EFFECT OF INSULIN RESISTANCE ON THE OCCURRENCE OF PREGNANCY IN WOMEN TREATED EMPIRICALLY FOR UNEXPLAINED INFERTILITY

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Abstract

Context. The detrimental effects of hyperinsulinemia on human ovaries during follicular development process have been shown in various studies, particularly in women with polycystic ovary syndrome. To our knowledge there is no study regarding the effect of insulin resistance (IR) on the intrauterine insemination (IUI) success in women with unexplained infertility (UEI).

Objective. To evaluate the effects of IR on the occurrence of pregnancy among infertile women undergoing ovulation induction (OI) with IUI for UEI.

Design. Prospective cohort study.

Subjects and Methods. 173 patients who met the criteria for UEI and under the age of 35 were included in this study. All women underwent OI with IUI and, subsequently, they were divided into two groups based on the presence of pregnancy. Data reviewed for analysis were demographic, clinical, and laboratory features.

Results. There were 37 (21.4%) cycles with pregnancy and 136 (78.6%) cycles without pregnancy. Median infertility duration was significantly lower in the pregnant group when compared with non-pregnants ($p=0.018$). The mean number of previous cycles per patient was also lower in this group ($p=0.028$). No significant differences were found between the groups in terms of other variables. Levels of insulin, fasting glucose and HOMA-IR were also similar between the two groups.

Conclusions. IR calculated by using HOMA-IR index has no positive or negative effect on the occurrence of pregnancy in women undergoing OI therapy with IUI for UEI.

Key words: HOMA-IR, intrauterine insemination, unexplained infertility, ovulation induction, pregnancy.

INTRODUCTION

Infertility continues to be an important problem for some couples. Nowadays, women get educated

for a longer time, take a more active role in business life, get married in older ages and become pregnant. Unfortunately, ovocyte quality and quantity decreases as the woman's age increases. Obesity, infections, systemic diseases, drugs, smoking and alcohol use can also reduce fertility in both women and men (1). Therefore, more couples seek adjunct treatments to achieve pregnancy such as ovulation induction (OI), intrauterine insemination (IUI), or *in vitro* fertilization (IVF).

Infertility has many underlying causes that we know or do not know. Unexplained infertility (UEI) represents a large group of infertile couples in which the mechanism that causes infertility is not known or not explained with available methods. The incidence of UEI ranges between 10%-30% among infertile couples, depending on diagnostic criteria used or population studied (2). It is a diagnosis of exclusion. At a minimum, the diagnosis of UEI implies evidence of normal sperm parameters, ovulatory function, a normal uterine cavity, and bilateral tubal patency (3). The treatments recommended today for UEI are as follows; IUI, controlled ovarian hyperstimulation (COH) with clomiphene citrate (CC) or gonadotropins (Gn), COH with IUI, and IVF. COH with IUI is one of the first choice treatments (4). Although IUI treatment is a widely applied method, literature data on pregnancy success significantly differ. Very low (4%) and very high (40%) pregnancy rates are reported (5, 6).

Insulin resistance (IR) is a common condition seen in all societies and is defined as the lack of necessary insulin response to a certain amount of glucose. IR can also be defined as any effect reduction that can occur in those stages from the release of insulin-producing pancreatic beta-cells to the anticipated effects on target cells (7). Metabolically, IR can be considered as reduced effect of insulin on metabolic events or

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reduced normal sensitivity to insulin at cellular level (8). IR is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population (9). In 1980, Burghen *et al.* (10) firstly reported an association of polycystic ovary syndrome (PCOS) with hyperinsulinism. Hyperinsulinemia and IR are encountered in 30% of women with non-obese PCOS and in 75% of women with obese PCOS (11). The etiology of IR in PCOS has not been fully elucidated, besides IR; hyperinsulinemia is associated with increased steroid production in the theca cells and release of the luteinizing hormone (LH) in patients with PCOS. Insulin increases the effect of LH on preovulatory follicles and this leads to premature luteinization and premature follicle death (11, 12).

