and management of PCOS recommend that medical professionals take into account ethnic differences in the presentation of PCOS: Caucasian women have a higher BMI, particularly in North America and Australia, whereas East Asian women have a lower BMI and less hirsutism.

4.2.4. Reproductive endocrine features of infertile women with PCOS

The mean total Testosterone concentration in our study was 0.287 ± 0.136 ng/mL, which is significantly lower than other studies. Taking into account the characteristics of the study sample, the difference in our Testosterone levels was reasonable, given that our research sample of PCOS patients had very few clinical signs of hyperandrogenism.

The serum AMH concentration in our study was 7.42 ± 3.70 ng/mL. This result was comparable to those of previous studies conducted on the Vietnamese population, but lower than those conducted on Caucasian women. Differences in study population, ethnicity, and AMH quantification methods may affect differences in AMH concentration-related study outcomes

4.2.5. Ultrasound characteristics of infertile women with PCOS

In this study, the mean volume of the left ovary was 8.42 ± 3.56 ml; mean volume of the right ovary was 9.41 ± 3.87 ml. 83.0% of the women had polycystic ovaries on both sides. Multiple studies based on the Rotterdam consensus criteria suggested a lower cutoff of ovarian volume spanning from 6.40 to 7.50 ml in order to increase the diagnostic sensitivity of PCOS.

4.2.10. Metabolic profiles of infertile women with PCOS

Prevalence of MetS in our study was 12.3%. The findings were comparable to those of other studies conducted in the country. Cao Ngoc Thanh et al. (2019) found that the prevalence of MetS in women with PCOS was quite low at 12.5%. Other studies in Asia have demonstrated that the prevalence of MetS varies by country: 14.5 % in Korea; 14.5% in women with PCOS; this prevalence in Taiwan was 16%; 24.9% among Hong Kong Chinese PCOS women. We know that the incidence of MetS in women with PCOS varies considerably across countries and races, most likely as a result of differences in diet, lifestyle, and genetics.

Dyslipidemia: 43.9% of women had disorders of at least one parameter of blood lipid balance, with an increase in LDL-cholesterol being the most common, followed by an increase in TG, and a decrease in HDL-cholesterol being the least common. Despite low prevalence of obesity, the dyslipidemia status of women with PCOS in Vietnam is alarming, according to the findings of this study. In order to prevent future cardiovascular complications, the implementation of lipid balance and close monitoring is required, along with measures to control dyslipidemia.

4.2.13. Predictors of metabolic syndrome in infertile women with PCOS

Obesity (including BMI and waist circumference) was identified as a significant predictor of MetS via univariate logistic regression analysis. Increased waist circumference was the most fundamental criterion in the definition of MetS, as it was associated not only with impaired glucose tolerance and type 2 diabetes, but also with hypertension and dyslipidemia. There is evidence that altered visceral adipocyte function leads to aberrant morphological and functional development of visceral fat in women with PCOS. Obesity, particularly abdominal obesity, is therefore the most significant predictor of MetS that can be detected in the early phases of PCOS, even before the onset of the disease.

Similar to the research of Le Minh Tam (2018) and Sun Y. et al (2018), increased TG was the strongest predictor of MetS based on lipid profile.

4.3. EFFECTIVENESS OF SOME INTERVENTIONS IN INFERTILE WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

4.3.1. Changes in clinical features following metformin therapy

Our research revealed that metformin treatment enhanced menstrual cycle regularity, statistically significant weight loss and BMI, and a slight reduction in mFG scores. Metformin has been shown to be beneficial for weight loss, lowering androgen levels, restoring menstrual cycles, and inducing ovulation in PCOS patients. Patel R. et al. (2017) revealed that metformin reduced BMI, waist-to-hip ratio, SBP, and DBP relative to placebo, but did not affect mFG scores. A meta-analysis by Morley LC et al. (2017) comparing metformin to placebo or no treatment found that metformin increased the rate of menstrual regularity based on seven studies. Theoretically, metformin could ameliorate hyperandrogenism and its clinical manifestations, such as acne and hirsutism, because it decreases ovarian androgen production, ovarian P450c17a activity, and free Testosterone levels, resulting in a reduction of the mFG score within a few months.

4.3.2. Changes in endocrine and metabolic characteristics following metformin therapy

After three months of metformin treatment, LH and Testosterone levels decreased statistically. Other metabolic and endocrine parameters were not significantly altered. In addition to enhancing BMI, hirsutism, and regular menstruation, decreasing free Testosterone concentration, fasting blood insulin, and the HOMA index, Oner G et al. reported that metformin 1500 mg/day reduced total cholesterol concentration. Evidence-based guidelines for the assessment and management of PCOS have aggregated relevant clinical trials, demonstrating that the number of studies on metformin's effects is substantial despite their limited quality and reliability. Metformin is effective in improving weight, BMI, waist circumference, Testosterone, Cholesterol, and TG in general or in specific groups in women with polycystic ovary syndrome. There is stronger evidence of a metabolic benefit in obese women with PCOS.

