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Effect of Insulin-sensitizer Drug (Metformin) on Some Physiological Parameters in Some Infertile Obese Egyptian Women Undergoing ICSI

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Abstract

Background: Egyptian infertile obese women with polycystic ovary syndrome (PCOS) were markedly increased in ART Unit for infertility treatment undergoing ICSI. In a trial to enhance the ovulation, fertilization response and pregnancy outcome treated patients with Metformin as insulin sensitizing drug, 3 months before ICSI processing.

The aim of this study was to evaluate the effect of Metformin on the hormonal profile, lipid and lipoprotein profile and insulin resistance in obese PCOS patients undergoing ICSI.

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

Design: A prospective randomized study.

Patient and Methods: A total of 75 patients with obese PCOS were randomized to receive oral Metformin for three months. For each patient, FBS and Hb, hormonal profile included FSH, LH, FSH/LH ratio, E_2 and PRL, lipid and lipoprotein profile included total cholesterol, LDL, HDL and triglycerides evaluation were performed at baseline and after 3 months of treatment.

Results: There was a significant decrease in the FBS in cases treated with Metformin 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 were decreased significantly compared with obese PCOS group. Significant decrease was also observed in respect to LDL in cases of Metformin than cases of obese PCOS. Significant increase in the level of HDL was recorded. Significant decrease was detected in FSH, LH and FSH/LH ratio in Metformin treated group while no difference was observed in prolactin or estradiol hormonal profiles. Observed improvement was detected in the number of received HMG ampoules, mature oocytes, number of grade A embryos and in pregnancy outcome.

Conclusion: Metformin as lowering insulin resistance, improving the response in obese PCOS women undergoing ICSI, by enhance their resistance sensitivity in transadipose tissue leads to improvement the function of gonads and hormonal and lipids profile .This improvement was detected by decreased total cost for ICSI, lowering the number of both given stimulation ampoules and mature collected oocytes and finally the percent of successful pregnancy rate.

Keywords: PCOS, Obese, Metformin, 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; also it has long-term health concerns that cross the life span **(1)**.

PCOS may be an incidental finding on a routine ultrasound scan in women of normal weight with regular cycles, who do not exhibit signs of hyperandrogenism or conversely, these women may present a classical appearance of hirsutism obesity and oligomenorrhea or amenorrhea. The most important abnormality in patients with PCOS is anovulation 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 **(3).** 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.

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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 PCOS, pregnancy and may reduce fertility **(4)**.

Metformin (insulin-lowering drugs) is an oral iguanid, used extensively in type II diabetes. It inhibits hepatic glucose production and increases peripheral insulin sensitivity, but does not because hypoglycemia. It has been observed to improve the symptoms and reproductive function in some women with PCOS. Several studies have shown an increase in insulin sensitivity and pregnancy rate (PR), accompanied by decreased insulin and androgen levels in patients with PCOS taking Metformin **(5)**.

The present study aimed to evaluate the effect of Metformin on some sexual hormones, biochemical parameters and ovulation response (oocyte maturation, quality of embryos and pregnancy outcome) in obese PCOS women.

Subject and methods:

This prospective study was designed to predict the effect of Metformin as insulin sensitizer drug on some Egyptian infertile obese women among 75 infertile obese and PCOS women. The age of women ranges from 20-38 years at the start of the treatment. 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 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 >30-35 treated with 850 mg Metformin HCL daily (insulin sensitizer drug).

Duration:

Treatment duration takes 3 months. Necessary investigations to diagnose PCOS were done .

-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) was done by VIDAS measurement, using the ELFA technique (Enzyme Linked Fluorescent Assay) on day three of the menstrual cycles **(6)**.

According to the ART protocols, women had ovarian gonadotropin stimulation drugs consisted of human menopausal gonadotropins (HMG) 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. The development of other urinary FSH (uFSH) preparations (urofollitropin and highly purified urofollitropin), which contain significantly reduced or negligible quantities of LH, has resulted in higher pregnancy rates than with hMG **(7)**.

The number of ampoules of initial gonadotropin dose used for ovarian stimulation is 75-300 IU/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 and 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 hCG (10,000 IU) intramuscularly, when at least 4 follicles reached 18 mm. in diameter.

Assessment of oocyte maturation after oocyte retrieval (8).