Homeostatic model evaluation, which was first described in 1985, is one of the IR measuring methods and it detects β -cell function (13). Homeostasis model assessment (HOMA) is a test that allows the practical examination of large patient populations calculated by using blood glucose and insulin levels. We were inspired by the studies in literature which investigated the relationship between IR and follicle development. We aimed to investigate whether there is an effect of IR on the occurrence of pregnancy among infertile women undergoing OI with IUI for UEI or not.

MATERIALS AND METHODS

The study was carried out between July 2014 and January 2015 in the infertility outpatient clinic of the Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara with 173 patients admitted for infertility treatment. Institutional review board approved the study and written informed consent was taken from each woman. 173 cycles of 173 different patients induced by CC or Gn were followed up during the study period.

Age, gravida, parity, history of miscarriage, number of alive children, type and duration of infertility of patients were recorded. Women who had previously no pregnancy were regarded as primary infertile; if pregnancy occurs at least once it was considered as secondary infertility. Current diseases and past operations were questioned. All of the patients' general and gynecological examinations were performed after the initial evaluation, and their vital signs were recorded. Height and weight of the patients were measured using a professional calibrated device on an empty stomach in the morning. Body mass index (BMI) was calculated

as weight divided by height squared.

The routine infertility workup including baseline hormone levels [follicle stimulating hormone (FSH), LH, estradiol (E2), thyroid stimulating hormone, prolaktin (PRL)] on cycle day 2-3, ovarian antral follicle count and spermiogram were run and also noted for each couple. All patients underwent hysterosalpingography and both tubes showed patency. CC or recombinant FSH was used for controlled ovarian stimulation. Venous blood was taken into two biochemical tubes between 8:00 a.m. and 10:00 a.m. on IUI day following 8-12 hours fasting from the each patient. All OI cycles were followed by folliculometry with Logiq ProSeries 200 ultrasound device equipped with 6.5 MHz transvaginal probe. Follicle diameters were measured on two planes and their mean diameters were taken. At the same time endometrial thickness was also measured.

Serum levels of FSH, LH, E2, PRL, insulin and TSH were measured with UnicelDxI 800 Immunoassay System (Beckman Coulter, Fullerton, CA, USA). The serum level of glucose was determined with the use of AU680 Chemistry System (Beckman Coulter, Fullerton, CA, USA). IR was diagnosed with the Homeostasis Model Assessment (HOMA)-IR index, which is calculated by multiplying the insulin value (mU/mL) by the glucose value (mg/dL), and dividing the product by 405, with a cut-off point of 2.5.

Patient selection

Inclusion criteria

Inability to achieve pregnancy despite the regular and unprotected sexual intercourse for at least 12 months and:

1. The woman is between the ages of 18-35.
2. Having regular menstrual cycles.
3. FSH level on cycle day 2-3 is less than 12 IU/L.
4. Midluteal progesterone is $> 5\text{ng/mL}$.
5. HSG showing bilateral tubal patency.
6. Normal semen analysis according to the WHO 2010 criteria.
7. Primary or secondary infertility treated with COH (either CC or Gn).

Patients who meet the above criteria were regarded as having UEI.

Exclusion criteria

1. The woman age under the age of 18 or over the age of 35 years.
2. Having any endocrine disorders (PCOS, thyroid dysfunction, diabetes mellitus, hyperprolactinemia, hypogonadism).

3. Having other systemic medical conditions (life threatening conditions for mother, e.g., cardiovascular diseases, uncontrolled diabetes, chronic renal diseases).

4. Being under medical treatment which may interfere with glucose and insulin metabolism such as corticosteroid, metformin, or beta blocker use.

