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The relationship between hormones level and body mass index with insertion and deletion (D/I) polymorphism of ACE gene in infertile patients with polycystic ovary syndrome

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ABSTRACT

Background: The prevalence of polycystic ovary syndrome (PCOS) and infertility are rising due to changes in lifestyle. In this study, the polymorphism of insertion and deletion (I/D)in the angiotensin converting enzyme (ACE) gene in PCOS patients and the possibility of its association with PCOS and plasma level of hormones level as well as body mass index was investigated.

Materials and Methods: In this study, three polymorphisms in the ACE gene in Iranian women (Insertion-Insertion (II), Insertion-Deletion (ID), Deletion-Deletion (DD)) at three groups: PCOS, infertile patients and control as well as their relationship with hormones level and Body Mass Index (BMI) were examined. The methods included standard DNA extraction from peripheral blood and polymerase chain reaction (PCR) using specific primers for ACE gene. The data were then statistically analyzed.

Results: Results showed an higher prevalence of ID polymorphism in PCOS and infertility population with statistically significant association (p=0.013). There were also significant associations between the polymorphism with BMI, LH and progesterone level. The number of primordial follicles also found to be three times greater in PCOS group compared with normal ones.

Conclusion: The results showed significant association between polymorphisms of ACE gene, hormones level and BMI in PCOS which might be considered as possible prognostic marker for infertility of PCOS patients.

Keywords: Gene polymorphism, polycystic ovary syndrome (PCOS), Angiotensin converting enzyme gene (ACE gene), Infertility.
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INTRODUCTION

e Polycystic Ovary Syndrome (PCOS) or Stein-Leventhal Syndrome was rst diagnosed in women with sclerocystic in 1884 and the attention was not drawn by other variations up to 100 years later, so that the increase in Luteinizing Hormone (LH) was not examined in relation to morbidity of this disease until 1985.[1] PCOS syndrome is the most prevalent cause of infertility and endocrine gland disorders in women which ranging from 6 to 10% of fertile women population. e terminological reason of this syndrome is related to the presence of large ovaries with a great number of little cysts located in the outer layer of each ovary. e disease pose signi cant challenge for health professionals as it increases the risk of breast and endometrial cancer, type II diabetes mellitus, gestational hypertension, and obesity compare to healthy females. In fact, 40% of the patients are obese and 75% of them are infertile. e diagnosis usually established by history taking, ovarian sonography, and gonadal hormone analysis.[2]

Angiotensinogen is produced by the liver and released into the blood stream. It cause Juxtaglomerular apparatus to release renin which convert Angiotensin 1 into Angiotensin 2. released renin from kidneys converts angiotensinogen into angiotensin 1. When angiotensin 2 enters into blood circulation it stimulates adrenal cortex to produce aldosterone.[3] Changing activity of ACE would a ect the homeostasis of RAAS and potentially result in several chronic or degenerative disease. In this study, we evaluate the relationship between D/I type of ACE gene polimorphism toward PCOS risk since PCOS are related with increased risk of type 2 diabetes mellitus, breast cancer, and infertility. However, we only examine the impact of ACE gene polimorphism in relation to infertility related PCOS. Mattei et al determined the position of the ACE gene on chromosome 17q23 through topical hybridization and it was shown that this area was highly involved in the polymorphism but no recombination was observed in this gene.[4] Similarly, it was

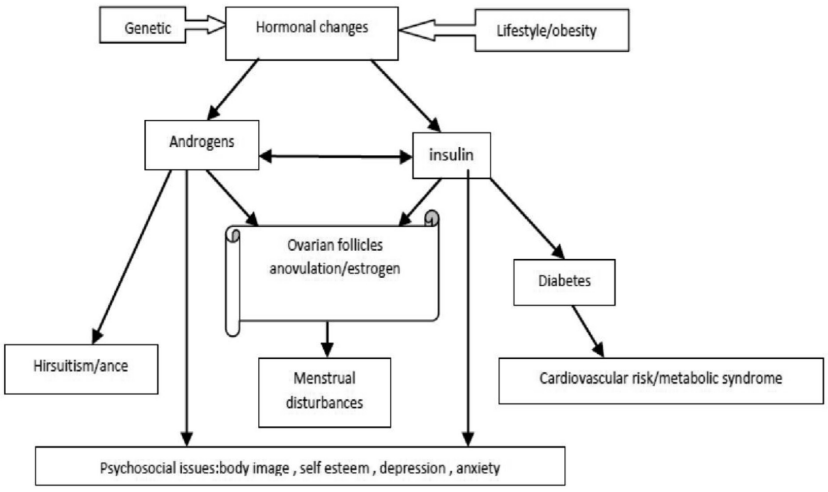

Figure 1 Etiology and clinical traits including metabolic and reproductive indices in PCOS[10]

