

## Effect of Transcutaneous Electrical Muscle Stimulation on Reproductive Dysfunction in Female Rats with Letrozole induced Polycystic Ovarian Syndrome.

Wessam E. Morsy and Manal S. Abd-El Hamid

Department of Physiology, Faculty of Medicine, Ain Shams University, Egypt.

[manalsaidsun@yahoo.com](mailto:manalsaidsun@yahoo.com)

**Abstract: Background and aim of work:** Polycystic ovarian syndrome (PCO) is a very common endocrine disease. Physical exercise and diet regimen appear to have positive effects on this syndrome. So much attention has been directed toward the use of transcutaneous electrical muscle stimulation (TEMS) in promoting exercise being fast and easy method. The study tried to evaluate the effect of this exercise type on reproductive dysfunction in rats with polycystic ovarian syndrome. **Materials and Methods:** Female white albino rats were allocated into three groups: Group I: Control rats, group II: Letrozole induced polycystic ovarian syndrome rats (PCO) where letrozole was given orally and daily in a dose of 1 mg/kg dissolved in 0.9% NaCl solution for 21 days and group III: Polycystic ovarian syndrome rats subjected to bilateral transcutaneous electrical muscle stimulation (PCO+TEMS) of the lower limbs for three weeks after the induction of polycystic ovarian syndrome. After 6 weeks from the beginning of the study, final body weight, body mass and Lee indices were determined. Plasma levels of LH, free testosterone, estradiol, progesterone, prolactin, fasting glucose and fasting insulin were measured. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Plasma catalase activity was determined. Histopathological ovarian examination was done. **Results:** The letrozole induced polycystic ovarian syndrome in group II showed significant increase in plasma free testosterone, luteinizing hormone, glucose and insulin levels with elevated insulin resistance score whereas estradiol, progesterone and catalase activity were significantly decreased compared to the control group. Furthermore, body weight, absolute retroperitoneal fat weight, ovarian weight and both final body mass and Lee indices were significantly increased in PCO group than the control group. Ovaries showed histological ovarian cysts and atretic ovarian follicles. Following transcutaneous electrical muscle stimulation (TEMS) of PCO rats in group III, plasma free testosterone, luteinizing hormone, glucose and insulin levels were significantly decreased with improved insulin resistance score whereas progesterone, estradiol were significantly increased compared to the PCO rats in group II. Meanwhile, catalase activity showed non-significant increase compared to non-treated PCO rats. In addition, body weight, retroperitoneal fat weight, ovarian weight and both final body mass and Lee indices were significantly decreased compared to the PCO group. Prolactin hormone level did not show any significant difference between three groups. In addition, ovarian morphology was reverted to normal. **Conclusion:** Letrozole successfully induced polycystic ovarian syndrome in adult female rats, however transcutaneous electric muscle stimulation as a passive exercise modality used in previous studies, succeeded to improve polycystic ovarian syndrome hormonal profile and the accompanied insulin resistance significantly, with partial improvement in the oxidant state.

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**Key words:** Polycystic ovarian disease, Amenorrhea, reproductive dysfunction, Infertility, Insulin resistance, hyperandrogenism, transcutaneous electrical muscle stimulation.

### 1. Introduction:

Polycystic ovarian syndrome is one of the most common causes of infertility (Baravalle et al., 2006), which is one of the main problems in today's medicine (Sarvari et al., 2010). According to Miri et al (2014) this syndrome is characterized by hyperandrogenism, ovulatory dysfunction, irregular menstrual cycles, imbalance of sex hormones and polycystic ovarian morphology, associated with metabolic disturbances, such as insulin resistance and obesity.

Ovarian hyperandrogenism which is the hallmark of PCOS (Benrick et al., 2013) is either genetically determined or due to extra-ovarian factors such as

hyperinsulinaemia or disturbances of the hypothalamic-pituitary-ovarian axis (Goodarzi and Azziz, 2006).

Excess androgens hinder gonadotrophin-induced estrogen and progesterone synthesis in ovarian follicles (Zelevnik et al., 2004) and so impair folliculogenesis with failure to select dominant follicle (Jonard and Dewailly, 2004). Also, excess androgens contribute to insulin resistance in PCOs (Diamanti-Kandarakis, 2008). Furthermore, insulin resistance amplifies ovarian androgen synthesis (Baillargeon and Carpentier, 2007) and adrenal androgen production (Yildiz et al., 2004). In addition, insulin resistance disrupts components of the hypothalamic-hypophyseal-

ovarian axis, increases LH pulse frequency favoring hyperandrogenemia (Rojas et al., 2014).

Obesity is the predominant metabolic aberration in PCOs (Gambineri et al., 2002) and is precipitated by excess androgens (Abbott et al., 2002). Obesity elevates oxidative stress, which contributes to insulin resistance (Urakawa et al., 2003). In PCO there is a shift to android body fat distribution (Sam, 2007). Central adiposity may play a key role in the perpetuation of androgen excess, ovulation and metabolic aberrations (Diamanti-Kandarakis, 2008). Moreover, in PCOs adipose tissue dysfunction has been observed with the over-production of pro-inflammatory adipokines such as TNF $\alpha$  (Chen et al., 2013).

The development of local and systemic oxidative stress in PCO was attributed to androgen excess, insulin resistance, obesity and abdominal adiposity (Murri et al., 2013). Correction of oxidative stress by exercise was suggested to have beneficial effects in PCOS (Wright and Sutherland, 2008). Aerobic exercise independent of weight loss improves insulin sensitivity and ovarian morphology in women with PCOS (Redman et al., 2011). Swimming training for 2 weeks significantly decreased testosterone level, insulin resistance and improved ovarian morphology in PCOS rat model with high-fat diet (Wu et al., 2014).