5. Oligo-amenorrhea.

6. Endometriosis.

7. Previous pelvic surgery, radiotherapy and chemotherapy.

8. Diminished ovarian reserve and family history of POI.

9. Those who have contraindicated hormone therapy or are allergic.

10. Oligoastoteratospermic male partner.

11. Patients without ovulation.

12. Smoking.

Ovulation induction protocol

The CC protocols were initiated on the 3-5th day of the cycle after application of basal sonography of ovaries showing no cysts retained from the previous cycle. The doses were determined based on the patient characteristics such as BMI and previous cycle features. Typically, a CC 50 mg tablet was prescribed orally after the initial examination, and the dose was increased up to 150 mg according to the ovarian response. CC (Klomen®, Kocak Farma, Istanbul, Turkey) was applied for 5 days. Serial ultrasound examinations were performed during ovarian induction. When at least one follicle reached a maximum diameter of 17-18 mm, 10000 IU of human chorionic gonadotropin (hCG) (Pregnyl®, Organon, Istanbul, Turkey) were administered intramuscularly. IUI procedure was performed after 24-36 hours hCG triggers. The Gn (Puregon®, Organon, Istanbul, Turkey) protocols were initiated on the 3rd day of the cycle after basal sonography of ovaries. The dosage of the drug was individualized according to the response of patient and/or the data from the previous cycles. The hCG (Pregnyl®, Organon, Istanbul, Turkey) with a dose of 10.000 IU was applied for the final maturation of oocyte, if a follicle of 17-18 mm or larger was detected and the IUI was planned for 34-36 hours later. Patients got rested for 30 minutes and sexual intercourse was suggested every two days after IUI. Crinone vaginal progesterone gel for luteal support was prescribed to all women.

Sperm preparation methods

Semen samples were collected from the

patients by masturbation in a private room nearby the laboratory. After liquefaction for 20 minutes at the vacuum furnace, the collected semen specimens (pre-washed) were assessed for conventional semen parameters including sperm concentration and sperm motility by the computer-assisted semen analyzer. The rest of the semen was processed using standard swim up method with a sperm preparation medium (SpermRinse Solution, Vitrolife, Gothenburg, Sweden). The swim-up process which is a method of floating of progressive motility sperm in the medium started with the placement of semen sample in a falcon conical centrifuge tube. Media and sperm were mixed at a ratio of 1/1. After pipetting, the sample was centrifuged at 1200-1500 rpm for 10 minutes. The supernatant was removed using a pipette. After the supernatant was dumped, 1 mL of the same medium was added and the same cycle was repeated for 5 minutes. The tube was placed at an angle of 45 degrees and 0.5-1 mL of medium was added onto the pellet. This was incubated for 1 hour at 37°C without deterioration of the angle. A small amount was taken from the supernatant, and post wash analysis was again performed by the computer-assisted semen analyzer and recorded. Finally, the sperm solution was withdrawn from the tube surface with a pipette to aid in totaling 0.5-1 mL of the tube without distortion of the angle, and it was transferred in to falcon 5 mL tube to be used for IUI. Sperm analysis was performed by the same andrology laboratory technician according to a quality control program at the andrology laboratory of the current hospital. Sperm analysis was assessed according to World Health Organization guidelines (14).

Evaluation of pregnancy

The patients who had no signs of menstruation were called for pregnancy test on the 14th day after IUI women and the pregnancy was evaluated by examining serum levels of β -hCG hormone. Patients with β -hCG >10 mIU/ mL were accepted pregnant. In patients with positive pregnancy test, the increase in β -hCG was evaluated after 2 days. Patients who had an increase in β -hCG rather than normal doubling in serial measurements (non viable pregnancy), whose β -hCG value is decreasing while their vaginal bleeding starts or those with pregnancy of unknown location (no visible intrauterine or extrauterine pregnancy on ultrasound) at the 6th weeks of gestation were accepted as biochemical pregnancy. Clinical pregnancy was diagnosed 5 weeks after the IUI by evidence of fetal heart activity.