shown that the diversity in ACE gene is accompanied by deletion and insertion of polymorphism for about 250 base pairs located in intron 16 at the *ACE* gene. Allele I in *ACE* gene polymorphism is associated with low activity of the ACE gene and increase the e ciency of muscles in response to physical practices and exercises.[5] e length of Allele-I was determined the same as iterative *Alu*-allele with 287 base pairs.[6]

It was reported that Allele D of ID ACE gene polymorphism might be led to increase the expression of the corresponding gene and this might be e ective in the regions of the same gene in the renin-angiotensin system.[7] It has been studied that those patients who had received alternative hormonal therapy resulted in favorable symptoms in terms of muscle response and contraction. Furthermore, the I-allele of the ACE gene was observed to in uence bone mineral density. It a ected mostly backbone with no impact observed in hip and pelvic which lead to the conclusion that this allele improves bone mineral density and muscle contraction, especially during postmenopausal period.[8]

ere are 13 polymorphisms that have been found in ACE gene.[9] e importance of ACE gene was described in experimental mice. ACE gene deletion or inactivation in experimental mice resulted in impairment of sperm movement. Nonetheless, ACE gene was also observed to have important role in PCOS development. erefore, we examined then e ect of ACE polymorphism toward PCOS risk as well as several other variables especially BMI and reproductive related hormone.

MATERIALS AND METHODS

121 subjects were enrolled which consist of 52 cases of infertile polycystic ovary syndrome patients, 30 cases infertile and 39 cases of control subjects

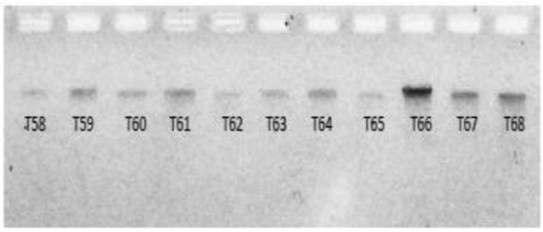

Figure 2 Con rmation for extraction of DNA in infertile group with PCOS syndrome (T) before proceeding to Polymerase Chain Reaction (PCR)

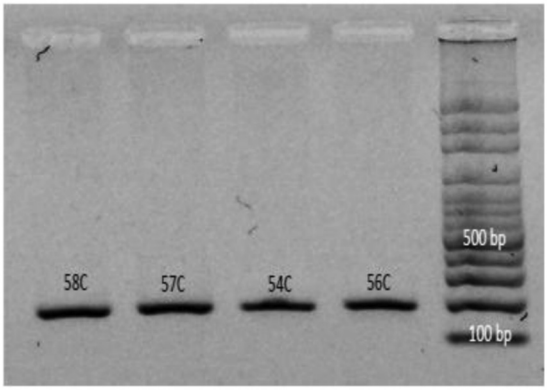

Figure 3 Selection of optimum temperature by using gradient PCR

obtained from Shariati hospital (Tehran,Iran), Cytogenome laboratory (Tehran,Iran) and infertility clinics within period of 2014 to 2016 years.

e study was approved by local institutional ethics committee and written consent was obtained from all subjects who were investigated in this project. Diagnosis of the disease was established under the supervision of gynecologist and according to the clinical signs, symptoms, and laboratory ndings.

Blood samples were obtained from subjects and the level of hormones that related to fertility including LH, FSH, TSH, Estradiol, Progesterone and Prolactin were examined. en, the values of these hormones were compared to normal and infertileindividual. BMI was also measured and its relationship to PCOS was analyzed.

DNA was extracted from venous blood according to manufacturer's procedure (ATP Bioscience, Iran). Electrophoresis by 2% agarose gel was performed to con rm the presence of extracted DNA (Figure 2). Oligonucleotide sequences of forward primer of ACE gene was 5´-GAGCCACTCCCATCCTTTCT-´3 and reverse primer was 5´-GTGGCCATCACATTCGTCAG-´3 (Takapouzist Co., Iran) .

