Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Analysis of the clinical efficacy of leuprolide acetate in the treatment of obese patients with endometriosis and its role on the expression of MIF gene

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ARTICLE INFO

Original paper

Article history:
Received: August 17, 2021
Accepted: Novomber16, 2021
Published: December 01, 2021

Keywords:
Endometriosis; Estrogen; GnRH Agonists; Over Weight; Real-time PCR

ABSTRACT

Endometriosis is an invasive but benign disease of women that develops in endometrial glands outside the endometrium and uterine muscle. It affects about 15-20% of women of childbearing age. One effective way to treat endometriosis is to use GnRH agonists, which inhibit estrogen production. However, one of the possible side effects of this treatment is obesity and BMI increasing, which is a concern for some patients. This study investigated the role of leuprolide acetate in treating overweight patients (BMI≥30) and their comparison with non-overweight patients (BMI<30) for six months. Also, the effect of this medicine was evaluated on the expression of the MIF gene, which is an effective gene in obesity. For this purpose, a clinical trial was performed on 75 women with endometriosis aged 18 to 35 years. These patients were divided into two groups. The first group consisted of 38 patients with BMI<30. The second group consisted of 37 patients with BMI≥30. Both groups were treated with leuprolide acetate at a dose of 3.75 mg/month (intramuscularly) for six months. In addition to clinical evaluations, the expression of the MIF gene was assessed by the real-time PCR technique. The results showed that treatment with leuprolide acetate during six months in both groups reduced dysmenorrhea, dyspareunia, and chronic pelvic pain (P<0.05). Although this decrease was greater in the BMI <30 group, the difference was not significant. Also, after collecting the side effects of the medication, it was found that hypoestrogenism, such as cramps and spotting, was more in the first group; Endogenous complications such as oily skin, acne, and hirsutism were also more common in the second group. The results of MIF gene expression showed that the expression level before and after the start of the experiment in the second group (BMI≥ 30) is higher than the first group (BMI <30). The results also showed that the two groups increased the expression of the MIF gene after treatment with leuprolide acetate. This increase was statistically significant in the second group (P = 0.042). Generally, it was found that this medication causes more weight gain in obese people and increases the risk of obesity-related diseases among these patients. Therefore, it is recommended that this treatment be used with caution in obese patients with endometriosis.

DOI: http://dx.doi.org/10.14715/cmb/2021.67.4.31

Introduction

Endometriosis is an inherited disease of multiple genes with complex and multifactorial etiology. This disease is estrogen-dependent and appears to regress in the absence of estrogen (1). However, despite the evidence of this disease in menopausal age, it seems that there are other sources for estrogen production. Recent findings indicate that estrogen is also produced in peripheral tissues (skin and adipose tissue) (2). Endometriosis tissue can also make local estrogen. The enzyme aromatase stimulates this type of local estrogen (3). At least ten other promoters affect the amount of aromatase gene transcription and translation in different body tissues. Each body tissue that can induce aromatase can individually produce significant amounts of this estrogen under the influence of its unique activators (4). Despite healthy endometrium, endometriosis tissue also can express this gene and produce aromatase and eventually estrogen. Prostaglandin E2 causes the expression of this gene and ultimately the production of local estrogen in endometriosis tissue (5). Therefore, one of the ways to treat endometriosis is to use GnRH agonist drugs that inhibit estrogen production (6). Leuprolide acetate is a synthetic nonapeptide analogue of the gonadotropin-releasing hormone (GnRH or LH-RH) (7).

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Leuprolide lowers estrogen levels in women and is used to treat the symptoms of endometriosis or uterine fibroids. Common side effects of leuprolide acetate include nausea, pale skin, pain around the injection site, headache, sweating, urinary incontinence, joint pain, weight gain, and obesity (8). Obesity is associated with an increase in excess adipose tissue and various metabolic disorders, and its prevalence seems to be increasing in the world. Obesity is a significant risk factor for multiple diseases, including type II diabetes and cardiovascular disease (9). There is ample evidence that, although obesity is asymptomatic, it is associated with chronic inflammation. Chronic inflammation is characterized by cytokine production changes, increased reactants levels in the acute phase, and the strengthening of inflammatory signaling pathways (10, 11). There is also evidence that as obesity increases, the number of macrophages entering adipose tissue increases (12). Thus, macrophages entering adipose tissue are a significant source of cytokines that increase obesity-induced inflammation and promote insulin resistance and other metabolic disorders (13). Local interactions of adipocytes and macrophages penetrating adipose tissue, its effect on the function of monocytes, and T lymphocytes have been reported in previous studies. On the other hand, there is evidence that obesity and weight gain affect the pro-inflammatory status of mononuclear cells (14).

