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Effect of Cholesterol lowering Agent (Lipolax) on Some Physiological Parameters in Some Infertile Obese PCOS Egyptian Women Undergoing ICSI

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ABSTRACT

Background: Infertile obese women with polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting women and characterized by hyperandrogenism, chronic anovulation with either oligomenorrhea or amenorrhea and hyperandrogenism, and its morbidity may include hyperinsulinemia, insulin resistance. Lipolax was used to enhance the ovulation response especially when beginning the intra cytoplasmic sperm injection (ICSI) protocol. This study aimed to enhance the sexual hormones, lipid profile, ovulation response and pregnancy outcome in infertile obese PCOS women's treated with Lipolax drug (fenofibrate) before undergoing to ICSI.

Setting: This study was conducted in International Islamic Center for Population Studies and Research-Assisted Reproduction Unit, Al-Azhar University during the period between 2009 and 2011.

Patients and Methods: A total number of 75 infertile patients with obese PCOS were randomized to receive oral Lipolax (fenofibrate) for three months before undergoing to ICSI. For each patient FBS and Hb, hormonal profile included FSH, LH, FSH/LH ratio, E_2, PRL and lipid profile evaluation were performed at baseline and after 3 months of treatment.

Results: There was a significant decrease in the FBS, in cases treated with Lipolax when compared with control or obese PCOS, no significant differences were noticed in the level of Hb% in both groups. Total lipids, triglycerides and total cholesterol decreased significantly compared with obese PCOS group. Significant decrease was also observed in respect to LDL in cases of Lipolax treated group than cases of obese PCOS, significant increase in the level of HDL was recorded. A significant enhance was detected in increase FSH hormone level and decrease LH, FSH/LH ratio and E_2 hormonal level after Lipolax treatment, no difference was observed in prolactin hormonal profile. Observed improvement was detected in the number of HMG ampoules, mature oocytes, and number of grade A embryos and also in pregnancy outcome.

Conclusion: Lipolax (lowering cholesterol agent) improving the response in obese PCOS women undergoing ICSI, by enhance their resistance sensitivity in transadipose tissue leads to improve the function of gonads and sexual hormones and lipids profile which leads to decreased total cost of ICSI by lowering the number of stimulation ampoules and increase mature collected oocytes and finally the percent of successful pregnancy rate.

Keywords: PCOS, Obese, Lipolax, ICSI, Lipid profile, Physiological parameters.

Introduction:

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age**,** it is a complex disorder with multiple components, including reproductive, metabolic, and cardiovascular manifestations, and also it has long-term health concerns that cross the life span[(1)].

Polycystic ovaries, ovulation-related infertility, excessive secretion of androgenic hormones that cause hirsutism and acne, high cholesterol level, type 2 diabetes, insulin resistance, also obesity and oligo- or amenorrhea. The most important abnormality in patients with PCOS is one of an ovulation manifested by oligomenorrhea or secondary amenorrhea [(2)].

The sonographic appearance of PCOS may occur together, or in isolation with a biochemical status, which involves metabolic and hormonal changes. Concentrations of luteinizing hormone (LH) are elevated in 45-75% of cases and raised testosterone levels are seen in 80% of patients. The above hormonal levels are the usual indicators of this syndrome. Obesity is very common among women with PCOS and 30-60% is overweight to some degree. A polycystic ovary has abnormal number of eggs that can be viewed near its surface resembling cysts (Fig 1and 2) [(3)].

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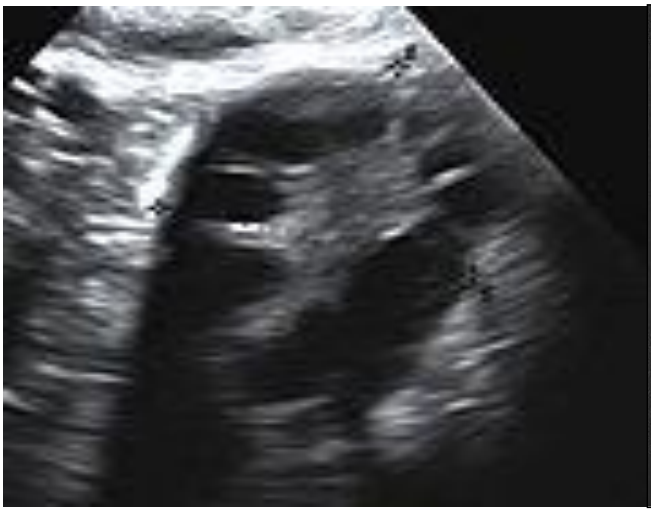

Fig (1): A polycystic ovary shown on an ultrasound image before treatment.