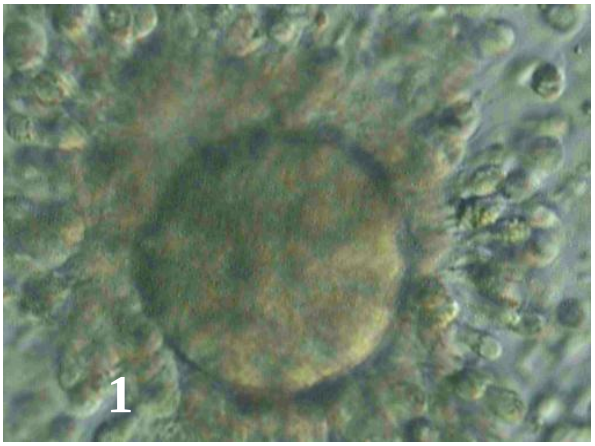

Fig. 1: Oocyte after retrieval covered with cumulus masses when the light path was properly angled had a transparent appearance, which was easily recognized from the darker granulose cells and the oocyte was clearly visible. (X 200).

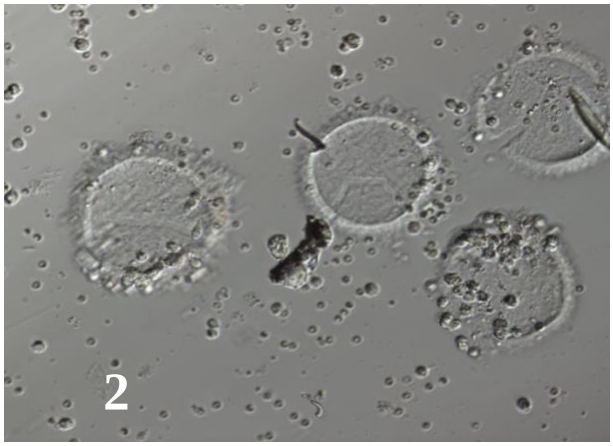

Fig. 2: Empty follicle syndrome (atretic nonviable), artesia occurs in all oocytes from early immature to post mature stages. (X 200).

The oocytes were assessed for maturity (quality) and oocyte grading (8).

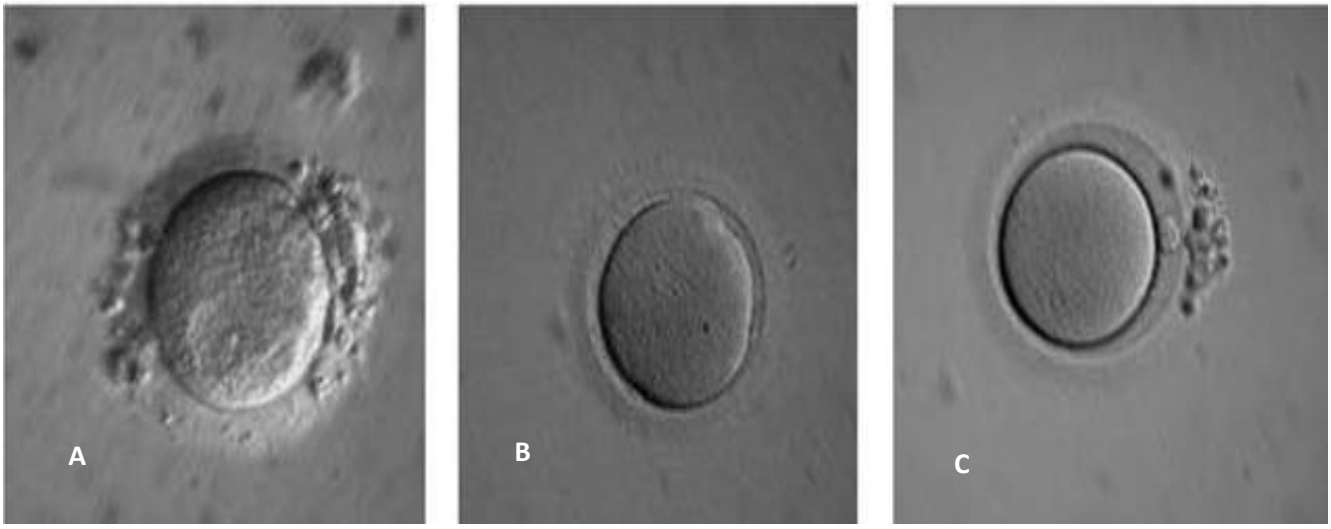

Fig 3: (A) A germinal vesicle (GV). (B) Metaphase I oocytes. (C) A typical metaphase II (8).

Assessment of oocyte grading (8).

Grade	Characteristics
Grade 1 (immature oocyte, prophase I)	Shows a centrally located germinal vesicle. No polar body present.
Grade 2 (nearly mature, metahase I)	No polar body, no germinal vesicle.
Grade 3 (mature/ preovulatory, metphase II)	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 (Fig.4).

