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Research Article

Serum Hormone Levels, T Lymphocyte Subsets, and Achievement of Pregnancies in Patients with Premature Ovarian Insufficiency following Administration of Ethinylestradiol/Drospirenone

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Primary ovarian insufficiency (POI) is closely associated with ovarian hormone deficiency, amenorrhea, menopause, and infertility in women. Hormone replacement therapy remains the mainstay of treatment and management of POI. A combined oral contraceptive (Yasmin) containing 0.02 mg ethinylestradiol and 3 mg drospirenone has been shown to be a well-tolerated and effective combination that provides high contraceptive reliability and good cycle control. Herein, we aim to examine clinical efficacy of ethinylestradiol/drospirenone in treating patients with POI and its effects on serum hormone levels, body mass, lipid metabolism, and T lymphocyte subsets. Retrospective analysis of clinical records and follow-up data from 80 patients with POI was performed. The control group contained 40 patients with POI receiving oral administration of 0.035 mg ethinylestradiol and 2 mg cyproterone acetate once a day for consecutive 21 days with drug withdrawal of 7 days in 3 months. The observation group contained 40 patients with POI receiving oral administration of 0.02 mg ethinylestradiol and 3 mg drospirenone once a day for consecutive 28 days in 3 months. There was no significant difference on serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T), concentrations of high- and low-density lipoprotein cholesterol (HDL-C and LDL-C), and triglycerides (TG), body mass, the numbers of CD^{+3}, CD^{+4}, CD^{+8} T cells, and ratio of CD^{+4}/CD^{+8} between the control and observation group ($P > 0.05$) before treatment. After treatment, serum levels of FSH, LH, and T, concentrations of LDL-C, and TG, body mass, and CD^{+8} T cells were reduced but the concentration of HDL-C, CD^{+3}, and CD^{+4} T cells and ratio of CD^{+4}/CD^{+8} were increased in both two groups ($P < 0.05$); these changes were more significant in the observation group compared with the control group ($P < 0.05$). Besides, the total response rate of the observation group was 90.00%, which is higher than that of the control group, 77.50% ($P < 0.05$). The pregnancy rate of the observation group was 45.00%, which is higher than that of the control group, 30.00% ($P < 0.05$). Taken together, these results suggest that the combined oral contraceptive (Yasmin) containing 0.02 mg ethinylestradiol and 3 mg drospirenone restores hormone levels, improves body mass and lipid metabolism, and sustains autoimmune function for patients with POI, suggesting ethinylestradiol/drospirenone treatment is effective in treating POI.

1. Introduction

Premature ovarian insufficiency (POI) refers to ovarian dysfunction or premature menopause which is defined as cessation of menstruation before the age of 40 [1]. Clinically, POI is associated with raised follicle-stimulating hormone, raised gonadotropins, and oestrogen deficiency. The disorder usually leads to infertility, menopausal symptoms, decreased bone density, psychological distress such as depression and anxiety [2, 3], and even increased risks of cardiovascular and neurological diseases [4]. According to recent evidence, the etiology for POI has been attributed to genetic defects, iatrogenic factors involving radiotherapy, chemotherapy, and surgery, and autoimmune; however, the majority of cases remain unexplained [5]. In recent years, stem cell therapy has attracted extensive attention in the field of regenerative

medicine, and it has been used in the treatment of reproductive system diseases, but there is no data to confirm whether the treatment can improve the therapeutic effect [6]. Many health complications correlated with POI are directly associated with ovarian hormone deficiency, mainly estrogen deficiency, and some reports reveal that appropriate physiologic estrogen and progestin replacement relieve menopausal symptoms, although trial data from Women's Health Initiative (WHI), which is a study of natural menopause women, indicated that multiple increased health risks related to use of estrogen/progestin therapy (EPT), but the results of the study to evaluate the HRT safety in natural postmenopausal female cannot be simply applied to female with POI. As for now, hormone replacement therapy (HRT) is the most efficient treatment for female with POI [2, 7, 8]. There are many drug delivery routes, doses, and types of oestrogen and progestin preparations for the treatment of female with POI [8]. In this study, clinical efficacy of ethinylestradiol/drospirenone in treating patients with POI and its effects on serum hormone levels, body mass, lipid metabolism, and T lymphocyte subsets.