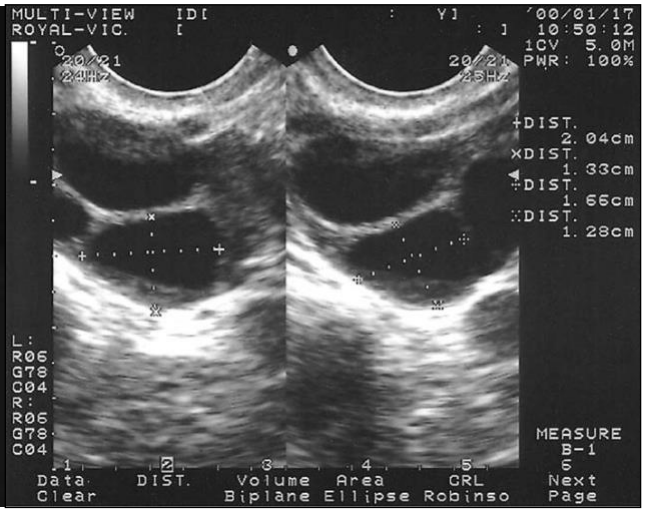

Fig (2): Transvaginal ultrasound scan of polycystic ovary after treatment.

Obesity has been defined as a body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters) greater than 30. While beginning obese increases risks associated with pregnancy and may reduce fertility. On the other hand some obese PCOS women's have been normal fertile [(4)].

Lipolax[Fenofibrate, its chemical names is: 2-{4-(4-chlorobenzoyl)phenoxy}-2methyl propanoic acid 1-methylethyl ester] is used along with a proper diet to help lower "bad" cholesterol and fats (such as LDL, triglycerides) by decreasing their synthesis and raise "good" cholesterol (HDL) in the blood by reducing its release into the circulation and increasing catabolism. It belongs to a group of drugs known as fibrates. It works by increasing the natural substance (enzyme) that breaks down fats in the blood. Lowering triglycerides in people with very high triglycerides blood levels may also decrease the risk of pancreas disease (pancreatitis) [(5)].

The present study aimed to evaluate the enhance in fertility outcome after ICSI cycle in women suffering with obesity and PCOS treated with cholesterol lowering agent, on some sexual hormones, biochemical parameters and ovulation response (oocyte maturation, quality of embryos and pregnancy outcome).

Subject and Methods:

This prospective study was designed to predict the effect of Lipolax as a cholesterol lowering agent (Produced by SIGMA pharmaceutical industries–Egypt S.A.E.in cooperation with ELITE Pharma) on some Egyptian infertile obese women among 75 infertile obese PCOS women. The age of women ranges from 20-38 years at the start of the treatment and necessary investigations to diagnose PCOS has been done.

This study was done at assisted fertilization attending the ART Unit in the International Islamic Center for Population Studies and Research, Al-Azhar University, Cairo, Egypt.

The cases of the study were classified into four groups:

Group I :

Control group consisted of 25 cases (not obese or PCOS) with BMI < 30.

Group II :

Consisted of 25 cases of obese PCOS females with BMI > 30-35.

Group III :

Consisted of 25 cases of obese PCOS females with BMI >30-35treated with Lipolex (Fenofibrate)(cholesterol lowering agent) 200mg daily.

Duration: Treatment duration takes 3 months.

Physiological parameters:

-The menstrual cycle regulation percentage was calculated to evaluate the oligo menstruation (infrequent or very light menstruation) or irregular menstruation (lack of ovulation)

-Changes of body mass index (kg/m^2) calculated according to the following equation:

% of BMI changes =

$$\frac{\text{final weight- initial weigh}}{\text{Initial weigh}} \times 100$$

Biochemical assays:

Serum glucose was estimated according to enzymatic colorimetric method described by Tietz[6]**.** Total lipids were assayed by the method of Kaplan [7]. Serum total cholesterol (T.C) was performed according to Henry *et al*.[8]**.** Serum triglycerides (T.G) were determined according to the method of Fossati and Prencie[9]**.** Serum high density lipoproteins cholesterol (HDL-cholesterol) was assayed according to Burstein [10]. The concentration of low density lipoproteins cholesterol (LDL-cholesterol) in serum was estimated by the following equation used by Friedewald*et et al*.[11]**:**

LDL - cholesterol (mg/dl) = Total cholesterol –

HDL cholesterol - (T.G / 5).