Much attention has been directed toward the use of transcutaneous electrical muscle stimulation (TEMS) in promoting exercise as it elicits an exercise response without loading the limbs or joints, improves sedentary adult exercise capacity (including cardiovascular fitness and muscle strength subjects) and is well tolerated with good compliance in obese persons (Banerjee et al., 2005). In addition, transcutaneous electrical muscle Stimulation (TEMS) leads to faster and greater weight loss from the fat compartments of the body (Sharma et al., 2011). In experimental studies, muscle contractions elicited by electrical stimulation to lower limb muscle showed a positive effect on insulin sensitivity in condition of fatty liver (EL-Kafoury et al 2011) and in soleus muscle and mesenteric adipose tissue (Johansson et al., 2013). Therefore, it was intriguing to study the ability of transcutaneous electrical stimulation as an alternative to active exercise regime to improve reproductive and metabolic parameters in polycystic ovarian syndrome rat model. Also to explore how insulin sensitivity may be contributed in these changes.

## 2. Materials and Methods:

### *Animals:*

Female white albino rats weighing (160- 210 gm) were purchased from the Vacsera Animal House (Helwan) and housed in the Physiology department animal house (College of Medicine, Ain Shams University, Egypt) under standard condition of

boarding. Regular meals were introduced daily with free access to water.

### **Experimental Protocol:**

At the beginning of the study, rats were weighed then their naso-anal length was recorded to calculate the body mass index (BMI) and the Lee index (Bernardis et al., 1978) (Novelli et al., 2007).

### *Experimental Groups:*

Rats were allocated into three groups;

**1. Group I:** Control rats; which received 0.9% NaCl solution orally, once daily for 21 days, then sacrificed 3 weeks later.

**2. Group II:** Polycystic ovarian syndrome rats (PCO); where experimental polycystic ovarian syndrome was induced by letrozole given in a dose of 1 mg/kg dissolved in 0.9% NaCl solution administered orally, once daily for 21 days (Baravalle et al., 2006), then sacrificed 3 weeks later.

**3. Group III:** Polycystic ovarian syndrome rats subjected to bilateral transcutaneous electrical muscle stimulation (PCO+TEMS) of lower limb muscles for three weeks after induction of polycystic ovary syndrome.

### *Induction of polycystic ovarian syndrome:*

Experimental polycystic ovarian syndrome was induced by the aromatase inhibitor, letrozole (*Femara tablets*, each tab contains 2.5 mg, Novartis Co.), in a dose of 1 mg/kg dissolved in 0.9% NaCl solution administered orally for 21 days (Baravalle et al., 2006). This dose was prepared by dissolving 5mg of letrozole in 10 ml of normal saline (0.9% NaCl). Control rats received equivalent volume of normal saline orally for 21 days.

### *Transcutaneous electrical muscle stimulation:*

In group III (PCO+TEMS), rats were subjected to bilateral transcutaneous electrical stimulation, over the anterior surface of the thigh, to the lower limb quadriceps muscle. Rats were first allowed to adapt to the sensation associating the electric stimulation of the muscle to achieve the physiological effect of exercise with minimal element of stress.

Adaptation was performed one week before treatment whereas the exercise intensity was gradually increased. TEMS duration was increased from 5 minute per session in the first session to 10 minutes per session reaching finally 45 minutes in the fifth session. Then the duration was maintained at that duration (45 minutes per session) during the 3 weeks treatment period.

In addition, mild anesthesia using ether was used in the first 2-3 sessions by using a mask containing cotton pad moistened with ether. The control rats as well were exposed to mild anesthesia only for the same periods as test rats.

Alpha wave healthtronic device (model B.B-1006) was used to produce passive exercise by sending

electrical impulses or signals to the selected muscle or muscle group to contract and relax.

Each device has three pairs of leads; each pair of leads was covered with wet cotton, electrically connected, and applied bilaterally over the thigh to the lower limb quadriceps muscle of fixed rats.

The power knob was turned slowly in a clockwise direction and the leads were moved over the muscle searching for the motor endpoint where better response occurred and rhythmical muscle movement felt and seen (which actually occurred when the power knob was at No<sub>4</sub>). The exercise cycle was repeated automatically once every two seconds, where the frequency was adjusted at intermittent position. The leads were fixed in their places by cotton coated plaster straps (EL-Kafoury et al., 2011).

#### **Determination of phases of Estrous Cycle by vaginal smear:**

The estrous cycle of rats is ideal for investigation of changes occurring during the reproductive cycle. It lasts for four days, and is divided into pro-estrous, estrous, met-estrous and di-estrous phases, which are determined according to cell types observed in the vaginal smear (Marcondes et al., 2002).

At the end of the experimental period, vaginal smear was done every morning by inserting the tip of the cotton of ear swab sticks into the rat vagina. The obtained vaginal material was spread on a glass slide and left to dry for few minutes and, then, unstained native material was examined using the microscope without the aid of the condenser lens. Three types of cells could be recognized (epithelial cells, cornified cells & leukocytes). The proportion among these cells was used for the determination of phases of estrous cycle; A pro-estrous smear consists of a predominance of nucleated epithelial cell; an estrous smear primarily consists of non nucleated cornified cells; a met-estrous (di-estrous-I) smear consists of the same proportion among leukocytes, cornified, epithelial cells and a di-estrous II smear consists mainly of a predominance of leukocytes (Mandle, 1951).

Rats were sacrificed on the day of di-estrous-I, according to Hatsuta et al., (2004), where both progesterone and estradiol could be detected and estimated easily in the blood sample.

#### **Determination of Body Mass Index (BMI):**

BMI was calculated as follows:

BMI =	Body weight (in gm)
	Square length (in cm <sup>2</sup> )

Rats were considered obese when the BMI became above the normal range for adult rats, which is from 0.45 to 0.68 gm / cm<sup>2</sup> (Novelli et al., 2007).