2. Materials and Methods

2.1. Study Subjects. A total of 80 patients diagnosed with POI with complete clinical data, and follow-up data were retrospectively analyzed, and they were arranged into the control group with 40 cases and the observation group with 40 cases according to different treatment methods. The control group was treated with ethinylestradiol and cyproterone acetate, with age ranging from 30 to 40 years (34.21 ± 2.43 years). The observation group was treated with drospirenone ethinylestradiol tablets (II), with age ranging from 31 to 39 years (34.12 ± 3.36 years). Eligible study subjects should meet the following inclusion criteria: (a) menstrual cycle was changed for more than seven days, and the number of changes was more than two times; (b) follicle stimulating hormone (FSH) > 25 U/l; (c) anti-Müllerian hormone (AMH) < 1 ng/ml; (d) the informed content was obtained from included patients or their family members. POI patients complicated by immunological diseases such as systemic erythema and idiopathic thrombocytopenic purpura, with surgical histories involving ovariectomy, salpingectomy, uterine artery embolization, and oophorocystectomy, genital tract malformations, medical history of hormone drugs within 3 months, serious cardiac, liver, and kidney dysfunction, mental or cognitive disabilities, poor compliance to treatment protocols, and withdrawal from the study were excluded from this study. A one-year follow-up was performed to evaluate achievements of pregnancies. The study protocol was approved by the Ethics Committee of our hospital.

2.2. Treatment Protocols. The control group contained 40 patients with POI receiving oral administration of ethinylestradiol and cyproterone tablets (Zhejiang Xianju Pharmaceutical Co., Ltd., National Medicine Permission Number: H20065479, specifications: 0.035 mg ethinylestradiol and 2 mg cyproterone acetate) once a day for consecutive 21 days with drug withdrawal of 7 days in 3 months. The observation group contained 40 patients with POI receiving oral administration of drospirenone ethinylestradiol tablets (II) (Bayer Weimar GmbH und Co. Ltd., Registration Number: H20140972, specification: 0.02 mg ethinylestradiol and 3 mg drospirenone) once a day for consecutive 28 days in 3 months.

2.3. Blood Sample Collection. Two tubes of fasting blood were collected from each patient, and blood collection was performed twice a day before and after treatment. In brief, 5 ml of venous blood was extracted from each patient fasting for at least 8 hours by vacuum blood sampling needle each time and stored in a refrigerator under 4°C for 45 minutes. The agglutinated blood was centrifuged in a centrifuge with 3500 r/min for 15 minutes. The serum extracted from testing tube, which was immediately stored in a low-temperature refrigerator (MDF-U5412, SANYO, Japan) at -80°C.

2.4. Hormonal and Biochemical Measurements. Serum levels of testosterone (T), luteinizing hormone (LH), and FSH were measured by chemiluminescence immunoassay using commercial available kits (Shanghai Westang Bio-Tech Co., Ltd., China). Serum levels of triglycerides (TG) were determined by oxidase method, HDL-cholesterol (HDL) by synthetic polymer/detergent HDL-C assay (SPD method), and LDL-cholesterol (LDL) by surfactant LDL-C assay (SUR method) using commercial available kits (Shenzhen Jvfeng Co., Ltd., China). CD^{+3}, CD^{+4}, and CD^{+8} T cells were sorted out by XL-type flow cytometer (Beckman Coulter, USA) using commercial available kits (Sangon, Shanghai, China), and the ratio of CD^{+4}/CD^{+8} was calculated.

2.5. Body Mass Measures. The patient stands upright on the floor of the height meter, with the heel close together, the tip toe of the foot is 60 degrees, the arms naturally droop, and the eyes are looking at the front horizontally, getting the data with cm measurement unit; the patient naturally stands on the weight pound, getting the stable data with 0.1 kg accurate unit, and body mass index (BMI) formula = body mass/height^2, comparison on the pregnancy rate in the two groups during the follow-up period of 12 months.

2.6. Assessment of Clinical Response. Normal menstrual cycles and hormone levels [9] were defined as excellent response. Relative improvement in menstrual cycles and hormone levels were defined as good response. No improvement in clinical symptoms was defined as no response. Total response rate = (excellent + good) response/40 × 100%.

2.7. Statistical Analysis. All data were processed by the SPSS 23.0 software. The measurement data were defined as mean ± standard deviation and analyzed by the t-test. The counting data were described by ratio or percentage and analyzed by the chi-square test. A level of $P < 0.05$ was considered statistically significance.