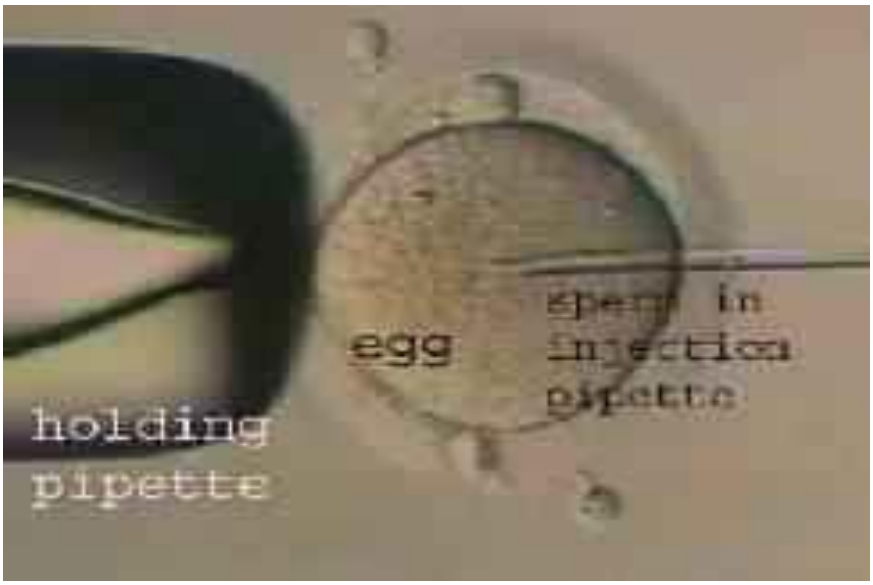

Fig. 4: A single sperm is being injected into an egg during an ICSI procedure (9).

- Fertilization and embryo cleavage after ICSI

After injection of a single spermatozoon into the ooplasma, 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 (Fig 5) **(10)**.