#### **Determination of Lee index:**

It is calculated by dividing the cube root of the body weight (in grams) by the naso-anal length (in millimeters) and multiplying the whole expression by 10000 (Bernardis et al., 1978; Novelli et al., 2007).

$$\text{Lee index} = \sqrt[3]{\frac{\text{Body weight in gram}}{\text{Naso-anal length in mm}}} \times 10000$$

With normal body composition in rats, the Lee index is in the range of 295 to 310; obesity is indicated by higher values (Bernardis et al., 1978).

#### **Experimental procedures**

##### **Blood Sampling**

After 6 weeks from the beginning of the study, the overnight fasted rats were sacrificed. The day of sacrifice was decided to be on the di-estrous-I phase, as shown by the vaginal smear. Fifteen minutes later, the rats were anaesthetized with intra-peritoneal injection of thiopental sodium (Pharco Pharmaceuticals, Egypt), in a dose of 40 mg/kg B.W and final body weight and length were recorded (for calculation of the BMI and the Lee index).

Blood samples were taken from the abdominal aorta and collected in heparinized plastic tube which was then centrifuged at 4000 rpm for 10 minutes to separate plasma. The plasma was then pipetted into clean storage tubes and stored at -20°C for later determination of free testosterone, LH, estradiol, progesterone, prolactin, insulin & catalase.

After taking the blood samples the retroperitoneal fat was excised, washed by saline, dried using filter paper and weighed.

Lastly the left ovary was dissected out, cleaned from fat and fibrous tissue, dried with filter paper and weighed in a precision 5 digit Melter balance (AE = 163), while the right ovary was stored in 10% formalin solution for histological examination.

##### **Biochemical analysis**

##### **Measurement of plasma estradiol, progesterone, prolactin, LH & free testosterone:**

Assays of plasma levels of selected hormones were carried out by enzyme-linked immunosorbent assay "ELISA" techniques using kits supplied by DRG diagnostics. The hormone concentration of each sample was calculated automatically by the analyzer and expressed in mIU/ml for both estradiol & LH (Kosasa, 1981), and in ng/ml for progesterone & prolactin (Filicori et al., 1984) and ng/dl for free testosterone (McCann and Kirkish, 1985).

##### **Determination of plasma insulin:**

Plasma insulin was measured using Immunospec insulin quantitative test kit which is based on an

enzyme-linked immunosorbent assay as described by Eastham (1985).

#### **Determination of plasma glucose:**

It was assayed by enzymatic oxidation according to (Tietz, 1995) using glucose reagent kits (Spectrum Diagnostics) supplied by Egyptian Company for Biotechnology (S.A.E). The glucose level value was converted from mg/dl to mmol/L to be used in calculation of HOMA-insulin resistance score.

#### **Measurement of insulin resistance by HOMA score:**

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma insulin ( $\mu\text{U/mL}$ ) x fasting plasma glucose (mmol/L)/22.5, as described by Matthews et al (1985).

#### **Evaluation of anti-oxidative stress by measuring plasma catalase enzyme activity:**

This was performed using a colorimetric method using kits supplied by Bio-diagnostic, Egypt (Aebi, 1984). Catalase reacts with a known quantity of  $\text{H}_2\text{O}_2$ . The reaction was stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, remaining  $\text{H}_2\text{O}_2$  reacts with 3, 5-dichloro-2hydroxybenzen sulfonic acid (DHBS) and 4 aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. Catalase activity (U/L) was calculated as follows:

$$\frac{^A\text{Standard} - ^A\text{Sample}}{^A\text{Standard}} \times 1000$$

#### **Examination of histopathological changes in the ovarian tissue:**

The ovary was fixed in 10% formalin solution immediately after removal. The specimens were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections, 5  $\mu\text{m}$  thick were cut and stained with Hematoxylin and Eosin and examined by using the light microscope for evaluation of histological changes in the ovarian tissue (Bancroft and Gamble, 2008).

#### **Statistical Analysis:**

Results were expressed as mean  $\pm$  SEM. The statistical significance of differences between means was determined by Student's 't' test for unpaired data at a level of significance  $P < 0.05$ . All Statistical data, statistical significance were analyzed by ANOVA, using SPSS program (Statistical Progression for Social Science) statistical package (SPSS Inc.) version 8.0.1

### **3. Results**

Results encountered in the present study are displayed in tables (1,2), figures (1,2,3) and photos (1,2,3). Results are expressed as Mean  $\pm$  SEM.

#### **Changes in body Weight, body mass index, Lee index, ovarian weight and retroperitoneal fat weight:**

As shown in table (1) and figure (1), compared to the control group the letrozole induced- polycystic ovarian syndrome group {PCO group} exhibited a significant increase in body weight gain%, % of change in body mass index and % of change in Lee index ( $p < 0.001$  for all). Transcutaneous electrical muscle stimulation of PCO rats for 3 weeks significantly decreased body weight gain % ( $p < 0.005$ ), body mass index gain % and Lee index gain ( $p < 0.001$  for both) compared to PCO group.

Moreover, absolute retroperitoneal fat and ovarian tissue weights were significantly higher in PCO groups ( $P < 0.001$  and  $P < 0.05$ ) compared to control group and decreased significantly ( $P < 0.001$  and  $P < 0.05$ ) following transcutaneous electrical muscle stimulation.