3. Results

3.1. Ethinylestradiol/Drospirenone Treatment Significantly Restored Serum Hormone Levels of POI Patients. The serum

Table 1: Comparison of serum hormone levels before treatment and after treatment in the two groups.

Group	Case	Time	LH (mlU/ml)	FSH (mIU/ml)	T (g/dl)
Control group	40	Before treatment	10.56 ± 2.15	26.42 ± 5.24	75.21 ± 6.47
		After treatment	8.87 ± 2.25	19.25 ± 2.41	71.35 ± 4.32
t			4.127	8.169	6.594
P			0.033	0.001	0.001
Observation group	40	Before treatment	10.49 ± 1.84	26.33 ± 5.62	74.38 ± 6.21
		After treatment	6.13 ± 1.35[a]	15.73 ± 2.22[a]	50.54 ± 4.47[a]
t			8.065	15.468	21.837
P			0.001	0.001	0.001

The letter of a indicates $P < 0.05$ compared with the control group; t test was performed for statistical comparison; FSH: follicle growth hormone; LH: luteinizing hormone; T: testosterone.

Table 2: Comparison of body mass and lipid metabolism before treatment and after treatment in the two groups.

Group	Case	Time	HDL-C (mmoL/l)	LDL-C (mmoL/l)	TG (mmoL/l)	Body mass (kg/m^2)
Control group	40	Before treatment	1.56 ± 0.65	4.12 ± 1.24	1.95 ± 0.33	30.36 ± 3.52
		After treatment	2.01 ± 0.41	3.11 ± 0.56	1.35 ± 0.12	26.82 ± 3.29
t			3.941	4.528	5.719	10.227
P			0.037	0.029	0.014	0.001
Observation group	40	Before treatment	1.55 ± 0.64	3.23 ± 1.22	1.98 ± 0.31	32.31 ± 3.36
		After treatment	2.53 ± 0.24[a]	2.33 ± 0.45[a]	1.01 ± 0.16[a]	22.34 ± 3.32[a]
t			5.067	4.314	5.296	17.534
P			0.003	0.012	0.001	0.001

The letter of a indicates $P < 0.05$ compared with the control group; t test was performed for statistical comparison; HDL-C: high density lipid-cholesterol; LDL-C: low density lipid-cholesterol; TG: triglycerides.

levels of LH, FSH, and T in the observation group were not remarkably different from those in the control group before treatment ($P > 0.05$). The serum levels of LH, FSH, and T were reduced after treatment in both two groups. The serum levels of LH, FSH, and T in the observation group were lower than those in the control group ($P < 0.05$, Table 1), suggesting that ethinylestradiol/drospirenone treatment significantly restored serum hormone levels of POI patients.

3.2. Ethinylestradiol/Drospirenone Treatment Significantly Improved Body Mass and Lipid Metabolism in POI Patients. POI is associated with the occurrence of osteoporosis, abnormal lipid metabolism, and cardiovascular disease in women. In this part, we compared the effects of ethinylestradiol and ethinylestradiol/drospirenone treatment on body mass and lipid metabolism in POI patients. Before treatment, there was slightly difference in body mass and lipid metabolism in the two groups ($P > 0.05$). After treatment, body mass and lipid metabolism in the two groups were improved ($P < 0.05$). It was revealed that the improvement in the observation group was greater than that in the control group ($P < 0.05$, Table 2).

3.3. Ethinylestradiol/Drospirenone Treatment Significantly Increased T-Lymphocyte Subpopulations. Previous evidence showed that POI may result from autoimmune dysregulation characterized by reduced T cells. The numbers of CD^{+3} and CD^{+4} T cells and the ratio of CD^{+4}/CD^{+8} were increased in the two groups but the number of CD^{+8} T cells was declined after treatment ($P < 0.05$). The observation group had more CD^{+3} and CD^{+4} T cells, higher ratio of CD^{+4}/CD^{+8}, and fewer CD^{+8} T cells than the control group ($t = 4.169$, $P = 0.018$; $t = 3.475$, $P = 0.023$; $t = 3.846$, $P = 0.0.017$; $t = 4.218$, $P = 0.012$, Table 3). These data showed that ethinylestradiol/drospirenone treatment could sustain autoimmune function in POI patients.