Macrophage migration inhibitory factor (MIF) is a cytokine regulator of immunity that plays an essential role in local and systemic inflammation and immune responses (15). Studies show that MIF is secreted in significant amounts by several cell types: monocytes, endothelial cells, keratinocytes, anterior pituitary cells, and osteoblasts, indicating multiple physiological functions (15, 16). The results of several clinical studies have demonstrated the role of MIF as a biomarker for the pathogenesis of systemic infections, sepsis, autoimmune diseases, cancer, type II diabetes, and obesity (16, 17). Reports have shown that the transcriptional activity of this gene is affected by its polymorphisms and is associated with the risk of obesity (17, 18). There is also evidence linking the pathogenesis of obesity to MIF. On the other hand, some biological roles of intracellular MIF in the process of adipogenesis are known. MIF plays a pro-inflammatory role in the production and secretion of cytokines, and pro-inflammatory cytokines are said to be involved in adipogenesis (18). Therefore, the role of MIF in adipogenesis and obesity is its unique feature among other pro-inflammatory cytokines (15).

In the current study, the clinical efficacy of leuprolide acetate was considered in treating obese patients with endometriosis. Also, its role was evaluated on the expression of the MIF gene among these patients.

Materials and methods

Study population

The present study was performed as a clinical trial on patients aged 18 to 35 years referred to obstetrics and gynecology clinics. Patients who were of childbearing age and had a particular monthly bleeding cycle (18 to 35 days), pelvic pain, and dysmenorrhea for at least two weeks per month for the past three months were included in the study. Patients were not included in the study from the beginning with vaginal bleeding for no reason, ovarian cyst greater than 2 cm, treatment with hormone therapy in the past three months, osteoporosis, smoking, allergies to Gonadotropin-Releasing Hormone (GnRH) Analogues medications, history of seizures, pulmonary, cardiac, liver, and kidney disease, cerebrovascular disease, and pregnancy. Written consent was obtained from all participants in the study before the start of the study.

A total of 75 patients with endometriosis participated in this study. These people were divided into two groups. The first group consisted of 38 people with endometriosis who had a body mass index (BMI) of less than 30. The second group consisted of 37 patients with endometriosis with a BMI equal to or greater than 30. Both groups were treated with leuprolide acetate at a dose of 3.75 mg (intramuscularly) per month for six months.

Analysis of body composition

The 1500 Bodystat was used to assess body composition. This device has four cables but only to the clamps that are connected to the electrodes. Consumption electrodes are attached to the right hand and foot. It is noteworthy that the analyzers of this device work with batteries that produce a signal from the body and measure impedance at a constant frequency of 50 kHz. Gender, weight, height, and

level of physical activity are among the information that enters the device. After the test, the device provides a complete analysis of body composition, including the percentage and amount of fat, percentage and amount of muscle mass and percentage of total body water, and comparison with average values in a short time.

RNA extraction and Real-time PCR evaluation

After preparing 5 ml of peripheral blood at the beginning and end of the experiment from the patients participating in this study, mRNA extraction was performed using a highly accurate RNA isolation kit (Roche Diagnostics, Germany). Expression of MIF and β-actin genes was measured using the real-time reverse transcriptase (RT-PCR) technique. For sequential amplification of RT-PCR, 2µl of each cDNA sample was used for 20µl of PCR-mix, and PCR reactions were performed in the step of an ABI real-time system (Applied Biosystems, Foster City, Canada).

The β-actin gene sequence, which was used as an internal control, and the MIF gene, was obtained from the NCBI gene bank, and their specific primers were designed by an express primer program. Primer sequences were blasted at NCBI and Gene Runner to confirm the specificity and accuracy of the designed primers. The sequences of primers are listed in Table 1.