#### **Changes in hormonal profile:**

As shown in table (2) and figure (2), compared to control letrozole treatment for 21 days induced polycystic ovarian syndrome evidenced by a significant increase in plasma levels of luteinizing hormone, free testosterone ( $P < 0.005$  for both), with concomitant significant decrease in plasma levels of estradiol ( $P < 0.005$ ) and progesterone ( $P < 0.01$ ). Transcutaneous electrical muscle stimulation of PCO rats for 3 weeks lead to a significant decrease in plasma levels of luteinizing hormone, free testosterone with concomitant significant increase in plasma levels of estradiol and progesterone ( $P < 0.05$  for all) compared to PCO group.

Prolactin hormone level did not show any significant difference between three groups.

#### **Insulin resistance and oxidative stress markers:**

As shown in table (2) and figure (3), Insulin hormone, glucose levels and HOMA-insulin resistance score were significantly increased in PCO group compared to control group ( $P < 0.05$ ), ( $P < 0.005$ ) and ( $P < 0.001$ ) respectively. Meanwhile, they decreased significantly following transcutaneous electrical muscle stimulation treatment ( $P < 0.05$ ), ( $P < 0.01$ ) and ( $P < 0.001$ ). Concerning plasma catalase activity, the anti-oxidant enzyme, it showed a significant decrease in PCO rats compared to control rats ( $P < 0.05$ ) while a non-significant increase was encountered following transcutaneous electrical muscle stimulation compared to PCO group.

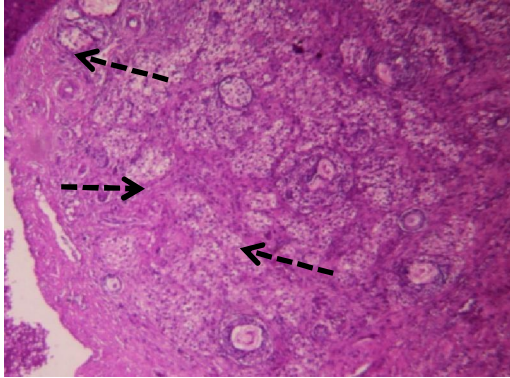
#### **Results of histopathological examination of ovaries:**

As shown in photo (1) the ovarian tissue in control rats showed different normal ovarian follicles (dashed arrows), normal stroma and corpus luteum formation (thick arrow). However, the ovary of letrozole induced PCOS rats showed atretic ovarian follicles, ovarian cysts abundance (C) with a

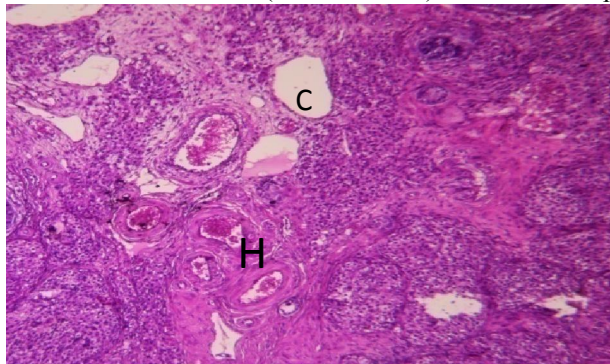
vascularized granulosa cell wall (H) and absence of corpus luteum (photo 2).

Following transcutaneous electrical muscle stimulation reappearance of developed antral follicle

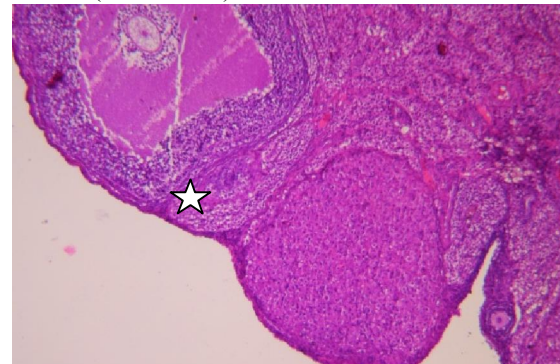
with oocyte surrounded by granulosa cells (star) was detected together with corpus luteum presence (photo3), with a structure relatively near to normal.



**Photo (1):** Ovarian sections from control group rats showing no histopathological alterations, with different normal ovarian follicles (dashed arrows) and normal corpus luteum (thick arrow).



**Photo (2):** Ovarian sections from letrozole induced PCO rats showing atretic follicles with cyst formation (C), interstitial hemorrhage (H) with absence of corpus luteum. (H & E X 100)



**Photo (3):** Ovarian sections from transcutaneous electric muscle stimulation PCO treated rats showing no histopathological changes and normal ovarian follicle cells with reappearance of corpus luteum (star). (H & E X 100)

**Table (1): The changes in body weight (gm), body mass index (gm/cm<sup>2</sup>), Lee index, retroperitoneal fat weight (gm) and ovarian weight (mg) in the three studied groups.**

	Control group	Poly-cystic ovarian syndrome group(PCO)	TEMS treated PCO group
	N=6	N=6	N=6
Initial body weight (gm)	188.3±6.28	182.5±4.23	186.6±6.4
Final body weight (gm)	227.5±8.3*	250.0±7.18 <sup>a</sup>	230.8±5.97*
%change in body weight	20.78±1.7	37.08±3.36 <sup>a</sup>	23.96±2.61 <sup>b</sup>
Initial BMI(gm/cm <sup>2</sup> )	0.56±0.02	0.5±0.035	0.56±0.02
Final BMI(gm/cm <sup>2</sup> )	0.60±0.02*	0.63±0.04*	0.61±0.02*
% of change in BMI	9.5±0.99	23.79±2.56 <sup>a</sup>	11.09±0.91 <sup>b</sup>
Initial Lee index	311.48±5.51	298.1±9.1	311.89±4.57
Final Lee index	315.9±5.03*	315.8±9.6*	317.38±4.06*
% of change in Lee index	1.44±0.29	5.95±0.54 <sup>a</sup>	1.77±0.26 <sup>b</sup>
Retroperitoneal fat(gm)	2.23±0.23	5.7±0.69 <sup>a</sup>	2.75±0.33 <sup>b</sup>
Left ovarian weight(mg)	87.33±15.39	137.8±16.56 <sup>a</sup>	89.9±8.51 <sup>b</sup>

All values are expressed as mean ± SEM

\*: Significance from respective initial value by Student's "t" test for paired data.

a: Significance from the control group by Student's "t" test for unpaired data.

b: Significance from the polycystic ovarian syndrome group by Student's "t" test for unpaired data.