3.4. Ethinylestradiol/Drospirenone Treatment Remarkably Improved Clinical Response of POI Patients. The total response rate in the observation group was 90.00%, including 24 cases (60.00%) defined as excellent response, 12 cases (30.00%) defined as good response, and 4 cases (10.00%) defined as no response. The total response rate in the control groups was 77.50%, including 20 cases (50.00%) defined as excellent response, 11 cases (27.50%) defined as good response, and 9 cases (22.50%) defined as no response. The total response rate in the observation groups was higher than that in control group ($\chi^2 = 6.284$, $P < 0.05$, Figure 1).

3.5. Ethinylestradiol/Drospirenone Treatment Yielded Better Achievement of Pregnancies for POI Patients. After 1-year follow-up, there were 18 pregnant women out of 40 cases in the observation group, and the pregnancy rate was 45.00%. There were 12 pregnant women out of 40 cases in the control

Table 3: Comparison of T cell subpopulations before treatment and after treatment in the two groups.

Group	Case	Time	CD^{+4} (%)	CD^{+8} (%)	CD^{+3} (%)	CD^{+4}/CD^{+8}
Control group	40	Before treatment	36.56 ± 10.52	40.14 ± 11.05	54.33 ± 12.85	0.82 ± 0.36
		After treatment	40.29 ± 10.43	35.37 ± 10.48	59.94 ± 12.57	1.21 ± 0.39
t			7.143	8.017	6.247	6.475
P			0.001	0.001	0.001	0.001
Observation group	40	Before treatment	36.46 ± 10.03	40.12 ± 11.06	53.91 ± 12.87	0.84 ± 0.34
		After treatment	48.14 ± 11.04[a]	30.19 ± 10.10[a]	64.65 ± 10.99[a]	1.63 ± 0.35[a]
t			10.542	19.102	21.719	6.839
P			0.001	0.001	0.001	0.001

The letter of a indicates $P < 0.05$ compared with the control group; t test was performed for statistical comparison.

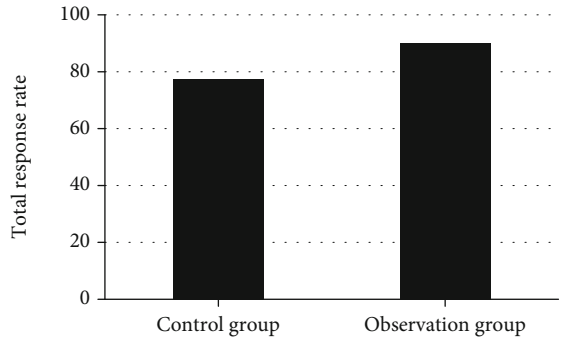

Figure 1: The total response rate in the observation and control group. Chi-square test was performed for statistical comparison.

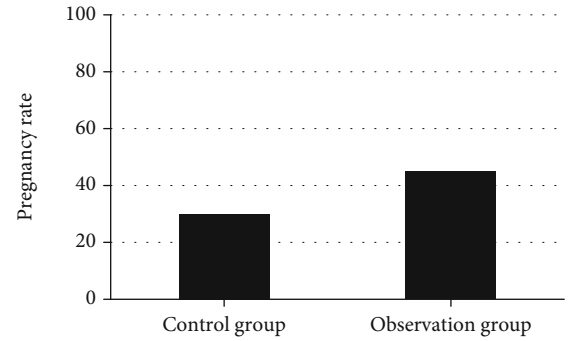

Figure 2: The pregnancy rate in the observation and control group. Chi-square test was performed for statistical comparison.

group, and the pregnancy rate was 30.00%. The pregnancy rate was higher in the observation group than that in the control group ($\chi^2 = 3.425$, $P < 0.05$, Figure 2).