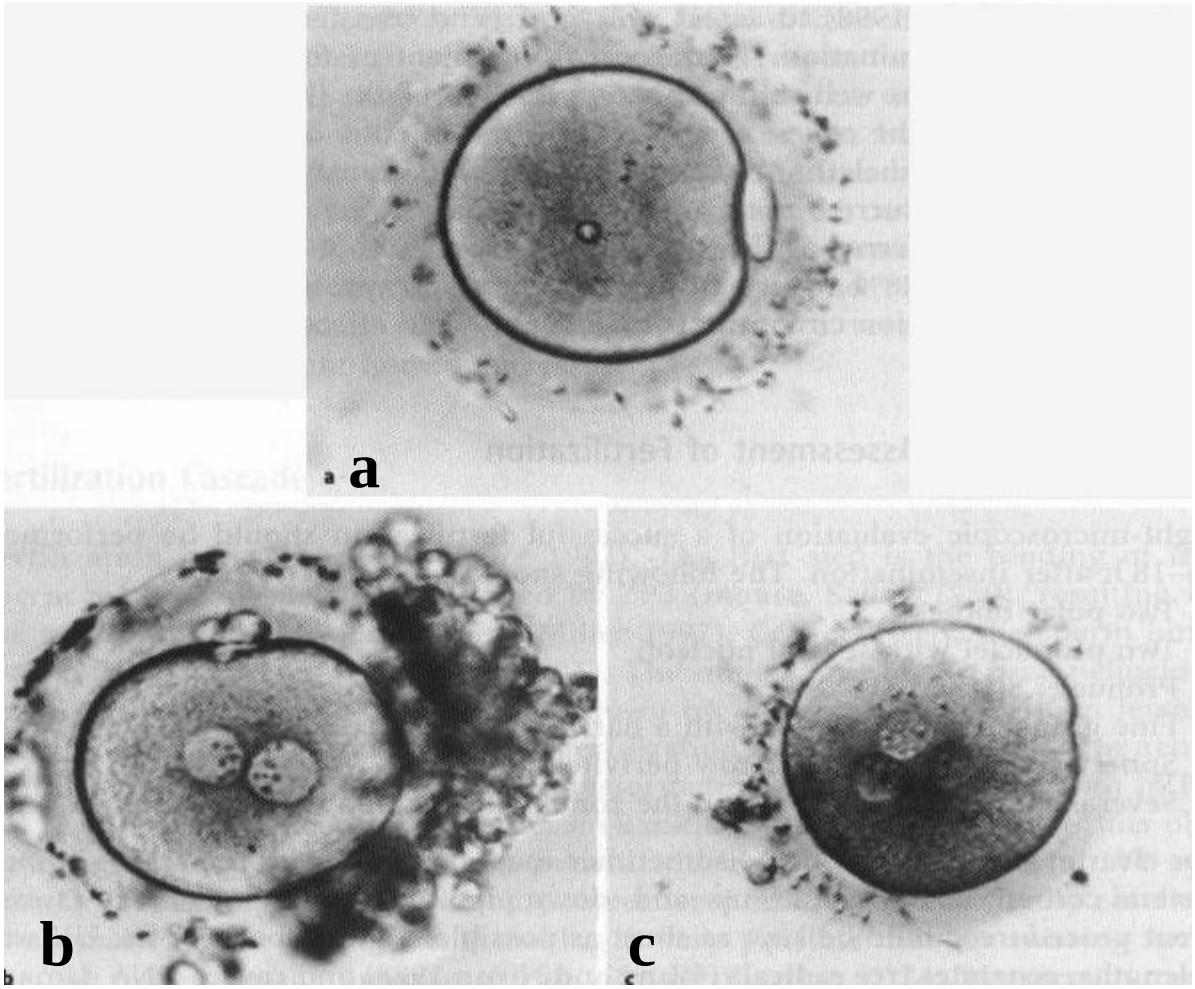

Fig. 5: a-c: Oocytes 16-18 hr. after insemination.(a) Unfertilized oocyte, one polar body, no pronucleus; (b) Fertilized oocyte, two pronuclei, two polar bodies; (c) Polyspermy, three pronuclei (10).

The cleaving embryos are scored according to equality of blastomeric size and proportion of nucleate fragments (Fig. 6).

1- Grade A: 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; refractivity 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 **(11)**.