DNA was ampli ed by PCR for 35 cycles with denaturation at 94°C for 40s, annealing at 56°C for 40s and extension at 72°C for 40s (Convergency , Germany) using 30 µl PCR mixture which consist

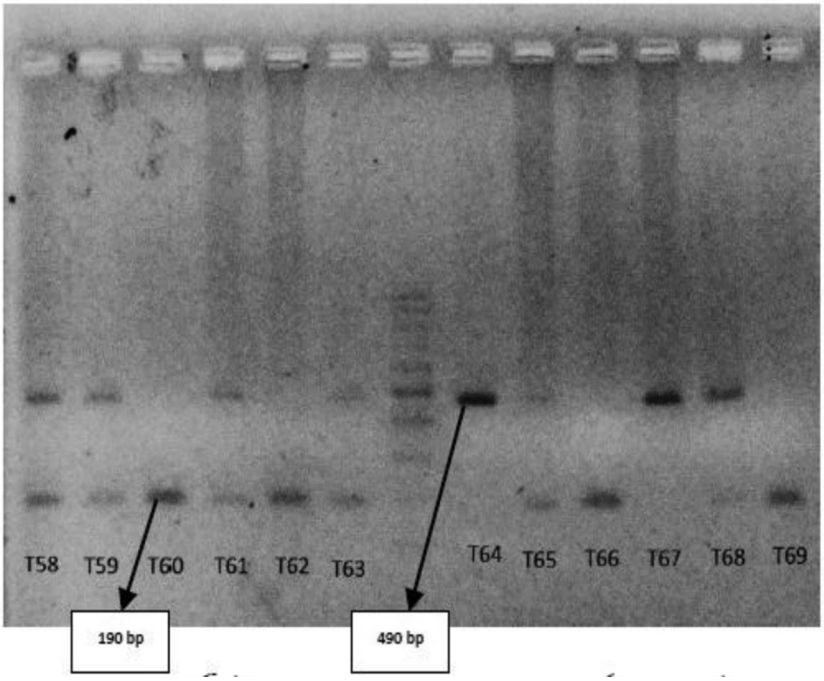

Figure 4 Analysis of infertile group with PCOS (T) on agarose gel.

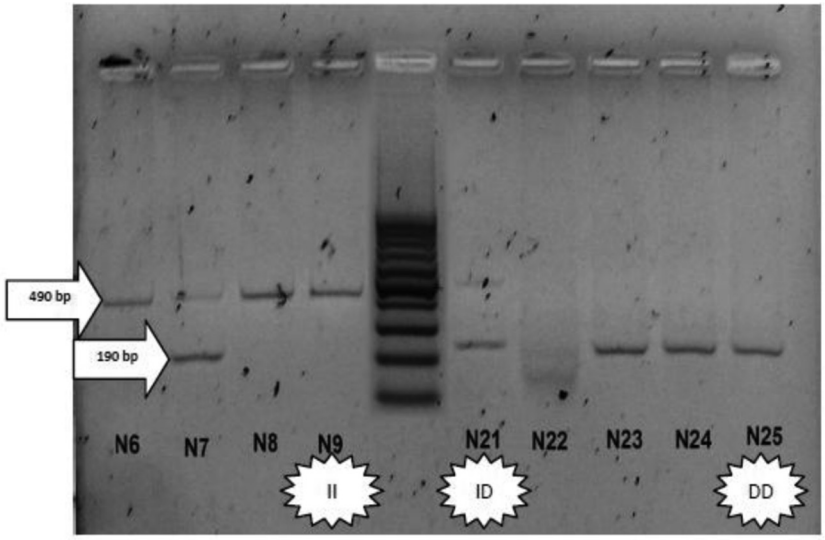

Figure 5 Comparison of control group (N) with ladder on agarose gel

Table 1 **Results of conducting statistical analysis (Chi-square) in the studied groups**

Studied items	infertile patients a□ected to polycystic ovary syndrome			infertile			control		
Numbers	52			30			39		
ACE gene Polymorphism	DD	ID	II	DD	ID	II	DD	ID	II
	16	30	6	6	20	4	20	13	6
	p-value = 0.013 (pcos and infertile comparison to control)								
	p-value = 0.048 (pcos and infertile and control comparison togthers)								

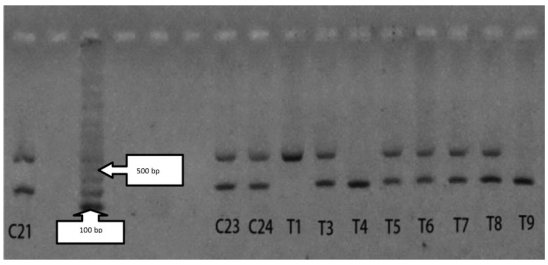

Figure 6 Analysis of infertile group (C) as positive control group along with infertile group PCOS (T) on agarose gel

of both primer,10x PCR bu er, DNA, dNTPs, Taq polymerase enzyme, MgCl2 and ddH2O (Figure 3).

e PCR products were then separated by electrophoresis on agarose gel.