Preparation for ovarian stimulation:

Preliminary evaluations including general, local vaginal examination, ultrasound evaluation were done. Hormonal profile including estradiol (E_2), prolactin (PRL), luteinizing hormone (LH) and follicular stimulating hormone (FSH) were done by VIDAS measurement, using the ELFA technique (Enzyme Linked Fluorescent Assay) on day three of the menstrual cycles [12].

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According to the ART protocols, women had ovarian gonadotropin stimulation drugs consisted of human menopausal gonadotropins **(**HMG) (Menotrophin) which contain equal concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Until recently, all available human FSH pharmaceutical preparations were extracted from postmenopausal urine. While HMG may be used as a source of FSH, it has low specific activity and contains significant amounts of LH (as well as other proteins), which is thought to be associated with poor oocyte quality, reduced fertilization rates, lower embryonic viability, and early pregnancy wastage [13].

The number of ampoules of initial gonadotropin dose used for ovarian stimulation is 75-300IU/ml adjusted according to:

1 - The patient's age.

2 - Body mass index.

3- Baseline serum FSH concentrations on day 2 or 3 of menstruation.

4- Previous response to ovarian stimulation.

But it was costly and associated with risks including multiple pregnancy and ovarian hyperstimulation syndrome**.**

Cycle Monitoring:

During treatment, the ovarian response is monitored by:

1. Vaginal ultrasound measurements of follicular growth were done starting on the sixth or seventh day of stimulation and repeated every two or three days according to follicular diameter. At each scan, the size and number of follicles were determined and recorded.
2. In normal responders, ovulation was triggered by administration of choriogonadotropin alpha (hCG) (10,000 IU) intramuscularly when at least 4 follicles reached 18 mm. in diameter.

Assessment of oocyte grading after oocyte retrieval [14].

Grade	Characteristics
Grade 1 (immature oocyte, prophase 1)	Shows a centrally located germinal vesicle. No polar body present.
Grade 2 (nearly mature, metahase 1)	No polar body, no germinal vesicle.
Grade 3 (mature/ preovulatory, metphase 11)	Sometimes appearing loosely aggregated extruded polar body, no nucleus. Clear ooplasm, homogeneously granulated.
Grade 4 (postmature)	Polar body is still intact or fragmented. Ooplasm may be slightly darkened, mainly granulated. Oocyte is still round.
Grade 5 (atretic nonviable	Atresia oocurs in all oocytes from early immature to postmature stages. Polar body and nucleus are degenerated, if present. Ooplasm is dark and vacuolated. Uneven surface and very irregular shape of the oocyte; a preivitelline space is obvious Clearly visible dark (brush-like) zona pellucida.

ICSI procedure:

ICSI procedure involves the injection of a single motile spermatozoon into the oocyte. The procedure is carried out in a plastic microinjection dish containing microdroplets covered with mineral oil. A fraction (1µl) of the sperm suspension is added to the periphery of the central polyvinyl pyrrolidone (PVP) droplet **[15].**

Fertilization and embryo cleavage after ICSI:

After injection of a single spermatozoon into the ooplasm, oocytes are considered normally fertilized when two individualized or fragmented polar bodies are present together with two clearly visible pronuclei (2-PN) that contain nucleoli. The fertilization rate after ICSI is usually expressed per number of injected oocytes and ranges from 57% to 67% according to the sperm origin **[15]**.

The cleaving embryos are scored according to equality of blastomeric size and proportion of nucleate fragments.

1-GradeA: Even, regular spherical blastomeres; moderate refractivity (i.e., not very dark), intact zona, no, or very few fragments (less than 10%).

2-Grade B: Uneven or irregular shaped blastomeres; mild variation in refractivity; no more than 10% fragmentation of blastomeres.

3-Grade C: Fragmentation of no more than 50% of blastomeres; remaining blastomeres must be at least in reasonable (Grade 2) condition; refractility associated with cell viability, intact zona pellucida.

4- Grade D: More than 50% of the blastomeres are fragmented, gross variation in refractivity; remaining blastomeres appear viable.

5- Zygote with two pronuclei on day 2 (delayed fertilization).