Statistical analysis

Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were used to demonstrate the mean $\pm$ standard deviation and median (range) for constant variables, whereas nominal variables were expressed as case number and percentages. As to the analysis of the data, normality in the repeated measures was tested with Kolmogorov-Smirnov test. To determine the differences between the groups, parametric (Independent samples t test) and non-parametric analyses (Mann-Whitney U) were performed after normality analysis. Nominal variables were analyzed with a Pearson chi-square test. The Receiver Operator Characteristics (ROC) curve analysis was used to find out discriminative factors between the two groups. Spearman correlation analysis was used to determine the relationship between HOMA-IR and other variables. The data were analyzed at 95% confidence level and $p < 0.05$ was accepted as meaningful.

RESULTS

One hundred seventy-three consecutive women with UEI who met the inclusion criteria were enrolled in this prospectively designed study. Pregnancy was achieved in 21.4% (n: 37) of the patients, whereas pregnancy was not achieved in 78.6% (n: 136) after COH with IUI treatment. Thirty-seven pregnancies were detected, of which 30 were clinical and 7 were biochemical. Pregnant and non-pregnant patients were

individually compared. Demographics and laboratory features of the patients were depicted in Table 1. There were no significant differences between the two groups in terms of age, BMI, obstetric characteristics and infertility type. The median infertility duration was statistically significantly higher in the non-pregnant group than in the pregnant group ($p: 0.018$). All but one hormone levels were similar among the groups. Basal LH value was 6.0 ± 3.1 mIU/mL in non-pregnant women and 7.4 ± 4.3 mIU/mL in pregnant women, and this difference was statistically significant ($p: 0.039$).

CC was used as an induction agent in 127 cases (73.41%) and Gn was used in 46 cases (26.59%) of 173 OI cycles applied to 173 cases. No significant difference was observed between the groups in terms of treatment agent used. When we evaluated previous cycles of the patients, it was noted that more induction cycles were applied to the non-pregnant group than to the pregnant group ($p: 0.028$). Previous and current cycle characteristics were shown in Table 2.

Mean fasting blood glucose level was similar between the 2 groups. Also, fasting insulin levels were similar among the groups. No significant difference was observed between the pregnant and non-pregnant groups with regard to mean HOMA-IR levels (2.2 ± 1.7 vs. 1.8 ± 1.7 , $p: 0.186$). When the cut-off value of HOMA-IR index for IR was taken as 2.5, IR was detected in 37.8% of the pregnant group and 33.8% of the non-pregnant group ($p > 0.05$). The mean BMI of the patients was 25.0 ± 4.5 in patients with HOMA-IR < 2.5 and 28.0 ± 4.5 in patients with HOMA-IR ≥ 2.5 ($p = 0.004$). Conversely, mean FSH value of patients

Table 1. Demographics and baseline hormone levels of the patients

Variables	Pregnant (n:37)	Non-pregnant (n:136)	P value
Age (years)	25.9 $\pm$ 5.0	26.5 $\pm$ 4.6	0.506
BMI (kg/m ²)	26.1 $\pm$ 3.4	25.9 $\pm$ 4.9	0.839
Gravida	0(0–4)	0(0–4)	0.374
Parity	0(0–2)	0(0–2)	0.274
Live children	0(0–1)	0(0–2)	0.916
Abortion	0(0–4)	0(0–4)	0.081
Infertility duration (years)	2.5(1–7)	3(1–15)	0.018
Infertility type n (%)			
Primary	27(75)	106(77.9)	0.431
Secondary	9 (25)	30 (22.1)	
Basal FSH (mIU/mL)	6.5 $\pm$ 1.4	6.8 $\pm$ 1.7	0.445
Basal LH (mIU/mL)	7.4 $\pm$ 4.3	6.0 $\pm$ 3.1	0.039
Basal E2 (pg/mL)	45.9 $\pm$ 33.2	42.3 $\pm$ 16.5	0.357
TSH (mU/L)	2.2 $\pm$ 1.0	2.2 $\pm$ 1.0	0.937
PRL (ng/mL)	12.8 $\pm$ 4.7	13.1 $\pm$ 6.1	0.766

BMI: body mass index, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, E2: Estradiol, TSH: Thyroid Stimulating Hormone, PRL: Prolactin. $P < 0.05$ is considered statistically significant.