In the presence of D allele, the PCR procedure would produce 190 bp products. In contrast, if I allele was present, a 490 bp DNA will be produced. In case of heterozygous individual, both fragment will present and resulted in the formation of two band in electrophoresis.

Data were analyzed using the statistical package for social sciences (SPSS ver.20). For those data which were normally distributed student t-test and Analysis of Variance (ANOVA) were performed and non-parametric tests were used for data with abnormal distribution including Mann-Whitney, Chi-square, Fisher's exact test, and independent sample test. P value less than 0.05 was considered as signi cant.

RESULTS

e result of this study showed that all three possible genotypes were present in all three groups (Infertile with PCOS, Infertile, and control) with di erent proportion in each group (Table 1). More than half of subject with infertility and PCOS was observed to have ID genotype. e same cases also reported from Infertility groups. However, in control group, DD genotype was the dominant ones. e presence of the allele type was determined by PCR followed by electrophoresis as represented in Figure 4, 5, and 6. Comparison between Infertile group with or without PCOS to control resulted in statistically signi cant di erence (p-value= 0.013). Meanwhile, when all three groups were compared each other, the result also statistically signi cant but almost approach 0.05 (p=0.048)

6 hormones including FSH, LH, TSH, progesterone, prolactin, estradiol were analyzed in all three groups. e results are described in Table 2. e result shown that there were signi cant relationship between BMI, increase in LH and progesterone level with PCOS. e di erence in BMI only became statistically signi cant if both PCOS and

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Table 2 The results of statistical analysis of the studied hormones and Body Mass Index (BMI) in the studied groups

Studied parameters	Normal values	infertile patients a□ected to polycystic ovary syndrome	Infertile	Control
Number	-	52	30	39
Age	20-50years	4.9 (sd_1) ± 29.4	3.9 (sd) ± 30.8	4.2 (sd) ± 27.2
BMI	18.5 – 24.9	4.2 (sd) ± 27.3	4.1 (sd) ± 24.6	-
		p-value = 0.007 (pcos and infertile compared with control)		
FSH Level	10- 60 In some cases, it is slightly greater than 30. (At the middle of menstrual cycle, during pregnancy a er menopause)	3.7 (sd) ± 7.1	3.5 (sd) ± 7.2	1.95 (sd) ± 6.4
		p-value = 0.155 (pcos and infertile compared to control) p-value = 0.141 (comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.371 (comparison of infertile group with di erent polymorphism of ACE gene) p-value = 0.509 (pcos and infertile and control compared together)		
LH Level	5-20 In matured females	7.1 (sd) ± 8.3	2.9 (sd) ± 5.1	1.31 (sd) ± 5.4
		p-value = 0.019 (pcos and infertile compared to control) p-value = 0.876(comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.745 (comparison of infertile group with di erent polymorphism of ACE gene) p-value = 0.004 (pcos and infertile and control compared together)		
Estradiol Level	5-25 (a er menopause) 20-400 (before menopause)	35.1 (sd) ± 43.8	18.65 (sd) ± 49.7	-
		p-value = 0.393 (pcos and infertile comparison together) p-value = 0.738(comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.433 (comparison of infertile group with di erent polymorphism of ACE gene)		
Prolactin Level	Less than 500	257.9 (sd) ± 325.1	232.3 (sd) ± 296.7	-
		p-value = 0.62 (pcos and infertile comparison together) p-value = 0.716 (comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.599 (comparison of infertile group with di erent polymorphism of ACE gene)		
Progesterone Level	5-20 Before ovulation and at the middle of menstrual cycle	5.45 (sd) ± 5.59	8.65 (sd) ± 10.5	-
		p-value = 0.007 (pcos and infertile comparison together) p-value = 0.057(comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.820(comparison of infertile group with di erent polymorphism of ACE gene)		
TSH Level	0.4- 4.2	1.7 (sd) ± 2.6	1.2 (sd) ± 2.5	-
		p-value = 0.693 (pcos and infertile comparison together) p-value = 0.188 (comparison of pcos group with di erent polymorphism of ACE gene) p-value = 0.153 (comparison of infertile group with di erent polymorphism of ACE gene)		
Cycle Length	21-35days	2.5 (sd) ± 26.7	3.3 (sd) ± 27.1	2.6 (sd) ± 28.5