4.3.4. Changes in clinical features following inositol therapy

We found a statistically significant improvement in the menstrual cycle after three months of treatment with inositol. Weight, BMI, waist circumference, and mFG scores tended to decline, but this trend was not statistically significant ($p > 0.05$). After 8 weeks of treatment with myo-Inositol and an unrestricted diet, Genazzani A. et al. (2012) reported that the patients lost weight at a statistically significant level (decrease in BMI). Zarezadeh M. et al. (2021) discovered that inositol supplementation substantially decreased BMI. Women

with PCOS and overweight/obesity exhibited the most pronounced effect. Inositol in the form of myo-inositol has an even greater effect on reducing BMI. There was a significant decrease in Testosterone levels after 12 weeks of treatment with MI, as well as a decrease in mFG score that did not reach statistical significance (22.7 ± 1.4 to 18.0 ± 0.8) according to the study by Genazzani A. et al. (2008). During six months of treatment with MI, Papaleo et al. reported that the menstrual cycle was restored and preserved.

4.3.5. Changes in endocrine and metabolic characteristics following inositol therapy

After 3 months of treatment with inositol, the concentration of total Testosterone decreased significantly (- 0.032 ± 0.098 ng/mL, p = 0.033). Other endocrine and lipid parameters' changes were not statistically significant.

Unfer et al. (2017) demonstrated a significant decrease in fasting insulin concentration and HOMA index in the MI-supplemented group In addition, there was a trend toward a decrease in Testosterone levels in the MI group compared to the control group, but this difference did not reach statistical significance. These findings demonstrated the beneficial effects of MI in enhancing the metabolism and hyperandrogenism of PCOS-affected females. Similarly, Hayamizu K. et al. (2022) found that compared to the control group, Inositol improved fasting insulin concentration, AUC of glucose tolerance test, free Testosterone and SHBG concentration, as well as ovulation rate were also improved.

Inositol ameliorates insulin resistance and hyperinsulinemia caused by hyperandrogenism, anovulation, and metabolic abnormalities in obese and slim PCOS patients, thereby reducing hyperandrogenism-related symptoms, restoring menstruation, enhancing fertility, and enhancing metabolic parameters.

4.3.7. Comparison of metformin and inositol effectiveness

Our results revealed that the metformin group had a significantly higher menstrual regularity rate than the inositol group ($p < 0.05$). Changes in clinical and paraclinical parameters following treatment with two distinct drugs were not statistically significant ($p > 0.05$). Comparing the two groups, pregnancy rates were comparable while adverse events were significantly higher in the metformin group.

Thakur SS et al (2020) found that myo-inositol appeared to be less effective than metformin and the other group in restoring the menstrual cycle, but the difference was not statistically significant ($p > 0.05$). In addition, metformin improved acne and hirsutism symptoms, whereas myo-inositol had

no discernible effect on skin manifestations. After treatment, both metformin and inositol significantly decreased BMI, and the difference between the two groups was not statistically significant. Regarding the rate of spontaneous pregnancy after six months of treatment, the metformin group significantly improved while the inositol group did not. It should be noted, however, that the sample size of this study was extremely limited. The meta-analysis of Facchinetti F. et al. (2019) reported that there was no difference in the effectiveness of metformin and myo-inositol on short-term endocrine changes, and because myo-inositol was more tolerable, it is more acceptable for restoring androgen expression and metabolism in women with PCOS.

4.3.10. Efficacy of IUI following letrozole-induced ovulation in infertile women with PCOS

In our study, the rate of cycle completion after ovulation induction with letrozole was extremely high (97.9%), the average number of IVF days was 14.33 ± 3.03 days, the average number of mature follicles was 1.23 ± 0.60 follicles, and the mean endometrial thickness was 7.02 ± 2.40 mm, resulting in a clinical pregnancy rate of 25% and a progressive pregnancy rate of 25%. This result is comparable to those of Nguyen Thanh Tung and Kallol Kumar Roy et al (2012). Today, there is accumulating evidence to support the use of LET in women with PCOS, encouraging ART specialists to alter their practice attitudes and recommend Letrozole as the first-line ovulatory stimulant in this population.

Our study showed that the LET group had a higher cycle completion rate, fewer days of ovulation stimulation, and a considerably thinner endometrium ($p < 0.05$), while there was no difference in the pregnancy rate between the two groups ($p > 0.05$).

Alizzi et al. (2018) conducted a similar clinical trial in a group of women with PCOS and no evidence of insulin resistance, reporting that in both groups, pregnancy was achieved in 54/80 women (67.5%) and the ovulation rate reached 91.3% with the average number of cycles performed being 2.3 cycles, 70% of women developed a single mature follicle. Comparing the outcomes of ovulation induction cycles between the two groups, Alizzi et al. discovered that the letrozole group alone resulted in a single mature follicle rate of up to 97.9%, while more than 70% of the letrozole + FSH group developed two mature follicles ($p < 0.001$); however, the pregnancy rate was not statistically different between the two groups. This result was comparable to that of our study, which demonstrated that the letrozole + FSH protocol resulted in greater

multifollicles development than the letrozole alone regimen, but had no effect on the pregnancy rate.