Table 2. Comparison of markers of glucose metabolism between the two groups

Variables	Pregnant (n:37)	Non-pregnant (n:136)	P value
Insulin ($\mu\text{U/mL}$)	9.0 $\pm$ 6.7	8.5 $\pm$ 7.4	0.209
Glucose (mg/dL)	91.7 $\pm$ 9.5	90.7 $\pm$ 14.2	0.571
HOMA-IR index	2.2 $\pm$ 1.7	1.8 $\pm$ 1.7	0.186
IR n(%) (HOMA $\geq$ 2.5)	14(37.8)	46(33.8)	0.649

HOMA: Homeostasis model assessment, IR: insulin resistance.

Table 3. Cycle characteristics of the subjects

Variables	Pregnant (n:37)	Non-pregnant (n:136)	P value
Follicle size (mm)	18.6 $\pm$ 2.41	18.26 $\pm$ 1.59	0.561
Number of previous cycles	1.9 $\pm$ 1.0	2.4 $\pm$ 2.0	0.028
r-FSH dose (IU)	75(50-150)	75(37.5-150)	0.570
CC dose (mg)	50(50-150)	50(50-150)	0.482
Current treatment n(%)			
CC	29(78.4)	98(72.1)	0.440
r-FSH	8(21.6)	38(27.9)	

r-FSH: recombinant follicle stimulating hormone, CC: Clomiphene Citrate. $P < 0.05$ is considered statistically significant.

with HOMA-IR < 2.5 were significantly higher than the patients with IR (7.0 $\pm$ 1.8 vs. 6.4 $\pm$ 1.40; p : 0.032). Similar to FSH values, mean LH values were higher in patients without IR (HOMA-IR < 2.5) than in those with IR (p : 0.042). In the ROC curve analysis, HOMA-IR index was not found to be a significant parameter in predicting pregnancy. Only basal LH levels and previous cycle number were found to be discriminative factors between the two groups.

A statistically significant inverse correlation was found between HOMA-IR and age and FSH values in UEI patients (r : -0.151, p : 0.047 and r : -0.214, p : 0.005). However, HOMA-IR was positively correlated with BMI (r =0.481, p <0.001).

DISCUSSION

Despite the adequate investigation, UEI is defined as infertility in which an underlying cause is not found in women under the age of 35 after at least one year regular and unprotected intercourse. This period should be limited for 6 months in women over the age of 35 (15). Approximately 15% of infertility is attributed to unexplained factors. Due to the lack of a correctable pathology, the treatment method of patients with UEI was OI with or without IUI that empirically performed, and IVF (16). IUI is one of the most commonly used methods of infertility treatment. IUI, commonly combined with COH, is considered one of the first treatment options in subfertile patients with UEI and who have low monthly fecundability rates but are likely to have spontaneous conception.

The predictors of IUI success have been the subject of many studies. In this study, we intended to evaluate the effects of IR on the occurrence of pregnancy among infertile women undergoing OI with IUI for UEI. As far as we know, this is the first study evaluating the effect of IR on the IUI success in UEI patients.

The pregnancy rate was found to be 21.4% in this cohort. Demographics such as age, BMI, and obstetrical characteristics were similar in both groups. Thus, the effect of those variables on treatment outcome was minimized. Patients older than 35 years who are known to have low pregnancy rates in treatment cycles were also not included in the study. In addition, cycle monitorization, drug usage, sperm preparation methods, IUI timing, and IUI techniques were performed by similar methods.