4. Discussion

Women with POI experience sustained sex steroid deficiency for longer periods compared with women who experienced normal menopause. Management for POI includes hormonal replacement therapy up to at least age 50, counseling and emotional support, and calcium and vitamin supplements [10, 11]. Evidence suggests that pregnancies might occur in women with primary ovarian insufficiency when high FSH concentrations are declined by ethinyloestradiol or gonadotrophin-releasing hormone analogues, followed by ovulation induction using low dose gonadotrophins [12]. However, there are no published prospective trials supporting the hypothesis that reduction of FSH concentrations allows development of follicles. Recently, a novel hormonal approach to optimize fertility for POI patients has been reported [13]. Estrogen-progestogen therapy combined with dehydroepiandrosterone and melatonin could optimize fertility and result in successful pregnancy in POI patients. However, management of POI is still a challenging issue for clinicians, and women with POI have a higher risk of infertility. In this study, we retrospectively analyzed 80 POI patients among which 40 received oral administration of ethinyloestradiol and 40 received oral administration of ethinylestradiol/drospirenone. Results revealed that ethinylestradiol/drospirenone can reduce levels of serum FSH, LH, and T and improve clinical efficiency, body mass, and lipid metabolism.

POI patients were characterized by elevated serum FSH, LH, and T levels [2, 14, 15]. It was found that patients with oral administration of ethinylestradiol/drospirenone exhibited lower serum FSH, LH, and T levels than those with oral administration of ethinylestradiol alone. A previous randomized controlled trial showed the combined oral contraceptive Yasmin (drospirenone 3 mg plus ethinylestradiol 30 microg [DRSP 3 mg/EE 30 microg]) is a well-tolerated and effective combination that provides high contraceptive reliability and good cycle control [16]. Another randomized, open-label, controlled, multicentre trial performed in healthy Chinese women found that ethinylestradiol plus drospirenone showed a more favorable effect on weight and premenstrual symptoms than ethinylestradiol plus desogestrel [17]. Another finding of our study showed that ethinylestradiol plus drospirenone improved body mass and lipid metabolism of POI patients more significantly than ethinylestradiol alone. In a randomized study conducted by Gaspard et al. [18], they made a conclusion that the combined low-dose oral administration of Yasmin displayed a favorable lipid profile with increased total HDL cholesterol. Yasmin may be regarded as responsible for the stable LDL cholesterol levels due to its antiandrogenic or missing androgenic activity. Anttila et al. performed a pooled analysis and found a 24/4 regimen of drospirenone 3 mg/ethinylestradiol 20 μg is

associated with a bleeding profile and cycle control [19]. It is believed that ethinylestradiol plus drospirenone (Yasmin) could effectively inhibit androgen levels in the oarium, leading to the decrement in plasma free testosterone level and free androgen index. Previous evidence showed that POI may result from autoimmune dysregulation characterized by reduced T cells [20]. POI appears to be a complex disease entity with multiple underlying etiopathogenic contributions including the possibility of several distinctly different autoimmune mechanisms [21]. Another finding of our study is elevated T-lymphocyte subpopulations in POI patients following ethinylestradiol/drospirenone treatment, suggesting that ethinylestradiol/drospirenone treatment could sustain autoimmune function in POI patients.

In conclusion, ethinylestradiol plus drospirenone (Yasmin) could significantly restore hormone levels, improve body mass and lipid metabolism, sustain autoimmune function, and increase clinical curative effects, and it merits further promotion in clinic. However, sample size is relatively small, which is a limitation for the present study. Further investigation should be warrant in large sample size.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jiewen Tao and JingWang contributed to the work equally and should be regarded as co-first authors.

References

[1] J. S. Laven, "Primary ovarian insufficiency," *Seminars in Reproductive Medicine*, vol. 34, no. 4, pp. 230–234, 2016.

[2] S. D. Sullivan, P. M. Sarrel, and L. M. Nelson, "Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause," *Fertility and Sterility*, vol. 106, no. 7, pp. 1588–1599, 2016.

[3] A. Podfigurna-Stopa, A. Czyzyk, M. Grymowicz et al., "Premature ovarian insufficiency: the context of long-term effects," *Journal of Endocrinological Investigation*, vol. 39, no. 9, pp. 983–990, 2016.

[4] S. Tsiligiannis, N. Panay, and J. C. Stevenson, "Premature ovarian insufficiency and long-term health consequences," *Current Vascular Pharmacology*, vol. 17, no. 6, pp. 604–609, 2019.

[5] M. De Vos, P. Devroey, and B. C. Fauser, "Primary ovarian insufficiency," *Lancet*, vol. 376, no. 9744, pp. 911–921, 2010.

[6] J. Na and G. J. Kim, "Recent trends in stem cell therapy for premature ovarian insufficiency and its therapeutic potential: a review," *Journal of ovarian research*, vol. 13, no. 1, p. 74, 2020.