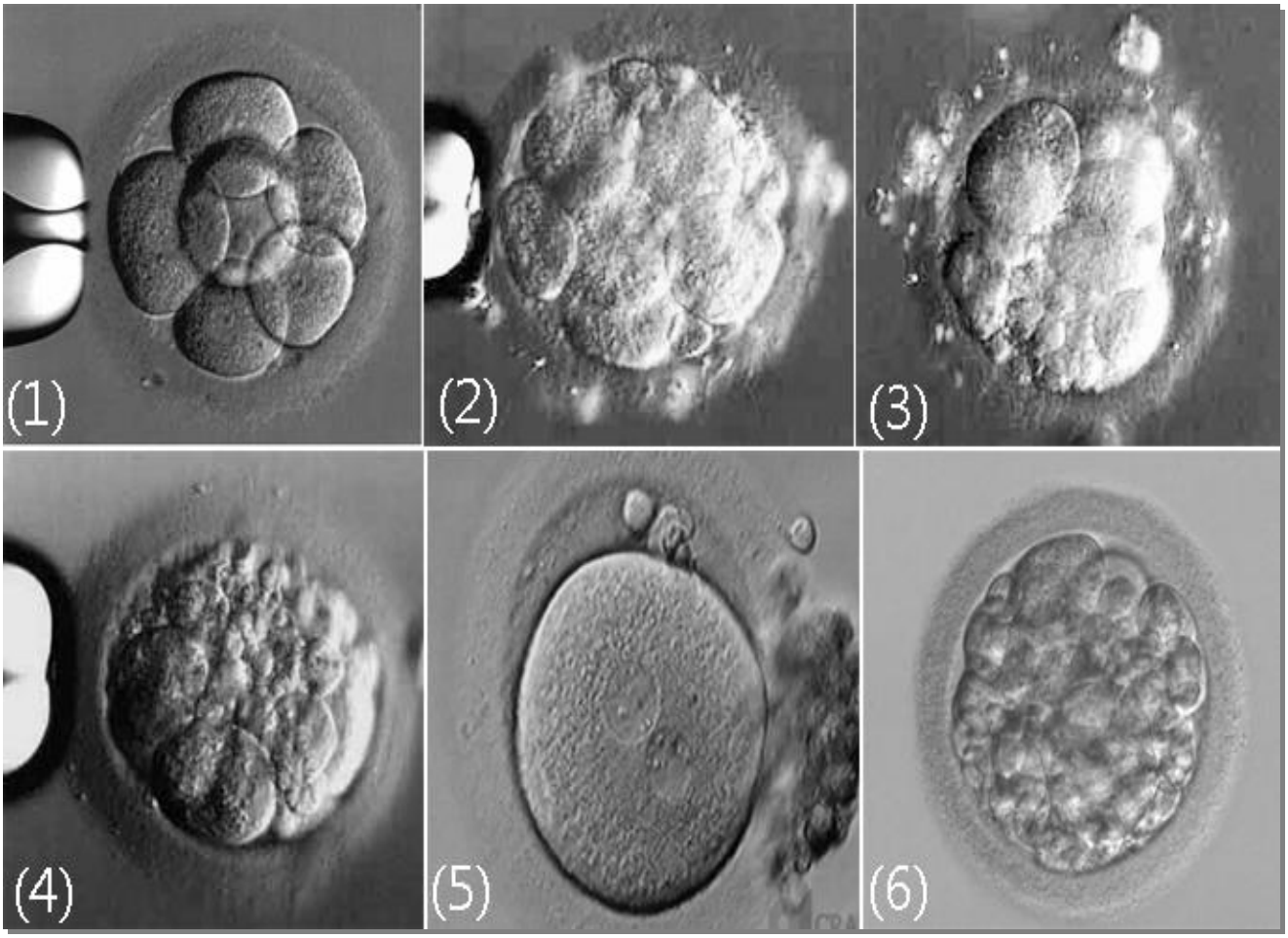

Fig. 6: The cleaving embryos are scored according to equality of blastomeric size and proportion of nucleate fragments (11).