Obesity is associated with limited ovulation, leading to limited fertilization and menstrual disturbance. This also causes insufficient implantation (17). Some studies report that overweight and obese subfertile women have reduced fertility treatment success, and their pregnancies are related to more complication (17, 18). Other studies have reported that weight loss helps to improve ovulation progress and infertility treatment outcomes (19, 20). One study showed that less follicular development occurred in obese women and higher dose medication in treatment was required (21). Moreover, IR is further increased in case of obesity. Short term weight loss (4-6 months) reduces IR, serum insulin, abdominal fat accumulation, and androgens, and would contribute positively to the lipid profile. We observed that the two groups were

similar in terms of BMI. We also sought to clarify whether IR is independent of BMI in UEI cases. There are many reports in the literature that BMI positively correlates with HOMA-IR (22, 23). Likewise, we found a significant positive correlation between BMI and HOMA-IR in women with UEI. Studies on infertility and IR are mostly related to PCOS patients (24). It is claimed that IR plays an important role in the pathogenesis of PCOS and may be obesity independent, although more pronounced in obesity (25).

One of the factors affecting success rates in infertile women undergoing OI with IUI for UEI is infertility duration. As the duration of infertility increases, success rates as well as pregnancy rates decrease per cycle. Makkar *et al.* evaluated 600 COH and IUI cycles to determine the prognostic factors associated with successful outcome. They reported that median duration of infertility was shorter in pregnant cycles than in non pregnant cycles (3 years vs. 4 years) (26). Another study analyzed 407 women undergoing 1010 cycles of IUI. The pregnancy rates was found to be 10.6% when infertility duration was <3 years and, it was found as 7.1% when infertility duration was ≥3 years (27). The following studies have found similar results (28), just as in ours.

There are different results in the literature on the effect of infertility type on the success of COH with IUI. Kamath *et al.* (28) reported that pregnancy rates in primary infertile patients were 8.9%, while those in secondary infertile patients were 12.6%. This difference was not statistically significant. Unlike that study, another group of researchers found that the pregnancy rate in the secondary infertile group (21.4%) was significantly higher than in the primary infertile group (7.9%) (29). Although our study includes more primary infertile patients, our pregnancy rates were higher than the others and there was no significant difference in the type of infertility among the 2 groups.

The FSH value measured on the cycle day 3 is an important test for the evaluation of ovarian reserve. When FSH value exceeds 15, pregnancy rates decrease significantly. The study of Merviel *et al.* (30) found that the basal FSH value did not predict IUI success. Similarly, we have not found basal FSH value as a parameter predicting the success of IUI. The LH plays an important role in the control of the menstrual cycle, especially in the facilitation of ovulation. It also allows the uterus to be prepared for implantation by stimulating estrogen and progesterone release in the ovaries. Some studies have shown that ideal LH level plays a significant role in follicle development, oocyte

formation and continuation of pregnancy (31). In our study, LH value was found to be 6.02 ± 3.1 mIU / mL in non-pregnant women and 7.4 ± 4.3 in pregnant women and a significant difference was found. We have also shown that LH value can be a predictor of pregnancy in advanced statistical evaluation.

Demonstrations of follicular development with transvaginal ultrasound (TVUS) and LH in urine are the methods used to predict ovulation (32). Monitoring with TVUS is a noninvasive office technique that involves a margin of error because the preovulatory follicle size varies widely. Vermesh *et al.* (33) have shown that preovulatory follicle size ranges between 18-30 mm. They expressed that the time of follicle rupture may be varied since the time of hCG administration. Andersen *et al.* (34) found that the mean time interval from hCG injection to follicular rupture was 38.3 h (34-46 h) when a leading follicle of at least 18 mm in diameter was observed sonographically. At the same time, the hCG injection time with good estrogen levels and follicle size > 16 mm increases pregnancy rates in the IUI cycle (35). In our study, we performed hCG application when the leading follicle reached 17-18 mm diameter and IUI procedure was performed after 34-36 h following hCG administration. E2 levels on hCG day were not measured because the vast majority of patients were in the CC cycle.