[7] L. Webber, R. A. Anderson, M. Davies, F. Janse, and N. Vermeulen, "HRT for women with premature ovarian insufficiency: a comprehensive review," *Human reproduction open*, vol. 2017, no. 2, article hox007, 2017.

[8] P. Machura, M. Grymowicz, E. Rudnicka et al., "Premature ovarian insufficiency - hormone replacement therapy and management of long-term consequences," *Przeglad menopauzalny= Menopause review*, vol. 17, no. 3, pp. 135–138, 2018.

[9] L. A. Cole, D. G. Ladner, and F. W. Byrn, "The normal variabilities of the menstrual cycle," *Fertility and Sterility*, vol. 91, no. 2, pp. 522–527, 2009.

[10] A. J. Fenton, "Premature ovarian insufficiency: pathogenesis and management," *Journal of mid-life health*, vol. 6, no. 4, pp. 147–153, 2015.

[11] S. Luisi, C. Orlandini, C. Regini, A. Pizzo, F. Vellucci, and F. Petraglia, "Premature ovarian insufficiency: from pathogenesis to clinical management," *Journal of Endocrinological Investigation*, vol. 38, no. 6, pp. 597–603, 2015.

[12] J. H. Check, "Mild ovarian stimulation," *Journal of Assisted Reproduction and Genetics*, vol. 24, no. 12, pp. 621–627, 2007.

[13] S. Dragojevic Dikic, M. Vasiljevic, A. Jovanovic et al., "Premature ovarian insufficiency - novel hormonal approaches in optimizing fertility," *Gynecological Endocrinology*, vol. 36, no. 2, pp. 162–165, 2020.

[14] S. Sahmay, T. A. Usta, T. Erel, N. Atakul, and B. Aydogan, "Elevated LH levels draw a stronger distinction than AMH in premature ovarian insufficiency," *Climacteric*, vol. 17, no. 2, pp. 197–203, 2014.

[15] S. N. Kalantaridou, K. A. Calis, V. H. Vanderhoof et al., "Testosterone deficiency in young women with 46,XX spontaneous premature ovarian failure," *Fertility and Sterility*, vol. 86, no. 5, pp. 1475–1482, 2006.

[16] S. Kelly, E. Davies, S. Fearns et al., "Effects of oral contraceptives containing ethinylestradiol with either drospirenone or levonorgestrel on various parameters associated with well-being in healthy women: a randomized, single-blind, parallel-group, multicentre study," *Clinical Drug Investigation*, vol. 30, no. 5, pp. 325–336, 2010.

[17] F. Guang-Sheng, B. Mei-Lu, C. Li-Nan et al., "Efficacy and safety of the combined oral contraceptive ethinylestradiol/-drospirenone (Yasmin) in healthy Chinese women: a randomized, open-label, controlled, multicentre trial," *Clinical Drug Investigation*, vol. 30, no. 6, pp. 387–396, 2010.

[18] U. Gaspard, J. Endrikat, J. P. Desager, C. Buicu, C. Gerlinger, and R. Heithecker, "A randomized study on the influence of oral contraceptives containing ethinylestradiol combined with drospirenone or desogestrel on lipid and lipoprotein metabolism over a period of 13 cycles," *Contraception*, vol. 69, no. 4, pp. 271–278, 2004.

[19] L. Anttila, W. Neunteufel, F. Petraglia, J. Marr, and M. Kunz, "Cycle control and bleeding pattern of a 24/4 regimen of drospirenone 3 mg/ethinylestradiol 20 mug compared with a 21/7 regimen of desogestrel 150 mug/ethinylestradiol 20 mug: a pooled analysis," *Clinical Drug Investigation*, vol. 31, no. 8, pp. 519–525, 2011.

[20] A. La Marca, A. Brozzetti, G. Sighinolfi, S. Marzotti, A. Volpe, and A. Falorni, "Primary ovarian insufficiency: autoimmune causes," *Current Opinion in Obstetrics & Gynecology*, vol. 22, no. 4, pp. 277–282, 2010.

[21] V. K. Tuohyand and C. Z. Altuntas, "Autoimmunity and premature ovarian failure," *Current Opinion in Obstetrics & Gynecology*, vol. 19, pp. 366–369, 2007.