Table1. Primer sequences of β-actin and MIF genes

Gene		Primer Sequence	Annealing Temp.	Amplicon Size
β-actin	Forward	5’-GCACTCTTCCAGCCTTCC-3’	60°C	435bp
	Reverse	5’-GCGCTCAGGAGGAGCAAT-3’		
MIF	Forward	5’-GCGCGTGCGTCTGTGCC-3’	57°C	247bp
	Reverse	5’-GACCACGTGCACCGCGATGTA-3’		

Materials for PCR reactions were purchased from Applied Biosystems, and each sample was performed three times in 20ul volumes and finally up to 40 cycles under real-time conditions. Ct values were normalized for relative standard curves on similar plates of the sample. The relative expression values of the target genes in each sample were normalized for the housekeeping [beta actin] gene. The information was expressed in the form of multiplicative changes in mRNA levels compared to the control group.

Statistical analysis

The findings of the study were analyzed using SPSS software version 13. The Chi-square test was used to compare the values of variables between groups.

Pain due to monthly bleeding, chronic pelvic pain, and pain during intercourse were evaluated before the study and then monthly for up to six months. After receiving a complete explanation, patients filled out a monthly form that contained severe dysmenorrhea pain, pelvic pain, dyspareunia, and drug side effects. Patients were asked about pain based on the 11-point Visual Analog Scale (VAS) from 0 (painless) to 10 (most severe pain). Patients were also examined monthly by a gynecologist and were asked about their recovery, drug side effects, and so on. Patients were also given forms to record adverse drug reactions during treatment.

The obtained information assessed the difference between gene expression levels among the groups. Data were expressed as cycle threshold values [ct] based on logarithmic PCR plots and values according to the manufacturer's protocol. The β-actin was considered a housekeeping gene, and the results were reported as the ratio of MIF expression values to β-actin. A correlation test evaluated the relationship between MIF gene expressions in peripheral blood cells between the studied groups. Values less than 0.05 were considered significant for all tests.

Results and discussion

Clinical evaluations

Among the 75 participants in this experiment, one from the first group (BMI <30) due to not referring for monthly examinations and filling out forms, four from the second group (BMI≥ 30), two patients due to lack of follow-up, and two patients due to androgenic effects of the drug were excluded from the study. Finally, 37 patients in the first group and 33 patients

in the second group completed the study. The mean and standard deviation of the age of patients in the first group was 32.3±5.8 years, and the second group was 31.9±6.4 years, which was not statistically different from each other.

The Kolmogorov–Smirnov test did not confirm the normal distribution of the data. Friedman test showed that the mean scores of pain assessment test (dysmenorrhea, dyspareunia, and chronic pelvic pain) in the six times measured in each group had a decreasing trend. The results of the $\chi 2$ test and the amount of P-value are summarized in Table 2.

Table 2. Evaluation of pain intensity based on Visual Analog Scale (VAS) in both groups

	First Group			Second Group		
	Dysmenorrhea	Dyspareunia	Pelvic Pain	Dysmenorrhea	Dyspareunia	Pelvic Pain
Start	4.58 ± 2.08	2.77 ± 1.28	2.80 ± 1.14	4.66 ± 2.12	3.11 ± 0.60	3.00 ± 0.50
1st month	2.52 ± 2.06	1.25 ± 1.05	1.58 ± 1.05	2.55± 1.42	1.88 ± 0.33	1.77 ± 0.66
2nd month	1.52 ± 1.48	0.63 ± 0.89	1.13 ± 0.96	2.00 ± 0.86	2.11 ± 0.60	1.88 ± 0.60
3rd month	1.16 ± 1.32	0.47 ± 0.77	0.88 ± 0.82	2.55 ± 0.88	2.22 ± 0.44	2.11 ± 0.60
4th month	0.83 ± 1.08	0.33 ± 0.67	0.77 ± 0.76	2.66 ± 0.86	2.44 ± 0.52	2.11 ± 0.60
5th month	0.83 ± 1.05	0.38 ± 0.80	0.80 ± 0.74	2.66 ± 0.86	2.44 ± 0.52	2.11 ± 0.60
$\chi 2$ test	148.3	146.54	136.92	29.8	30.74	32.05
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Five months after the test, the mean chronic pelvic pain, dyspareunia, and dysmenorrhea were lower in the first group. But no significant difference between the two groups was confirmed by the Wilcoxon test (P<0.05).

The results of collecting forms related to adverse drug reactions are summarized in Table 3. No pathological signs were observed in the monthly examination of patients.