El-Sayed A. et al. (2021) compared to the LET + FSH group, the letrozole group alone had a higher rate of single follicle development and a lower rate of multiple follicles development, while the endometrium was thinner than in the FSH group. These results were comparable to those of our study, as the pregnancy rate was not significantly different. Therefore, it is recommended to use letrozole alone due to its cost-effectiveness without altering the pregnancy rate.

CONCLUSION

1. Phenotypes, reproductive endocrinology and metabolic profile on infertile women with polycystic ovarian syndrome

- Infertile women with Polycystic Ovarian Syndrome were slim with average BMI of 21.10 ± 2.43 kg/m; 89.5% of women had menstrual disorders. Mean mFG was 1.79 ± 2.79, AMH concentration was high (7.42 ± 3.70 ng/mL).

- The incidence of glucose intolerance was 5.8 %. 12.3% of women had metabolic syndrome and 18.1% had insulin resistance.

- Predictors of metabolic syndrome in infertile women with polycystic ovary syndrome include increased waist circumference and increased TG.

2. Effectiveness of some interventions in infertile women with polycystic ovarian syndrome

- Metformin treatment for 3 months improved menstrual cycle, decreased weight and BMI, decreased mFG score, and decreased basal LH and Testosterone levels significantly ($p < 0.05$). In the overweight/obese group, weight, BMI, and waist circumference improvements were greater. The spontaneous conception rate was 21%. Side effects were seen in 29.2% of women.

- Inositol treatment for three months significantly enhanced menstrual cycle, reduced hips circumference, and decreased testosterone levels ($p < 0.05$). In the overweight/obese group, Inositol improved weight, BMI, waist circumference, and hips circumference, while in the non-overweight/normal weight group, it improved the menstrual cycle. The rate of natural conception was 18.9%. 9.4% experienced side effects.

- Metformin treatment was associated with a higher rate of regular menstruation than inositol treatment.

- The cycle completion rate for IUI following ovulation induction with letrozole was 90.4%; the average number of follicles was 1.43 ± 1.19 follicles; the single follicle development rate was 68.7%; and the pregnancy rate per cycle was 26.50%.

- Compared to letrozole alone, letrozole plus FSH had a lower cycle completion rate, higher multifollicles development rate, longer ovulation stimulation days, and thicker endometrium ($p < 0,05$). The pregnancy rate was not significantly different between the two regimens (27.7% vs 25%, $p > 0.05$).

SUGGESTIONS

- A strategy for screening and prophylactic treatment of glucose intolerance, metabolic disorders, and dyslipidemia is required to prevent pregnancy complications and long-term complications in women of reproductive age with polycystic ovary syndrome.

- Metformin and inositol can be used to improve weight and waist circumference in infertile obese women with polycystic ovary syndrome.

- The letrozole regimen should be used as the first-line therapy for ovulation induction in infertile women with polycystic ovary syndrome. Prefer to use letrozole alone due to its cost-effectiveness without affecting the pregnancy rate.

SCIENTIFIC PUBLICATIONS RELATED TO THIS THESIS

1. Le Viet Nguyen Sa, Le Minh Tam, Cao Ngoc Thanh (2019), "Updated evidence on the efficacy of metformin and inositol in the treatment of polycystic ovary syndrome", *Journal of Medicine and Pharmacy*, volume 9, issue 6+7, p. 195-201.

2. Le Viet Nguyen Sa, Nguyen Thi Nhu Quynh, Le Thi Thuan My, Cao Ngoc Thanh, Le Minh Tam (2021), "Effect of phenotypes on assisted reproductive outcomes in women with PCOS", *Journal of Obstertrics and Gynecology*, Vol 19 (1): 54 – 60.

3. Le Viet Nguyen Sa, Le Minh Tam, Nguyen Vu Quoc Hue, Cao Ngoc Thanh (2022), "The value of AMH in predicting ovulation induced by Aromatase inhibitors and pregnancy outcomes in women with polycystic ovary syndrome", *Hue University of medicine and pharmacy International PhD Students' Symposium 2022*.

4. NSV Le, MT Le, ND Nguyen, NQT Tran, QHV Nguyen, TN Cao (2021), "A cross-Sectional Study on Potential Ovarian volume and Related Factors in women with PCOS from Infertile Couples" – *International journal of women health*. 13, pp. 793-801.

5. Le Viet Nguyen Sa, Le Minh Tam, Tran Thi Nhu Quynh, Cao Ngoc Thanh (2022), "The effect of Metformin on clinical features**,** endocrine and metabolic profiles of infertile women with PCOS" ,*Journal of Clinical Medicine*, No 83, p. 50-56.