N: Number of rats.

**Table (2): Levels of plasma LH (mIU/ml), free testosterone (ng/dl), estradiol {E<sub>2</sub>} (mIU/ml), progesterone (ng/ml), prolactin (ng/ml), insulin (ng/ml), glucose (mg/dl), HOMA-IR score and plasma catalase enzyme (U/L) in the three studied groups.**

	Control group N=6	Poly-cystic ovarian syndrome group (PCO) N=6	TEMS treated group N=6
<b>LH (mIU/ml)</b>	9.59±0.43	13.11±1.15 <sup>a</sup>	8.08±0.37 <sup>b</sup>
<b>Free testosterone (ng/dl)</b>	2.94±0.07	3.15±0.01 <sup>a</sup>	2.99±0.02 <sup>b</sup>
<b>Estradiol {E<sub>2</sub>} (mIU/ml)</b>	5.28±0.83	2.16±0.46 <sup>a</sup>	4.18±0.55 <sup>b</sup>
<b>Progesterone (ng/ml)</b>	29.31±2.16	19.49±2.66 <sup>a</sup>	26.20±1.52 <sup>b</sup>
<b>Prolactin (ng/ml)</b>	0.78±0.15	0.66±0.05	0.87±0.1
<b>Insulin (ng/ml).</b>	1.83±0.29	3.02±0.5 <sup>a</sup>	1.61±0.19 <sup>b</sup>
<b>Glucose (mg/dl)</b>	80.33±2.57	101.6±7.19 <sup>a</sup>	81.8±1.9 <sup>b</sup>
<b>HOMA-IR score</b>	7.93±1.1	16.01±1.9 <sup>a</sup>	7.27±1.0 <sup>b</sup>
<b>Catalase (U/L)</b>	0.41±0.19	0.03±0.01 <sup>a</sup>	0.19±0.05

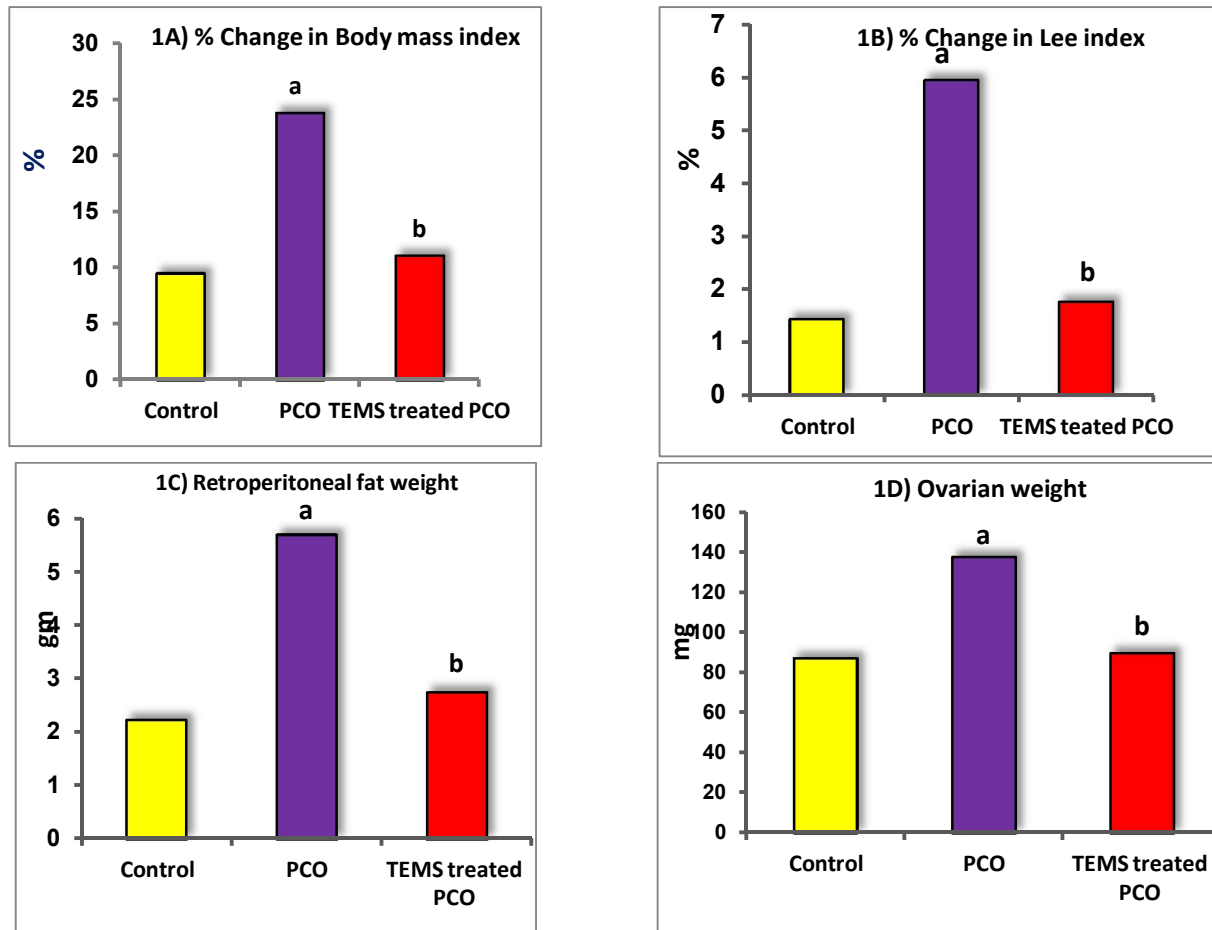
All values are expressed as mean ± SEM

\*: Significance from respective initial value by Student's "t" test for paired data.