- Outcome of ICSI

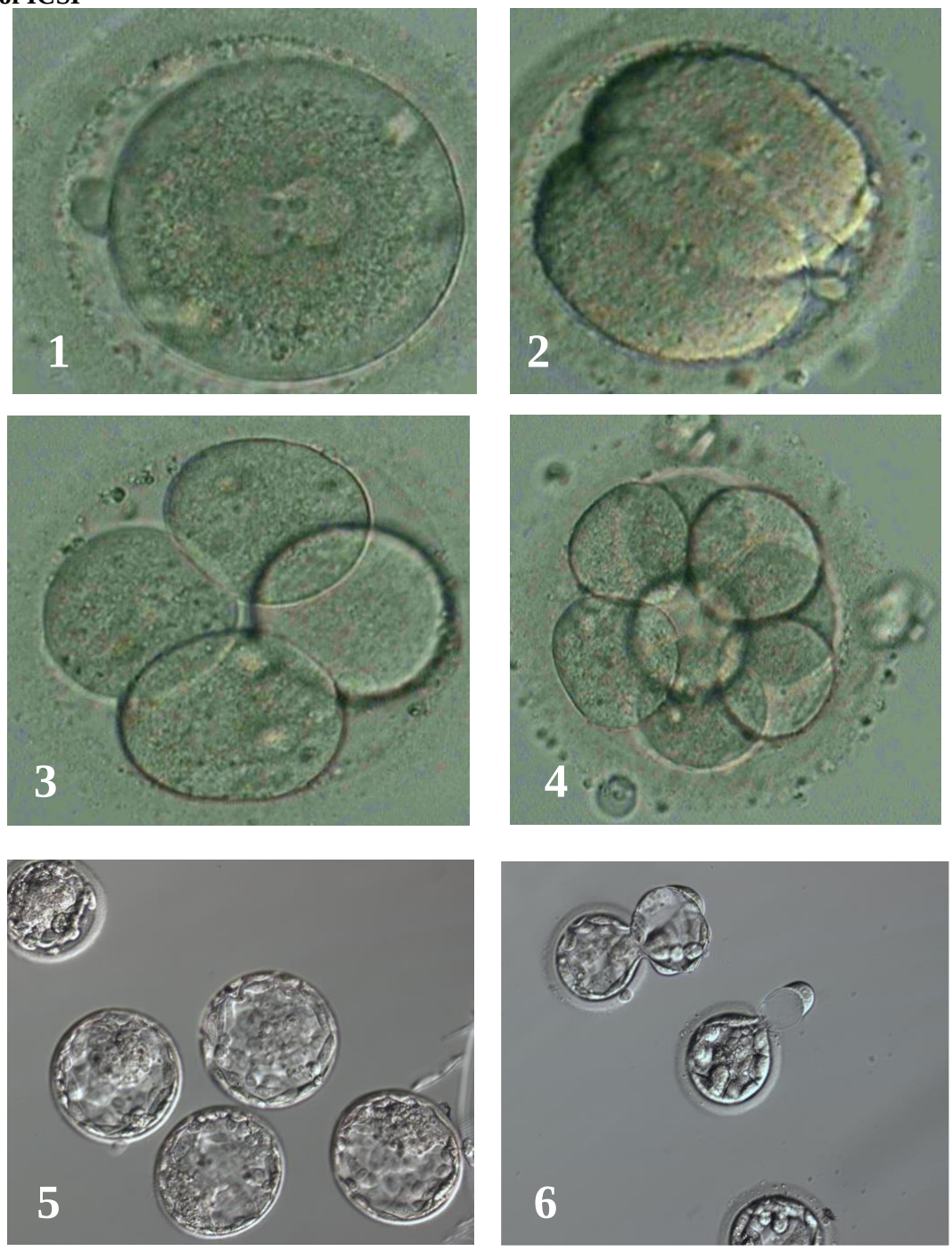

Fig. 7: 1- procuclei stage after 18 hrs. from ICSI: normally fertilized when two individualized or fragment polar bodies are present together with two clearly visible procuclei that contain nucleoli. 2- 2-cell stage after 24 hrs. from ICSI: 3- 4 cell stage after 48 hrs. (on day 2) from ICSI. 4- 8 cell stage after 75 hrs (on day 3) from ICSI. 2, 3 and 4 (grade A or excellent- quality embryos are defined as embryos with equal blastomeric and absent of nuclear fragment). 5- Blastocyst stage on day 5 from ICSI: grade A or excellent-quality embryos are defined as embryos with differentiation of the cavity with ectoderm and endoderm cell layer. 6- Hatched embryos on day 5 from ICSI: grade A or excellent quality embryos. (X 200).

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 100mg progesterone in oil intramuscular injection for 14 days, and then beta hCG (human chronic gonadotrophin) 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 **(7)**.

- Biochemical assays:

Serum glucose was estimated according to enzymatic colorimetric method described by **Tietz (12)**. Total lipids were assayed by the method of **Kaplan (13)**. Serum total cholesterol (T.C) was performed according to **Henry *et al* (14)**. Serum triglycerides (T.G) were determined according to the method of **Fossati** and **Prencie (15)**. Serum high density lipoproteins cholesterol (HDL-cholesterol) was assayed according to **Burstein (16)**. The concentration of low density lipoproteins cholesterol (LDL-cholesterol) in serum was estimated by the following equation used by **Friedewald *et al*. (17)**:

LDL - cholesterol (mg/dl) = Total cholesterol – HDL cholesterol – (T.G / 5).

Results:

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

	Control	**Obese PCOS**	**Obese PCOS & Metformin**
No of cases	25	25	25
% of regular menstruation	100 %	----------	----------
% irregular menstruation	--------- --	38% oligo 62% irreg	36% oligo 64%irreg

- Oligo menstruation or oligomenorrhea (few menstrual periods).

- Irregular menstruation or anovulation (lack of ovulation).

The present data in Table 1 showed the percentage of menstrual cycle variation among the subjects, the normal non obese group recorded 100% regular menstrual cycle while the obese PCOS group reported variation between 38 % oligo menstruation and 62 % irregular menstruation. However the group treated with oral 850 mg Metformin daily for 3 months still recorded variation between 36 % oligo and 64 % irregular menstruation.

Table (2) shows changes of body mass index (kg/m2)

	Control	**Obese PCOS**	**Obese PCOS & Metformin**
No of cases	25	25	25
BMI (mean± S.E)	25.4±1.73	36. 5±3.35**	30.3±3.39*
% of BMI change	---------- -	43.1 %	35.8 %

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

Table (2) showed significant ($P \leq 0.05$) enhance in the body mass index which recorded after Metformin treatment in comparison with control or obese PCOS groups.

Table (3): shows change of fasting blood glucose level (mg/dl) and hemoglobin concentration (%) before and after treatment.

	Control	Obese PCOS	Obese PCOS & Metformin
Fasting blood glucose level at the beginning of the experiment	89.24±1.5	108±1.7**	101.28±3.5**
Glucose level after 3 months	89.1±2.4	110.4±1.24**	80±1.27*
Hemoglobin at the beginning of the experiment	13.24±1.02	11.15±1.32*	11.58±0.77*
Hemoglobin after 3 months	13.3±0.99	11.15±2.4*	11.58±0.77*

Data recorded as mean ± S.E *P.value<0.05 significant ** P.value<0.01 highly significant.

The data in table (3) showed high significant increase ($P \leq 0.01$) in fasting blood glucose level in obese PCOS patients before treatment when compared with control women. After treatment, the Metformin treated patients recorded a significant decrease ($P \leq 0.05$) when compared with the control group. Hemoglobin level recorded a significant decrease ($P \leq 0.05$) in all groups in comparison with control group before or after treatment.

Table (4): shows change of lipid profile before and after treatment.