Table 3. Side effects of leuprolide acetate in two groups after 6th month

	First Group (n = 37)		Second Group (n = 33)	
Complication	number	percent	number	percent
Hot flashes	32	86.48	25	75.75
Headache	24	64.86	24	72.72
Spot stain	16	43.24	17	51.51
Weakness	7	18.91	6	18.18
greasy skin	2	5.40	18	54.54
Weight gain (10%)	15	40.54	30	90.90
Increased appetite	11	29.72	27	81.81
Joint and bone pain	13	35.13	24	72.72
Acne	2	5.40	5	15.15
Hirsutism	3	8.10	7	21.21

Analysis of body composition

The results of the analysis of body composition are given in Table 4. The results show a significant difference between the two groups in terms of variables (P <0.05). There was no significant difference in only the two cases; Resting Metabolic Rate (RMR) and Resting Metabolic Rate/Lean Body Mass (RMR/LBM).

Evaluation of MIF gene expression

The results of MIF gene expression showed that the expression level before and after the start of the experiment in the second group (BMI≥ 30) is higher than the first group (BMI <30). The results also showed that the two groups increased the expression of the MIF gene after treatment with leuprolide acetate. This increase was statistically significant in the second group (P = 0.042) (Figure 1). Endometriosis is an invasive but benign disease of women that develops in endometrial glands outside the endometrium and uterine muscle (19). It affects about 15-20% of women of childbearing age. Endometriosis is a multifactorial disease that is caused by a combination of genetic factors and environmental factors (20).

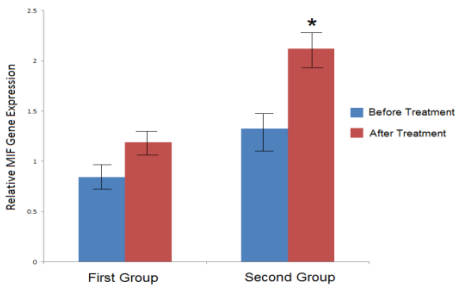

Figure 1. Relative MIF gene expression in two groups before and after treatment with leuprolide acetate; *: P<0.05

Table 4. The body composition variables in two groups

Variable	First Group (BMI<30)	Second Group (BMI≥30)
Body Mass Index (BMI) (Kg/m^2)	27.72 ± 10.06	34.20 ± 3.32 *
Basal Metabolic Rate (BMR) Prediction (Kcal/24h)	1448.50 ± 150.74	1611.2 ± 197.26 *
Fat Percent (%)	34.46 ± 3.89	40.84 ± 5.30 *
Fat Mass (Kg)	24.77 ± 3.71	36.02 ± 6.60 *
Muscle Mass (Kg)	47.15 ± 5.51	52.07 ± 6.78 *
Total Water of Body (Kg)	34.50 ± 4.02	38.12 ± 4.97 *
Visceral Fat (Kg)	5.38 ± 0.97	9.33 ± 2.24 *
Fasting Serum Glucose Level (mg/dl)	104.88 ± 17.76	127.80 ± 57.26 *
Triglyceride (TG) (mg/dl)	93.16 ± 20.32	134.0 ± 54.74 *
Total Cholesterol (T-Chol) (mg/dl)	163.55 ± 27.97	165.4 ± 41.40 *
High Density Lipoprotein (HDL) (mg/dl)	50.16 ± 12.66	44.13 ± 7.80 *
Hyper Sensitivity C-reactive Protein (Hs-CRP) (mg/L)	1.73 ± 1.86	6.3 ± 6.15 *
Insulin (µlU/ml)	10.0 ± 3.27	15.56 ± 6.77 *
Resting Metabolic Rate (RMR) (Kcal/24h/kg)	1499.06 ± 240.26	1726.18 ± 198.57
Resting Metabolic Rate/Kilogram (RMR/kg) (Kcal/24h/kg)	21.25 ± 3.47	19.57 ± 1.96 *
Resting Metabolic Rate/Lean Body Mass (Kcal/24h/kg)	32.39 ± 4.41	32.72 ± 4.42

*: P<0.05

Since the origin of endometriosis and its pathogenesis is highly debated, various etiologies have been proposed. The most accepted of these is the theory of menstrual blood return (21). This theory is also called implantation, in which menstrual tissue and blood are pushed back through the fallopian tubes and placed on the pelvic area, where it begins to grow and multiply in the same region, leading to endometriosis (3). Other theories include the theory of cellular metaplasia, embryonic residue theory, lymphatic metastasis, hormonal factors, environmental contamination, and genetic predisposition to endometriosis (5). This disease has a multifactorial and polygenic hereditary model and is known as a disease that has a family background (22).