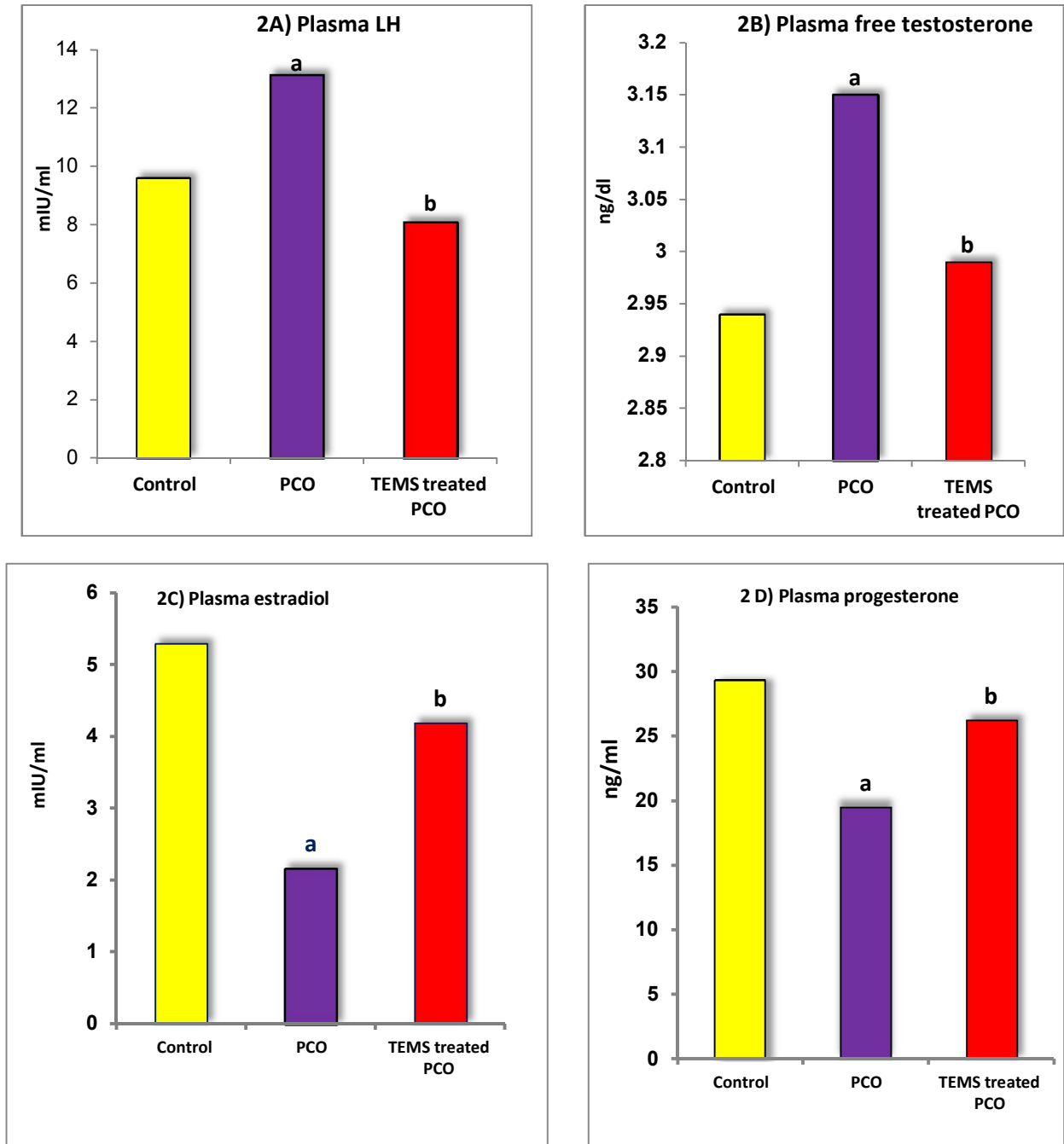
a: Significance from the control group by Student's "t" test for unpaired data.

b: Significance from the polycystic ovarian syndrome group by Student's "t" test for unpaired data.

