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ORIGINAL ARTICLE

Comparing the effect of sitagliptin and metformin on the oocyte and embryo quality in classic PCOS patients undergoing ICSI

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Abstract

Background Insulin resistance plays a major role in the pathogenesis of polycystic ovary syndrome (PCOS). Therefore, there is a growing interest in the use of insulin sensitizer drugs in the treatment of PCOS. Research in recent years has shown that sitagliptin has been reported to improve ovarian cycles and ovulation in PCOS patients.

Aims We aimed to compare the effects of metformin and sitagliptin on PCOS individuals undergoing ICSI.

Methods Sixty PCOS patients were divided into 3 groups: metformin, sitagliptin, and placebo group. Treatment was carried out 2 months before the start of the ovulation cycle and continued until the day of oocyte aspiration. The serum levels of total testosterone, estradiol, and fasting insulin along with the total number of retrieved, normal and abnormal MII, and fertilized oocytes, the number of transferred embryos (grades I, II and III), and biochemical and clinical pregnancy rates as well as the ovarian hyperstimulation syndrome (OHSS) were evaluated.

Results There was a significant reduction in the serum levels of Insulin and total testosterone in the treated groups compared with the placebo. The number of mature and normal MII oocytes increased significantly in the treated groups compared with the placebo. Moreover, the number of immature oocytes decreased significantly and the number of grade I embryos increases significantly in the sitagliptin group compared with the placebo group.

Conclusion We conclude that sitagliptin can improve the maturation of oocytes and embryos' quality more effectively than metformin, in PCOS patients undergoing ICSI.

Trial registration Trial registration is NCT04268563 (https://clinicaltrials.gov).

Keywords GnRH antagonist · ICSI · Metformin · PCOS · Sitagliptin

Introduction

Polycystic ovary syndrome (PCOS) is a common disorder which affects about 4% to 21% of women depending on definitions and populations studied [22]. Patients with PCOS should have at least two of three features: clinical or biochemical symptoms of hyperandrogenism, ovarian dysfunction, and polycystic ovaries according to the Rotterdam criteria [13]. Clinical features are broader and include the following: reproductive (infertility pregnancy-related risks) and metabolic (obesity, insulin resistance, type II diabetes, and cardiovascular risk factors) aspects [38].

PCOS is the cause of about 80% of anovulatory infertility in women [14]. PCOS women are commonly distinguished by an increased number of retrieved oocytes during the course of ovulation stimulation [40], although these oocytes are often of low quality, leading to poor fertilization, cleavage, and implantation rates [40].

Since PCOS tends to present as a spectrum of diseases, the Rotterdam criteria divided the disease into classic and non-classic phenotypes. Data derived from clinical populations suggest that women with "classic" PCOS are associated with more pronounced menstrual dysfunction, higher rates of insulin resistance and risk for metabolic syndrome, and more severe forms of atherogenic dyslipidemia, as compared with women diagnosed with

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non-classic. Therefore, we decided to limit this study to women with the classic type of the disease [11, 22].

Research in recent years has shown that insulin resistance plays a major role in the pathogenesis of PCOS. Therefore, there is a growing interest in the use of Insulin sensitizer drugs in the treatment of PCOS [6, 12]. Metformin is a synthetically derived biguanide and is the preferred first-line oral therapy for the treatment of diabetes mellitus type 2 [17, 27]. The liver is the primary site of action of metformin where it reduces hepatic glucose production and stimulates insulin-mediated glucose uptake by the liver and the skeletal muscle [2]. Previous studies have shown that metformin treatment in women with PCOS is effective in improving ovarian function. Metformin exerts these effects by reducing hyperandrogenism and insulin resistance in PCOS patients [10, 26]. Metformin may either directly inhibit androgen production in the ovarian cells or indirectly reduce androgens by improving insulin sensitivity [6, 35].