	Control	Obese PCOS	Obese PCOS & Metformin
Total lipid (mg/dl) at the beginning of the experiment	202.9±1.01	295.4±2.1**	290.4±1.8**
Total lipid (mg/dl) after 3 months	202.9±1.5	290.4±0.9**	279.5±2.4**
Triglycerides (mg/dl) at the beginning of the experiment	92.7±1.7	65±1.26*	65.5±2.69*
Triglycerides (mg/dl) after 3 months	92.7±2.7	65±2.26*	58.7±1.35*
Total cholesterol (mg/dl) at the beginning of the experiment	135.6±1.38	228.2±2.18**	228.2±1.18**
Total cholesterol (mg/dl) after 3 months	135.6±1.38	228.2±2.8**	200.8±2.17**
LDL (mg/dl) at the beginning of the experiment	91.72±3.05	150.8±1.65**	150.8±3.7**
LDL (mg/dl) after 3 months	91.72±1.05	150.8±2.65**	145.92±1.95*
HDL (mg/dl) at the beginning of the experiment	45.65±2.58	78.6±1.1**	78.6±2.64**
HDL (mg/dl) after 3 months	45.65±2.58	78.6±3.1**	80±3.06**
HDL/LDL at the beginning of the experiment	0.49	0.52*	0.52*
HDL/LDL after 3 months	0.49	0.52*	0.55*

Data recorded as mean± S.E *P.value<0.05 significant ** P.value<0.01 highly significant

Lipid profile (Table 4) showed a highly variation in obese PCOS or Metformin treated group rather than the control group. It was noticed that, total lipids, total cholesterol, LDL-cholesterol and HDL-cholesterol levels showed high significant increase ($P \leq 0.01$), while triglycerides showed a significant decrease ($P \leq 0.05$) even before and after treatment.

Table (5): shows change of hormonal profile before and after treatment.

	Control	Obese PCOS	Obese PCOS & Metformin
FSH(mIu/ml) at the beginning of the experiment	6.9±2.72	5.19±0.78*	5.96±6.94*
FSH (mIu/ml) after 3 months	6.12±1.72	5.24±0.81*	5.9±0.05*
LH (mIu/ml) at the beginning of the experiment	2.6±1.14	11±1.7**	11.5±1.2**
LH (mIu/ml) after 3 months	2.6±0.91	11.98±1.74* *	5±1.93*
FSH/LH(mIu/ml) at the beginning of the experiment	2.6±0.09	0.47±0.39**	0.51±0.15**
FSH/LH (pg/ml) after 3 months	2.6±0.06	0.43±0.38**	1.12±3.3*
E2 (pg/ml) at the beginning of the experiment	55.5±1.1	46.3±2.19	46.5±1.5
E2 (pg/ml) after 3 months	45.7±7.05	45.7±2.06	45.1±2.71
PRL at the beginning of the experiment	10.2±1.9	10.9±1.38	12.81±1.93
PRL after 3 months	11.14±1.9	10.44±1.5	11.86±2.09

Data recorded as mean± S.E ***P.value<0.05 significant.** **** P.value<0.01 highly significant.**

Table (5) recorded the hormonal profile variation between the control and subjects treated with Metformin. A significant reduction ($P \leq 0.05$) was recorded in FSH levels even before or after treatment, while a highly significant increase ($P \leq 0.01$) was reported in both LH and FSH/LH ratio in obese PCOS group before and after treatment, also in Metformin treated group before treatment. Conversely, with Metformin, LH level and FSH/LH ratio were decreased after treatment, but still significant increased ($P \leq 0.05$) when compared with the control group. No significant variation was recorded between groups in E_2 or PRL hormones before and after treatment**.**

Table (6) shows distribution of human menopausal gonadotropins (HMG) ampoules, oocyte retrieval and number of embryos.

	Control	Obese PCOS	Obese PCOS & Metformin
HMG ampoules	32.2	39.4	34.3
oocyte retrieval	8.7	6.4	8.5
number of embryos	2.9	2.13	2.5

Data recorded as mean.

The present data showed the outcome of patient's response during ICSI protocol treatment (Table 6). The

number of HMG ampoules reduces in Metformin obese PCOS treated group, reached near to the control group. A marked increase in the number of retrieved oocytes and embryos in Metformin obese PCOS treated group, reached near to the normal control group.

Table (7): shows distribution of ova maturation.

	Control	**Obese PCOS**	**Obese PCOS & Metformin**
Meta phase II	80 %	70 %	75 %
Meta phase I	20 %	25 %	20 %

Table 7 recorded the percent of ova maturation in obese PCOs and Metformin obese PCOs groups compared with control group as the following: obese PCOS groups had been lower than the control, a marked enhanced showed after Metformine treatment.