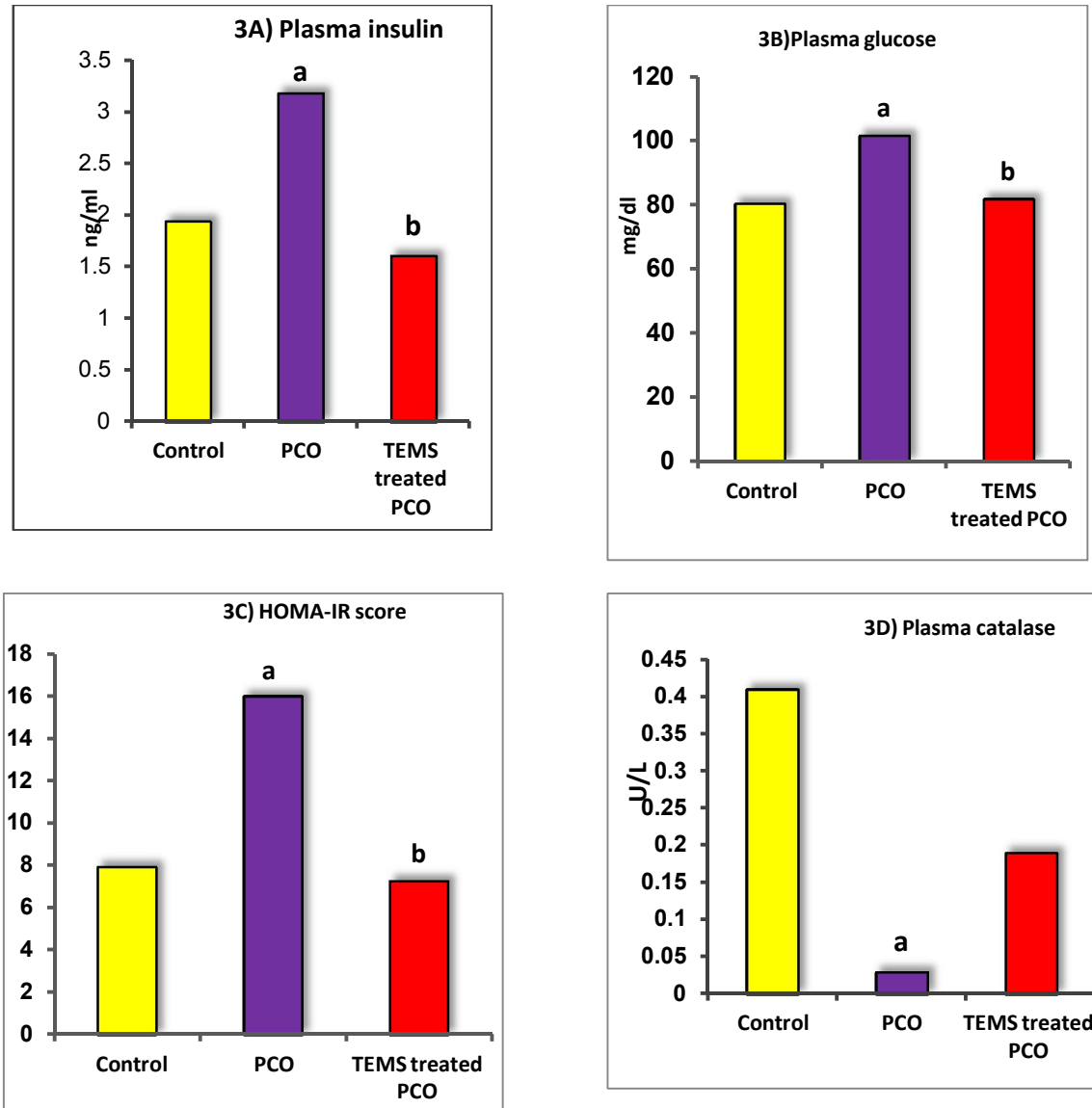
N: Number of rats.



**Figure (1): A) % change in body mass index, B) % change in Lee index, C) retroperitoneal fat weight (gm) and D) ovarian weight (mg) in the control group, polycystic ovarian syndrome group (PCO) and transcutaneous electrical muscle stimulation treated group (TEMS treated PCO). a: Significance from the control group by Student's "t" test for unpaired data. b: Significance from the polycystic ovarian syndrome group by Student's "t" test for unpaired data.**



**Figure (2):** Levels of plasma **A)** LH (mIU/ml), **B)** Free testosterone (ng/dl), **C)** Estradiol {E<sub>2</sub>} (mIU/ml) and **D)** Progesterone (ng/ml) in the control group, polycystic ovarian syndrome group (PCO) and transcutaneous electrical muscle stimulation treated group (TEMS treated PCO). **a:** Significance from the control group by Student's "t" test for unpaired data. **b:** Significance from the polycystic ovarian syndrome group by Student's "t" test for unpaired data.



**Figure (3):** Levels of plasma **A)** Insulin (ng/ml), **B)** Glucose (mg/dl), **C)** HOMA-IR score, **D)** Catalase enzyme (U/L) in the control group, polycystic ovarian syndrome group (PCO) and transcutaneous electrical muscle stimulation treated group (TEMS treated PCO). **a:** Significance from the control group by Student's "t" test for unpaired data. **b:** Significance from the polycystic ovarian syndrome group by Student's "t" test for unpaired data.

#### 4. Discussion:

The results of the present work elucidate the effects of 3 weeks transcutaneous electric muscle stimulation on the body weight, hormonal profile, oxidant state and insulin resistance in a letrozole induced polycystic ovarian syndrome rat model.

The polycystic ovarian syndrome induction in this study was confirmed by significant elevation in plasma free testosterone, luteinizing hormone levels with a significant decrease in plasma estrogen and progesterone levels as described by other studies

(Miri et al., 2014). Moreover, PCO is confirmed by the increased weight gain and ovarian structural changes in the form of increased ovarian weight, ovarian cysts abundance, high incidence of atretic follicles, diminished granulosa layer thickness and absence of corpora luteum. Similar hormonal and histological changes were detected after using letrozole to induce PCO (Baravalle et al., 2006; Jadhav et al., 2013 and Radha et al., 2014).

Polycystic ovarian syndrome model was induced by letrozole, a non-steroidal aromatase inhibitor that



blocks the conversion of androgens to estrogen, increasing androgen level and reducing estrogen level (Kafaliet al., 2004; Baravalle et al., 2006). In patients with PCO, hyperandrogenemia could be explained either by enhanced theca cells 17 $\alpha$ -hydroxylase enzyme gene activity (Diamanti-Kandarakis E., 2008) and their augmented sensibility to LH stimulation (Blank et al., 2006) or by elevated LH pulse frequency and amplitude which enhance theca androgen synthesis due to impaired negative feedback on gonadotropins (Jonard and Dewailly, 2004).