Khalil *et al.* (36) compared pregnancy rates in the first IUI cycle to the following up to six treatment cycles. They found that the pregnancy rate reached in the first IUI cycle was significantly higher than the remaining 5 cycles. The highest success rates were also found in the first cycles in the following studies (37, 38). In our study, the probability of pregnancy was found higher in the first cycles, and as the number of cycles increased, the likelihood of pregnancy decreased. As the number of cycles increases, it is evident that the age of men and women is progressing, which in turn will negatively affect success. An important point observed in the same way is that the number of patients decreases considerably as the cycle progresses.

The reported pregnancy rates in patients undergoing COH with IUI due to UEI range from 9% to 18% in the literature (39). When the efficacy of different treatment alternatives in UEI was examined, it was found that the highest chance of conception was in the Gn + IUI cycles with a % 17.1 success rate. Our study found a pregnancy rate of 21.4%. This may be due to the fact that UEI is a heterogeneous group or due to the stringent inclusion criteria of our study.

Metabolic factors such as hyperinsulinemia

and hyperandrogenemia have an important role on fertility and follicle development. Insulin receptors are found in the granulosa and theca cells of developing follicles and also in ovarian stromal cells. An experimental study showed that insulin but not insulin-like growth factor-1 (IGF1) promotes the primordial to primary follicle transition (40). However, IGFs were found to be involved in the increase in FSH-responsiveness of granulosa cells when follicles enter into the gonadotropin-dependent stages of follicular development in most mammals (41). It is well known that PCOS is associated with obesity and IR. IR accompanied by compensatory hyperinsulinemia is an important feature of both obese and lean PCOS women and is thought to be the underlying cause of hyperandrogenemia. Increased insulin and IGF levels play a significant role in the development of impaired follicular development, which is typical for PCOS patients (42). Increased insulin levels are thought to increase steroidogenesis and excessive androgen production in ovarian theca cells, which causes follicular atresia and provides the choice of dominant follicle. Hyperinsulinemia has an important role in the pathophysiology of PCOS and the view that correcting this disorder increases both ovulation and pregnancy rates has been widely accepted and researches have intensified in this respect.

Velazquez *et al.* (43) used metformin 1500 mg daily for 8 weeks in 26 non-diabetic PCOS patients, and found significant improvement in blood pressure, BMI, and some laboratory parameters. They concluded that metformin improved hyperinsulinemia and endocrinopathy in PCOS, provided regular menstrual cycles, and increases spontaneous pregnancies by positively affecting infertility. In a more comprehensive study, CC, metformin, or the combination of both was compared in terms of ovulation and pregnancy achievement, and live birth (44). According to this study, combined treatment increased ovulation, pregnancy and live birth rates. However, the increase in pregnancy and live birth rates was not statistically significant.

Dickerson *et al.* (45) showed that the level of IR is related to the treatment cycle outcome in non-PCOS women undergoing IVF. The total follicle count was significantly higher in women with a HOMA >2.5 following COH treatment. HOMA also positively correlated with total follicle count in those with IR (HOMA >2.5), which is linked to high insulin levels stimulating follicle development.

Although there are numerous publications in

the literature on the relationship between PCOS and IR, and their effects on pregnancy outcomes, our study is the first that investigated this relationship in women with UEI. Various methods are available for detecting IR. The euglycemic hyperinsulinemic clamp test is the gold standard among those methods. However, this method is invasive, expensive, and impractical in clinical use. In our study, we used fasting blood glucose, fasting insulin levels and HOMA-IR index values to detect IR. There is no international threshold set for the HOMA value. Although there are regional and racial differences among threshold values, it is generally accepted that a value higher than 2.5 is optimal threshold for IR (46). We found higher HOMA-IR levels in the pregnant group than in the non-pregnant group, but this did not reach statistical significance.

In conclusion, a certain level of insulin is obviously necessary for follicle development, ovulation and pregnancy. However, our results indicated that IR calculated by using HOMA-IR index has no value in predicting the occurrence of pregnancy in women undergoing OI therapy with IUI for UEI. Further studies are needed to determine the predictive role of IR in those with UEI.

Conflict of interest

The authors report no conflict of interest.

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