6- Nonviable: fragmented, lyses, contracted or dark blastomeres; no viable cells **[16].**

The number of embryos transferred should be limited in order to avoid multiple pregnancies. Luteal phase support was given to the patients in the form of daily 100 mg progesterone in oil intramuscular

injection for 14 days, then human chronic gonadotrophin (beta hCG) titer was performed for detection of chemical pregnancy and then it was confirmed by ultrasound examination at 5-6 weeks gestation by visualization of gestational sac. The luteal phase support should be continued for another eight weeks for pregnant cases [17].

Statistical Analysis

The data are expressed as means ± standard errors (SE). The t test was used to elucidate the differences between treated and control groups (Snedecor and Cochran) [18].

Results:

1-Physical parameters

Table (1) shows the menstrual cycle regulation of patients.

	Control	ObesePCOS	Obese PCOS and Lipolax
No of cases	**25**	**25**	**25**
% of regular menstrual	**100 %**	**----------**	**----------**
% Irregular menstrual	**----------**	**38%oligo 62%irrg**	**45%Oligo 55%irrg**

Oligo menstruation or oligomenorrhea (infrequent or very light menstruation).
Irregular menstruation or anovulation (lack of ovulation).

The present data in Table 1 showed the percentage of menstrual cycle variation among the control subjects which recorded 100% regular menstrual cycle while in obese PCOS group showed variation between 38 % oligo menstruation and 62 % irregular menstruation. In group treated with oral 200 mg Lipolax daily for 3monthes still recorded variation between 45 % oligo menstruation and 55 % irregular menstruation.

Table (2) shows changes of body mass index (kg/m^2) after treatment, in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	ObesePCOS	Obese PCOS and Lipolax
BMI (mean± S.E)	**25.4±1.73**	**36.35±3.35****	**30.08±3.04***
BMI change (before & after treatment)	**1.2 %**	**2.8 %**	**-17.25 %**
BMI change in comparison with the control	**----------**	**43.1** %	**35.66**%

***P.value <0.05 significant ** P.value <0.01 highly significant.**

Table (2) showed changes in the body mass index which recorded a highly significant increase ($P \leq 0.01$) in obese PCOS patients while in Lipolax treated group, a marked enhance was recorded a significant increase ($P \leq 0.05$) after treatment in a comparison with control group.

Table (3): Fasting blood glucose level (mg/dl) and hemoglobin concentration (%) before and after treatment in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
Fasting blood glucose level at the beginning of the experiment	89.24±1.5	108±1.7**a	111.6±0.9**a
Glucose level after 3 months of treatment	89.1±2.4	112.4±1.24**a	95.5±1.98*a **b
Hemoglobin at the beginning of the experiment	13.24±1.02	11.15±1.32*a	11.2±1.12*a
Hemoglobin after 3 months of treatment	13.3±0.99	11.15±2.4*a	11.3±1.22*a

Data recorded as mean± S.E a=in comparison with control b= in comparison with obese PCOS*P.value<0.05 significant ** P.value<0.01 highly significant.

Fasting blood glucose level showed in table (3), although there were a highly significant increase ($P \leq 0.01$) in obese PCOS patients before treatment when compared with control women but it is still within in the normal value. In Lipolax treated group, although there were a decrease in FBG level but it still recorded a significant increase ($P \leq 0.05$) when compared with the control group. A highly significant decrease ($P \leq 0.01$) in FBG level was recorded in Lipolax treated group compared with obese PCOS and still within in the normal value. Hemoglobin level recorded a significant decrease ($P \leq 0.05$) in all groups in comparison with control group before or after treatment but still in the normal value.

Table (4): shows change of lipid profile before and after treatment in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
Total lipids (mg/dl) at the beginning of the experiment	202.9±1.01	295.4±2.1**a	293.4±1.21**a
Total lipids(mg/dl) after 3 months of treatment	202.9±1.5	290.4±0.9**a	229.4±3.65*a **b
Triglycerides (mg/dl) at the beginning of the experiment	92.7±1.7	65±1.26**a	66±1.75**a
Triglycerides (mg/dl) after 3 months of treatment	92.7±2.7	65±2.26**a	55.3±1.56**a **b
Total cholesterol (mg/dl) at the beginning of the experiment	135.6±1.38	228.2±2.18**a	212.4±2.55**a
Total cholesterol (mg/dl) after 3 months of treatment	135.6±1.38	228.2±2.8**a	175.1±3.78*a **b
LDL (mg/dl) at the beginning of the experiment	91.72±3.05	160.8±1.65**a	160. ±2.56**
LDL (mg/dl) after 3 months of treatment	91.72±1.05	161.8±2.65**a	105.5±3.62*a **b
HDL (mg/dl) at the beginning of the experiment	45.65±2.58	53±1.1**a	52±3.43**a
HDL (mg/dl) after 3 months of treatment	45.65±2.58	66.4.6±3.1**a	70±2.36**a **b
HDL/LDL at the beginning of the experiment	0.49	0.32	0.33
HDL/LDL after 3 months of treatment	0.49	0.41	0.66

Data recorded as mean± S.E a=in comparison with control b= in comparison with obese PCOS*P.value<0.05 means significant **P.value<0.01means High sign.