Rojas et al. (2014) claimed that hyperandrogenemia is the main culprit of the clinical picture in PCO syndrome. Androgen excess hinders gonadotropin-induced estrogen and progesterone synthesis in the PCOS follicle (Foong et al., 2006) and impairs folliculogenesis (Jonard and Dewailly, 2004) leading to anovulation (Diamanti-Kandarakis, 2008).

Also, letrozole treated rats showed insulin resistance indicated by elevated insulin and glucose levels as well as high HOMA-insulin resistance score. In addition, the significant depression in the antioxidant catalase activity in these rats suggests a state of oxidative stress associated with the PCO. These results were consistent with other studies which linked insulin resistance and oxidative stress to this syndrome Lee et al. (2010) and Murri et al. (2013). The observed insulin resistance and oxidative stress in letrozole induced PCO rats could be explained by the increased androgen level. Testosterone seemed to interfere with insulin signaling in peripheral tissues and to precipitate insulin resistance (Diamanti-Kandarakis, 2008). In return, insulin resistance precipitates oxidative stress (Verit and Erel, 2008). Oxidative stress can induce both insulin resistance and hyperandrogenism in PCO patients (Desai et al., 2014).

In this study, the significantly increased body mass index, Lee index and retroperitoneal fat weight, all point to the associated abdominal obesity in PCO. Chen et al. (2013) confirmed the pivotal role of abdominal obesity in the pathogenesis of PCOS as well as the associated insulin resistance and hyperandrogenemia. Androgen was stated to drive pro-inflammatory cytokines secretion as tumor necrosis factor alpha (TNF- $\alpha$ ) from visceral adipose tissue in PCO which stimulates LH secretion promoting further androgen excess and inducing hyperinsulinemia and oxidative stress (Murri et al., 2013 and Rojas et al., 2014).

The presence of the hormonal and structural changes after letrozole treatment continued till the end of the study (6 weeks) despite stoppage of letrozole

administration in the last 3 weeks. This indicates that these changes did not return to normal spontaneously.

Transcutaneous electric muscle stimulation (TEMS) was used for three weeks following PCO induction to examine its efficacy as a treating procedure in PCO. TEMS succeeded to improve the hormonal profile of the PCO rats, indicated by decrease in plasma free testosterone, luteinizing hormone levels with a significant increase in plasma estrogen and progesterone levels. In addition, significant improvement in insulin sensitivity was ensued evidenced by decreased insulin levels and HOMA-IR score with positive effects on oxidative state whereas antioxidant catalase activity was increased though not reaching the matching values in the control group. This was accompanied by a restoration of the normal better ovarian follicles morphology and the reappearance of corpus luteum.

According to Mannerås-Holm (2010) exercise interventions can break this vicious circle of insulin resistance, androgen excess and ovarian dysfunction in PCOS. But because of the exhausting physical activity, the benefits of electrical stimulation became apparent, as it provides a safe, fast and effective method for exercising (Vrbova, et al., 2008). Electric muscle stimulation elicits rapid, rhythmical muscle contractions and can induce physiological responses consistent with physical exercise and increase oxygen demand in the lower limb muscles with higher levels of activity over time than any exercise regime due to the elimination of limiting effects by the central nervous and cardiovascular systems (Banerjee et al., 2005; Caulfield et al., 2004).

The beneficial effect of trans-cutaneous electric muscle stimulation (TEMS) in PCO noticed in this study could be possibly explained by the reduced body weight or by the decreased insulin resistance detected in our results. These findings were similar to EL-Kafoury et al. (2011) results where electrical muscle stimulation induced exercise was able to improve insulin resistance partially and reduced retroperitoneal body fat. This was ascertained by Watanabe et al (2012), who concluded that daily application of transcutaneous TEMS can improve the glucose tolerance and insulin sensitivity in rats.

Exercise has the ability to stimulate glucose transporter-4 (GLUT4) translocation, glucose uptake and improve insulin sensitivity (Deshmukh et al., 2008) and it also, reduces adiposity and adipocyte size (Bernick et al., 2013) which appears to ameliorate insulin resistance in PCOS (Diamanti-Kandarakis, 2008). Also, exercise lowers leptin levels and restores altered adipose tissue gene expression related to insulin resistance which can improve cyclicity (Bernick et al, 2013).

Also in this study, PCO hormonal profile was improved three weeks following the treatment as evidenced by lower plasma testosterone and LH levels and higher plasma estrogen and progesterone levels. This could be attributed to the exercise ability to regulate LH secretion, reduce hyperandrogenemia and restore normal ovarian morphology, ovulation and reproductive function (**Jedel et al., 2011; Qiu et al., 2009 and Harrison et al., 2011**).

Moreover, transcutaneous electrical muscle stimulation was able to improve the oxidant state similar to **Debevec et al. (2014)** assumption that moderate daily exercise training can attenuate the oxidative stress, which can add another strategy in PCO management as assumed by **Rzeczynska et al. (2011)**.

Concerning prolactin hormone, it does not show any significant difference between groups, this agrees with **Szosland et al (2015)** who stated that polycystic ovary syndrome is not associated with higher levels of prolactin. **Sheehan (2004)** reported that although PCOS and hyperprolactinemia are independent disorders, but hyperprolactinemia is considered an important differential diagnosis causing secondary amenorrhea, so it must be excluded.

Thus, we can conclude from the present study that, transcutaneous electric muscle stimulation, a passive form of exercise, can be included as a treatment modality in PCO management based on its ability to reduce body weight and hyperandrogenemia, and to alleviate insulin resistance and oxidative stress.

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#### Corresponding author:

Dr. Manal S. Abd-El Hamid  
Address: Department of Physiology, Faculty of Medicine, Ain Shams University  
Email: [manalsaidsun@yahoo.com](mailto:manalsaidsun@yahoo.com)

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