Infertile group were compared to control. Same situation also found in progesterone variable (p= 0.007). In LH variable, a slightly di erent situation was observed which was statistically signi - cant di erences were detected when both PCOS and Infertile group compared to control (P=0.019) but also detected when all three groups were compared with each other (P=0.007). Meanwhile, no statistically signi cant di erences were detected when all variables were compared between genotypes. In general, the statistically signi cant results were obtained when corresponding variables were compared among PCOS, Infertile, and control group. However, no statistically signi cant di erences were observed among di erent genotype groups.

it is important to encourage weight reduction in obese women with PCOS before considering therapy to induce ovulation.[29] In addition, Kiddy et al. also reported that the improvement in menstrual function and fertility may be the consequent upon an increase in insulin sensitivity which, directly or indirectly, a ects ovarian function.[30]

CONCLUSION

e results of this study clearly con rm that the ID polymorphism was predominant within infertile groups either with or without PCOS with statistically signi cant di erence with control group and highlighting its potential as PCOS and infertile biomarker. Furthermore, it also revealed the hormonal change resulted from PCOS including higher level of LH and Progesterone. In addition, there was also an association between BMI and PCOS with higher BMI was associated with higher risk of PCOS based on several literatures.However, further researches are required to con rm the association of ACE gene polymorphism with PCOS and infertility in other population in order to reveal its global impact and also to determine its interaction with other gene related to ovulation.