At the beginning of the experiment, total lipids, total cholesterol, LDL- cholesterol and HDL-cholesterol levels showed high significant increase ($P \leq 0.01$), while triglycerides showed significant decrease ($P \leq 0.05$) in obese PCOS when compared with non PCOS obese women. After treatment with Lipolax, marked improvement was recorded as a significant decrease ($P \leq 0.05$) in total lipids, total cholesterol, LDL- cholesterol, and triglycerides levels ($P \leq 0.01$) and a highly significant increase in HDL-cholesterol level when compared with control women.

After lipolax treatment, a highly significant decrease ($P \leq 0.01$) in total lipids, total cholesterol, LDL-cholesterol and a highly significant increase in HDL-cholesterol levels were recorded in comparison with obese PCOS group (Table 4).

Table (5): shows change of hormonal profile before and after treatment in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
FSH(mIu/ml) at the beginning of the experiment	6.9±2.72	5.19±0.78*a	5.6±1.28*a
FSH (mIu/ml) after 3 months of treatment	6.12±1.72	5.24±0.81*a	5.8±1.26*a *b
LH (mIu/ml) at the beginning of the experiment	2.6±1.14	11±1.7**a	10.68±8.21**
LH (mIu/ml) after 3 months of treatment	2.6±0.91	11.98±1.74**a	5 ±1.96*a **b
FSH/LH(mIu/ml) at the beginning of the experiment	2.6±0.09	0.47±0.39**a	0.52±0.42**a
FSH/LH (pg/ml)after 3 months of treatment	2.6±0.06	0.43±0.38**a	1.8±0.34*a **b
E_2 (pg/ml) at the beginning of the experiment	45.5±1.1	50.3±1.59*a	49.5±2.2*a
E_2 (pg/ml) after 3 months of treatment	45.7±7.05	50.9±2.65*a	45.8±2.06
PRL at the beginning of the experiment	10.2±1.9	10.6±1.41	10.4±1.38
PRL after 3 months of treatment	10.14±1.9	10.5±1.94	10.6±1.5

Data recorded as mean± S.E a=in comparison with control b= in comparison with obese PCOS*P.value<0.05 significant ** P.value<0.01 highly significant

Table (5) recorded the hormonal profile variation between the control group and obese PCOS treated with Lipolax. A marked enhance was recorded after treatment but still recorded significant decrease ($P \leq 0.05$) in comparison with control and significant increase ($P \leq 0.05$) in comparison with obese PCOS group. While a highly significant increase ($P \leq 0.01$) recorded in both LH hormone and FSH/LH ratio in obese PCOS group in comparison with control before and after Lipolax treatment. After treatment with Lipolax, LH hormone level and FSH/LH ratio decrease and still recorded significant increase ($P \leq 0.05$) than the control group and at ($P \leq 0.01$) when compared with obese PCOS group. On the other hand, a significant increase ($P \leq 0.05$) in E_2 was recorded in obese PCOS group and in Lipolax treated group at the beginning of treatment while a marked enhance recorded after treatment. No significant variation was recorded in PRL hormones before or after treatment.

Table (6): shows distribution of HMG (Human menopausal gonadotropins) ampoules of embryos in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
HMG ampoules	**32.2**	**39.4**	**33.5**
Oocyte retrieval	**8.7**	**6.4**	**8.7**
Number of embryos	**2.9**	**2.13**	**2.8**

Data recorded as average mean.

The present data showed the outcome of patient's response during ICSI protocol treatment in table (6). The number of HMG ampoules reduced after Lipolax obese PCOS treated group reached near to the normal control group. A marked increase in the number of retrieved oocytes and embryos in Lipolax obese PCOS treated group reached near to the normal control group.

Table (7): Shows distribution of ova maturation in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
Metaphase II	**80%**	**70%**	**78%**
Metaphase I	**20%**	**25%**	**20%**

Table (7) recorded the percent of metaphase II (mature oocyte) and metaphase I (immature oocyte) in obese PCOS and Lipolax obese PCOS treated groups compared with control group as the following: obese PCOS groups had been lower than the control, a marked enhanced showed after Lipolax treatment.