One effective way to treat endometriosis is to use GnRH agonists (such as leuprolide acetate), which inhibit estrogen production. However, one of the possible side effects of this treatment is obesity and BMI increasing, which is a concern for some patients (23, 24). This study investigated the role of leuprolide acetate in treating overweight patients (BMI≥30) and their comparison with non-overweight patients (BMI<30) for six months. Also, the effect of this medicine was evaluated on the expression of the MIF gene, which is an effective gene in obesity.

The results showed that treatment with leuprolide acetate for six months in both groups reduced dysmenorrhea, dyspareunia, and chronic pelvic pain. Although this decrease was greater in the BMI <30 group, the difference was not significant. This indicates that the medicine worked equally well in both groups. Also, after collecting the side effects of the medication, it was found that hypoestrogenism, such as cramps and spotting, was more in the first group; Endogenous complications such as oily skin, acne, and hirsutism were also more common in the second group. Numerous previous studies have confirmed these findings (7, 8, 25,26).

The results of MIF gene expression showed that the expression level before and after the start of the experiment in the second group (BMI≥ 30) is higher than the first group (BMI <30). The results also showed that the two groups increased the expression of the MIF gene after treatment with leuprolide acetate. This increase was statistically significant in the second group (P = 0.042). According to the results of this section and clinical evaluations, it was found that this medication causes more weight gain in obese people and increases the risk of obesity-related diseases among these patients.

As mentioned, the evidence obtained from this study showed a higher expression level of the MIF gene in obese individuals compared to those with a body mass index of less than 30. Similar findings have been reported from Dandona *et al*. (28) that increased plasma MIF concentrations and mRNA expression in obese individuals. The pre-inflammatory status of obesity partly justifies these findings. The positive

correlation of MIF gene expression level with body mass index, free fatty acid concentration, and CRP has been shown. The present study's findings also showed a positive relationship between MIF gene expression level and fasting glucose level. Similarly, in the obese group studied in this study, a positive correlation was found between fasting serum glucose levels, RMR / kg, TG, and RMRLBM.

In general, the results of this study showed that leuprolide acetate treats and improves the disease in patients with endometriosis. The treatment was the same in both groups with a BMI greater than or less than 30. But the medication further increased the expression of the MIF gene in the group with a BMI ≥ 30. Because this gene is associated with obesity, it seems that this medicine causes more weight gain in obese people. Therefore, it is recommended that this treatment be used with caution in obese patients with endometriosis. In the future, it is suggested that with more extensive studies and broader follow-up, the symptoms of endometriosis be evaluated after discontinuation of treatment.

References

1. Wuyung PE, Rahadiati FB, Hartono Tjahjadi SS, Kusmardi K, Kodariah R, Wiweko B. Histopathology and ARID1A Expression in Endometriosis-Associated Ovarian Carcinoma (EAOC) Carcinogenesis Model with Endometrial Autoimplantation and DMBA Induction. Asian Pac J Cancer Prev 2021; 22(2): 553.

2. Muangtan S, Suknikhom W, Sananpanichkul P. Epithelial ovarian cancer with endometriosis is not associated with menopausal status: a co-association study at Prapokklao Hospital. Asian Pac J Cancer Prev 2018; 19(5): 1337.

3. Marquardt RM, Kim TH, Shin J-H, Jeong J-W. Progesterone and estrogen signaling in the endometrium: what goes wrong in endometriosis? Int J Mol Sci 2019; 20(15): 3822.

4. Chantalat E, Valera M-C, Vaysse C et al. Estrogen receptors and endometriosis. Int J Mol Sci 2020; 21(8): 2815.

5. Liang Y, Xie H, Wu J, Liu D, Yao S. Villainous role of estrogen in macrophage-nerve interaction in endometriosis. Reprod Biol Endocrinol 2018; 16(1): 1-11.

6. Burns KA, Thomas SY, Hamilton KJ, Young SL, Cook DN, Korach KS. Early endometriosis in females is directed by immune-mediated estrogen receptor α and IL-6 cross-talk. Endocrinology 2018; 159(1): 103-118.

7. Abdou AM, Ammar IMM, Alnemr AAA, Abdelrhman AA. Dienogest versus leuprolide acetate for recurrent pelvic pain following laparoscopic treatment of endometriosis. J Obstet Gynaecol India 2018; 68(4): 306-313.