REFERENCES

1. Martin Dunitz,edited by Roy Homburg. polycystics ovary syndrome book. 2001. Taylor & Francis publication.
2. Jia H ,Wang B, Yu L, Jiang ZH. Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with polycystics ovary syndrome:a meta-analysis. Journal of the rennin-angiotensin-aldosterone system 2013;74:225-262.
3. http://www.geneclinics.org. Access: 25 Aug 2014.
4. Mattei M.G, Hubert C, Alhenc-Gelas F, Roeckel N, Corvol P, Soubrier F. Angiotensin-1 Converting Enzyme gene is on chromosome 17,Cytogenetic Journal 1989;51:1041-1048.
5. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, Soubrier F. Evidence from combined segregation and linkage analysis that a variant of the Angiotensin-1 Converting Enzyme (ACE) gene controls plasma ACE levels. American Journal of Human Genetics 1992;51:197-205.
6. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin- converting enzyme gene(DCP1) (Dipeptidyl Carboxypeptidase1). Oxford Journal 1992;20:1433-1437.
7. Suehiro T, Morita T, Inoue M, Kumon Y, Ikeda Y, Hashimoto K. Increased amount of the Angiotensin-Converting Enzyme (ACE) mRNA originating from the ACE allele with deletion. Human Genetics Journal 2004;115:91-96.
8. Woods D, Onambele G, Woledge R, Skelton D, Bruee S, Humphries SE, Mantgomery H. Angiotensin-1 Converting Enzyme genotype-dependent bene t from hormone replacement therapy in isometric muscle strength and bone mineral density. e Journal of Clinical Endocrinology and Metabolism 2001; 86:2200-2204.
9. Zhu X, Mc-Kenzie C.A, Forrester T, Nickerson DA, Broeckel U, Schunkert H, Deoring A, Jacob HJ, Cooper RS, Rieder MJ. Localization of small genomic region associated with elevated ACE. American Journal of Human Genetics 2000;67:1144-1153.
10. Teedel H, Deeks A, Moran L. Polycystics ovary syndrome: a complex conditions with psychological, reproductive and metabolic manifestations that impact on health across the lifespan. BMC Medicine Journal. 2010; 8:271-277.
11. Koika V, Georgopoulos NA, Piouka A, Roupas ND, Karela A, Armeni AK, Katsantoni E, Pandis D. Increased frequency of I/D genotype of the angiotensin-1 converting enzyme and association of the II genotype with insulin resistance in polycystic ovary syndrome. European Journal of Endocrinology 2012;166:695-702.
12. Sun L, Lv H, Wei W,Zhang D, Guan Y. Angiotensin-converting enzyme I/D and plasminogen activator inhibitor-1 4G/5G gene polymorphisms are associated with increased risk of spontaneous abortions in polycystic ovarian syndrome. Journal of endocrinological investigation 2010;33:77-82.
13. Deepika ML , Reddy RK, Rani UV, Balack Rishna N, Prasanna Ltha K, Jahan P. Do ACE I/D gene polymorphism serve as a predictive marker for age at onset in PCOS?. Journal of assisted reproduction and genetics 2013;30:125-130.
14. Bayram B, Kilicci C, Onlu H, Ozkurt M, Erkasap N, Yildirim E, Sahin F. Association of angiotensin converting enzyme (ACE) gene I/D polymorphism and polycystics ovary syndrome (PCOS). Gene Journal 2011; 489:86-88.
15. Karabulut A, Turgut S, Turgut G. Angiotensin converting enzyme gene insertion/deletion polymorphism in patients with polycystics ovary syndrome , Gynecological Endocrinology Journal 2010;26:393-398.
16. Celik O, Yesilada E, Hascalik S, Celik N, Sahin L, Keskin L, Ozerol E. Angiotensin-converting enzyme gene polymorphism and risk of insulin resistance in PCOS. Reproduction Biomedicine Online Journal 2010; 20:492-498.
17. Are S, Mottaghi S, Shari AM. Studying the correlation of rennin-angiotensin-system (RAS) components and insulin resistance in polycystic ovary syndrome (PCOS). Gynecological Endocrinology Journal 2013; 29: 470- 473.
18. Qin S, Zhou YJ, Liu Y, Shen HM, Li XD, Yan X, Tang HG. Expression and signi cance of ACE2-Ang-(1-7)-Mas axis in the endometrium of patients with polycystic ovary syndrome. Europe PMC Journal 2013; 28:223-230.
19. Alphan Z, Berberoglu Z, Gorar S, Candan Z, Aktas A, Aral Y, Ademoglu E. Increased total Renin levels but not Angiotensin-converting enzyme activity in obese patients with polycystic ovary syndrome. American Journal Experts 2013; 22:475-479.
20. Ann ET, Brain M, Kathryn AM, Ellen JA, Judith MA, David S. and Janet EH. Determinants of abnormal gondotropin secretion in clinically de ned women with polycystic ovary syndrome. e Journal of Clinical Endocronology& Metabolism1997; 82:102-108.
21. Joanne W, Nanette FS, Janet E. H, Marco F and William FCJR. Hyper function of the hypothalamic-pituitary axis in women with polycystic ovarian disease :indirect evidence for partial gonadotroph desensitization. e Journal of Clinical Endocronology& Metabolism1987;66:212-218.
22. Lisa MP, Christopher DW, Jenkins J and James T.P. True and sham acupuncture produced similar frequency of ovulation and improved LH and FSH ratios in women with polycystic ovary syndrome. e Journal of Clinical Endocronology& Metabolism 2011; 96:114-121.
23. Carmen LP, Marie LG, Joseph AA, William SE, and John CM. Polycystic ovary syndrome: evidence for reduced sensivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. e Journal of Clinical Endocronology & Metabolism 1997; 83:119-126.
24. Christine LC, Yong S, Amy GB, Mary E.F. Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. Fertility and Sterillity Journal 2002; 77:141-146.
25. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycytic ovary syndrome. International

Journal of Obesity & Related Metabolic Disorders 2002; 26:883.
26. Janet W Rich-Edwin, Marine B, Waiter C, David J, Meir J, Graham A, Jo Ann E. Adolescent body mass index and infertility caused by ovulatory disorder. American Journal of Obstetrics and Gynecology1994; 171:171-177.
27. Pasquali R, Casimirri F and Vicennati V. Weight control and its bene cial e ect on fertility in women with obesity and polycystic ovary syndrome. Human Reproduction Journal 1997; 12:82-87.
28. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, Polson DW, Reed MJ, Franks S. Di erences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263consecutive cases. Clinical Endocrinology Journal1990; 32:213-220.
29. Hamilton-Fairley D, Kiddy D, Watson H, Paterson C, Franks S. Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotropin. International Journal of Obstetrics & Gynecology 1992; 99:128-131.
30. Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ, Franks S. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clinical Endocrinology Journal 1992; 36:105-111.