Table (8): shows distribution of embryo grading.

	Control	**Obese PCOS**	**Obese PCOS & Metformin**
Grade A	75%	70%	80%
Grade B	25%	30%	20%

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

Table 8 recorded the percent of embryo grading after fertilization in ICSI. In obese PCOS group, lower grade A and higher grade B were recorded, while Metformin treated group showed higher grade A and lower grade B than the control group.

Table (9): shows distribution of pregnancy outcome.

	Control	**Obese PCOS**	**Obese PCOS & Metformine**
Positive pregnancy	35.6%	30.5%	45.4%
Negative pregnancy	64.4%	69.5%	50.6%

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

Discussion

Polycystic ovary syndrome patients can show abnormal patterns in pituitary hormones; luteinizing hormone (LH) and follicle stimulating hormone (FSH). In most healthy women, their levels of FSH and LH are about the same. Obese PCOS patients, on the other hand, will sometimes have elevated LH levels and consequently an elevated LH / FSH ratio,T level and DHEAS (hirsutism) are the hallmarks of the hypothalamic-pituitary ovarian dysfunction that is characteristic of PCOS. In this study, Metformin treated obese PCOS patients showed reduction of LH rather than untreated obese PCOS patients. As a result, LH/FSH ratio declined significantly in the Metformin treated group only (**18**).

Evidence of an association between PCOS and glycometabolic dysfunction has accumulated during the past few years, and that a large percentage of women with this condition have hyperinsulinism and insulin resistance, these may be due to rapid improvement of hyperandrogenemia and hyperandrogenism and effective in reducing insulin resistance in women with high baseline androgen levels similar to PCOS **(19)**.

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 (**20**).

Several investigators have suggested that hyper insulinemia increased androgen production by stimulating ovarian steroidgenesis (**5** and **21)**.

At higher concentrations, insulin binds to the insulin-like growth factor (IGF) receptors, transmits its signals to the ovary already in a hyper androgenic state because of an enzymatic dyes regulation of cytochrome P450c17-a, and inhibits the synthesis of the hepatic sex hormone-binding globulin (SHBG).

Metformin lowered FSH and LH levels and LH/FSH ratio. These effects should be viewed as beneficial, especially because increased LH/FSH ratio are considered the hallmarks of the hypothalamic–pituitary–ovarian dysfunction that is characteristic of PCOS. However, the primary actions of Metformin are exerted on the ovarian level by decreased LH also it decreases fasting glucose (**22)**.

Women with PCOS undergoing ICSI have been observed to benefit from Metformin .There is a clear benefit with respect to a reduced incidence of OHSS (ovarian hyper stimulation syndrome)**(23).**

Several lines of evidence support this concept, that, Metformin decreases ovarian theca–interstitial proliferation; second, surgical resection or ablation of a portion of the ovary (wedge resection or laparoscopic diathermy, which decreases ovarian androgen production) leads to marked decline of LH and LH/FSH ratio (**24)**.

In the present results, Metformin treatment decreases days of stimulation, numbers of HMG ampoules and number of the oocytes. It could be speculated that, Metformin normalized the hormonal milieu within antral follicles, 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**).

Metformin treatment decreases total lipids, total cholesterol and low-density lipoprotein (LDL) cholesterol in comparison with the control group. Triglycerides levels can be elevated due to impaired insulin signaling and intra –abdominal fatty adipose tissue deposition is highly related to insulin resistance in obese PCOS patients **(25)**. There are several lines of evidence suggesting that, women with PCOS are also at increased risk of cardiovascular disease due to dyslipidemia, insulin-resistant increased (3–7 times) and markers of abnormal vascular function. Another finding of our study was improvement .so; we recommended using hypoglycemic drugs for obese PCOS women who have hormonal dysfunction (**26**).

This mechanism of action may be due to impairment of local steroidogenesis mediated *via* an imbalance in the production of insulin like growth factors and a direct stimulatory effect on a local (intra-ovarian) protease inhibitor, plasminogen activator inhibitor-one (PAI-1) limiting follicle growth. It is thought that, Metfomin by suppressing hepatic gluconeogenesis and improving peripheral insulin resistance reduces ovarian hyperandrogenaemia and restores normal ovarian steroidogenesis and PAI-1 levels thus enhancing ovulation and improving fertility. Metformin has also been shown to reduce systemic luteinizing hormone (LH) and PAI-1 levels, both of which have been associated with an increased risk of miscarriage **(27)**.

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