8. Wang S-T, Johnson SJ, Mitchell D, Soliman AM, Vora JB, Agarwal SK. Cost–effectiveness of elagolix versus leuprolide acetate for treating moderate-to-severe endometriosis pain in the USA. J Comp Eff Res 2019; 8(5): 337-355.

9. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metabolism 2019; 92: 6-10.

10. Ercisli MF, Lechun G, Azeez SH, Hamasalih RM, Song S, Aziziaram Z. Relevance of genetic polymorphisms of the human cytochrome P450 3A4 in rivaroxaban-treated patients. Cell Mol Biomed Rep 2021; 1(1): 33-41.

11. Wang T, He C. Pro-inflammatory cytokines: The link between obesity and osteoarthritis. Cytokine Growth Factor Rev 2018; 44: 38-50.

12. Röszer T. Understanding the biology of self-renewing macrophages. Cells 2018; 7(8): 103.

13. Lauterbach MA, Wunderlich FT. Macrophage function in obesity-induced inflammation and insulin resistance. Pflugers Archiv 2017; 469(3): 385.

14. Johnson C, Drummer IV C, Shan H et al. A novel subset of CD95+ pro-inflammatory macrophages overcome miR155 deficiency and may serve as a switch from metabolically healthy obesity to metabolically unhealthy obesity. Front immunol 2021; 11: 3370.

15. Amosse J, Durcin M, Malloci M et al. Phenotyping of circulating extracellular vesicles (EVs) in obesity identifies large EVs as functional conveyors of Macrophage Migration Inhibitory Factor. Mol Metab 2018; 18: 134-142.

16. Li Y-H, Wen K, Zhu L-L et al. Tautomerase activity-lacking of the macrophage migration inhibitory factor alleviates the inflammation and

insulin tolerance in high fat diet-induced obese mice. Front Endocrinol 2020; 11: 134.

17. CHEN L, HUANG Y, LI L et al. 197-LB: The Regulation of HSL by Macrophage Migration Inhibitory Factor (MIF) Contributes to Adipocyte Hypertrophy and Development of Obesity. Am Diabetes Assoc; 2021.

18. Tang H, Tan X, Zhu L, Qin K, Gao H, Bai H. Swimming prevents nonalcoholic fatty liver disease by reducing migration inhibitory factor through Akt suppression and autophagy activation. Am J Transl Res 2019; 11(7): 4315.

19. Sananpanichkul P, Muangtan S, Suknikhom W, Bhamarapravatana K, Suwannarurk K. Does Endometriosis Hinder Successful Ovarian Debulking Surgery? Asian Pac J Cancer Prev 2018; 19(2): 509.

20. Zoure AA, Bayala B, Bambara HA et al. Epidemiological Situation and Medical Management of Gynaecological and Breast Cancers from 1998 to 2018 in West Africa: A Systematic Review. Asian Pac j cancer biol 2020; 5(4): 211-219.

21. Temtanakitpaisan A, Kleebkaow P, Aue-aungkul A. Epithelial Borderline Ovarian Tumor: Clinicopathological Features, Outcome and Prognostic Factors. Asian Pac j cance care 2018; 3(4): 75-75.

22. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol 2019; 15(11): 666-682.

23. Romanski PA, Bortoletto P, Magaoay B, Chung A, Rosenwaks Z, Spandorfer SD. Live birth outcomes in infertile patients with class III and class IV obesity following fresh embryo transfer. J Assist Reprod Genet 2021; 38(2): 347-355.

24. Furtado CLM, Iannetta R, Ferriani RA et al. Telomere length is not altered in girls with idiopathic central precocious puberty treated with a GnRH analog–leuprolide acetate. Gynecol Endocrinol 2020; 36(12): 1119-1123.

25. Poulos C, Soliman AM, Tekin S, Agarwal SK. Patient preferences for elagolix and leuprolide for treating endometriosis-related pain in the United States. Expert Rev Pharmacoeconomics Outcomes Res 2021; 21(5): 1091-1099.

26. Kazemi E, Zargooshi J, Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaei H, Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinforma 2021;22(4):bbaa338. https://doi.org/10.1093/bib/bbaa338

27. Dandona P, Aljada A, Ghanim H et al. Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. J Clin Endocrin Metabol 2004; 89(10): 5043-5047.