Table (8): shows distribution of embryo grading in normal, obese PCOS and Lipolax obese PCOS treated groups.

	Control	Obese PCOS	Obese PCOS and Lipolax
Grade A	**75%**	**70%**	**82%**
Grade B	**25%**	**30%**	**27%**

Grade A: fragmentation in the divided cells less than 25%.
Grade B: fragmentation in the divided cells between 25 and 50%.

Table (8) recorded the percent of embryo grading after fertilization in ICSI. In obese PCOS group, lower grade A and higher grade B embryos were recorded than the control group .While in Lipolax treated group, grade A showed higher percent and grade B was lower than the control group.

Table (9): Shows distribution of pregnancy outcome in normal, obese PCOS and Lipolax obese PCOS treated group.

	Control	Obese PCOS	Obese PCOS and Lipolax
Positive pregnancy	**35.6%**	**30.5%**	**48.5%**
Negative pregnancy	**64.4%**	**69.5%**	**50.2%**

In obese PCOS, patients were recorded a lower positive pregnancy and a higher negative pregnancy rates than the control group, while a marked improvement was found in obese PCOS after treatment with Lipolax as increased in positive pregnancy and decreased in negative pregnancy rates.

Discussion:

PCOS patients can show abnormal patterns in pituitary hormones as luteinizing hormone (LH) and follicle stimulating hormone (FSH). PCOS patients sometimes have elevated LH levels and consequently an elevated LH/FSH ratio. In this study, the group receiving lowering cholesterol drug showed a reduction of LH and LH/FSH ratio than obese PCOS or control group [(19)].

Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol. The excess of adipose tissue in obese patients may secrets more paradox which leads to excess androgens (responsible for hirsutism and virilization) and estrogens which inhibits FSH *via* negative feedback mechanism [(20)].

In the present results, Lipolax treatment decreases numbers of HMG ampoules and number of the oocytes. It could be speculated that, Lipolax favorably affecting oocyte maturation and development and obtained a metabolic improvement in embryo quality, leading to enhance pregnancy outcome and ultimately live birth rates [(21)]. The follicular hormonal milieu is an important regular of follicular and oocyte development, excess follicular androgen concentration, hyperinsulinism and elevated insulin resistance may affect oocyte quality [(22)].

The effects of lowering cholesterol drug on ovarian steroidogenesis might be due to several mechanisms. One possible explanation is the decreased availability of cholesterol, a substrate for T production. Lowering cholesterol drug might also decrease expression of several key enzymes involved in T production: cholesterol side-chain cleavage, 17α-hydroxylase/17, 20-lyase, and 3β-hydroxysteroid dehydrogenase (3βHSD). Such effects of lowering cholesterol drug were noted in adrenocortical cells and in cultures of ovarian theca–interstitial cells [(23)]. The mechanisms of these actions might be due to the inhibitory effects of lowering cholesterol drug on isoprenylation, leading to decreased function of small guanosinetriphosphatases, such as Ras, Rho, and Rac. Ras might increase expression of P450SCC, P450c17, and 3βHSD, and lowering cholesterol drug might abrogate Ras-induced steroidogenesis. In addition, lowering cholesterol drug induced inhibition of proliferation of theca–interstitial cells might reduces T output of the ovary by reducing the size of the theca–interstitial compartment [(24)].

Another important, but expected, finding is the demonstration of an improvement of lipid profile by lowering cholesterol drug. This effect is of particular value in obese PCOS, a condition characterized by elevated plasma levels of cholesterol, LDL and triglycerides with concomitantly reduced concentrations of HDL. Furthermore, women with obese PCOS have other cardiovascular risk factors, including increased thickness of arterial intima-media and greater prevalence of subclinical atherosclerosis. Use of lowering cholesterol drug in these patients is likely to offer significant protection from long-term cardiovascular morbidity [(21)].

We suggest that Lipolax decrease trunk obesity which leads to decrease resistance secretion and improve insulin resistance which leads to improve the function of the gonads. The body weight loss in PCOS is associated with beneficial effects on hormones, metabolism and clinical features. Endocrinological improvement can also be achieved by successful fertilization response and raise the percentage of pregnancy outcome. These were in harmony with Metformin therapy for PCOS patients [(25)], so the combination between the two drugs would be more benefits [(3, 21&26)].

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