Sitagliptin is the first dipeptidyl peptidase 4 (DPP4) inhibitor that enhances the effect of incretins such as GLP-1 (glucagon-like peptide-1) [9, 34],which are necessary for lowering blood glucose, regulation of insulin secretion, and regulation of fatty acid metabolism [3]. DPP4 inhibitors have been used in the treatment of type II diabetes for a long time. Although in recent years, DPP4 inhibitors such as sitagliptin have been reported to improve the ovarian cycles and ovulation in women with PCOS [30, 42], there have not been any related reports on comparing the effects of sitagliptin and metformin on the quality of oocytes and embryos in classic PCOS patients undergoing ICSI. Therefore, this research aimed to examine and compare the effects of sitagliptin with metformin on the PCOS women undergoing ICSI.

Materials and methods

Study population

Through a randomized clinical trial, placebo controlled pilot study, 60 infertile PCOS patients at the age of 25–35 years, candidate of ICSI, were enrolled. This study was conducted in the Infertility clinic of Mahdiyeh Educational Hospital, Tehran, and the ethics committee of Shahid Beheshti University of Medical Sciences, Iran, approved it (IR.SBMU.RETECH.REC.1396.424).

Patients were needed to fulfill the classic PCOS diagnostic criteria which was based on the NIH 2012 extension of ESHRE/ASRM 2003 [28] as follows: having at least clinical or biochemical hyperandrogenic symptoms and oligo-/amenorrhea cycles and with or without polycystic ovaries at ultrasound. Oligomenorrhea was defined as cycle intervals more than 35 days and amenorrhea as absence of menstruation for three consecutive months. Serum total testosterone > 1.22 ng/mL indicated biochemical hyperandrogenism and Ferriman and Gallwey score ≥ 8, and/or persistent acne was regarded as clinical hyperandrogenism.

The exclusion criteria for this study were as follows: hypersensitivity to either metformin or sitagliptin, presence of infertility factors other than anovulation, male infertility, systemicdisease or diabetes (type 1 or 2), consumption of medications affecting carbohydrate metabolism, hormonal analogues other than progesterone 2 months prior to study enrolment, and serum FSH level > 12 mIU/ml. The semen samples were assessed according to the WHO guidelines [7], and individuals with abnormal semen parameters were excluded from the study. A written informed consent was obtained from the patients, and they were all asked to avoid any changes in their diet and normal physical activity and also not to take any new pharmacotherapy during the study.

Treatment design

The power calculation in the present study was based on data from studies on metformin or sitagliptin [37, 10, 19, 21, 43]). Common findings for patients with PCOS undergoing IVF are low fertilization rates and impaired oocyte quality. Overall, considering a 95% confidence interval and 80% power, 60 patients were considered the sample population. Sample size was calculated by Minitab software version 19.2.0.

After being examined by a gynecologist, the subjects were randomly selected to receive metformin, sitagliptin, or placebo. Sixty patients were divided into 3 groups ($N = 20$): metformin group treated with metformin (Glucophage, Merck, West Drayton, UK; 500 mg two times daily), sitagliptin group who received sitagliptin (Januvia, Merck, West Drayton, UK; 50 mg two times daily), and the placebo treated group who were treated with oral rehydration solution (Poursina, Tehran, Iran; two times daily). In all of the groups, treatment was carried out 2 months before the start of the ovulation cycle and continued until the day of the oocyte aspiration. A midwife was hired to provide the patients with the drugs, and both patients and physicians were blinded to the treatment regimen. Patients were also asked to report any possible adverse effects during the treatment so that they could be evaluated for any presenting main complaints at the end of the treatment. A total number of 15 patients (5 per group) dropped out of the study which ultimately left us with 45 patients to be examined (Fig. 1).

Ovulation induction

After being pretreated for 2 months with placebo, metformin, or sitagliptin, the patients in all groups received injections of recombinant FSH (rFSH) (Gonal-F, Merck Serono Ltd., UK) from day 3 of the menstrual cycle. The menstrual cycle was induced by a progesterone (Medrofem®, Iran Hormone, Iran). When the leading follicle reached a 14-mm diameter on the ultrasound scan, injection of cetrorelix acetate 0.25 mg/day

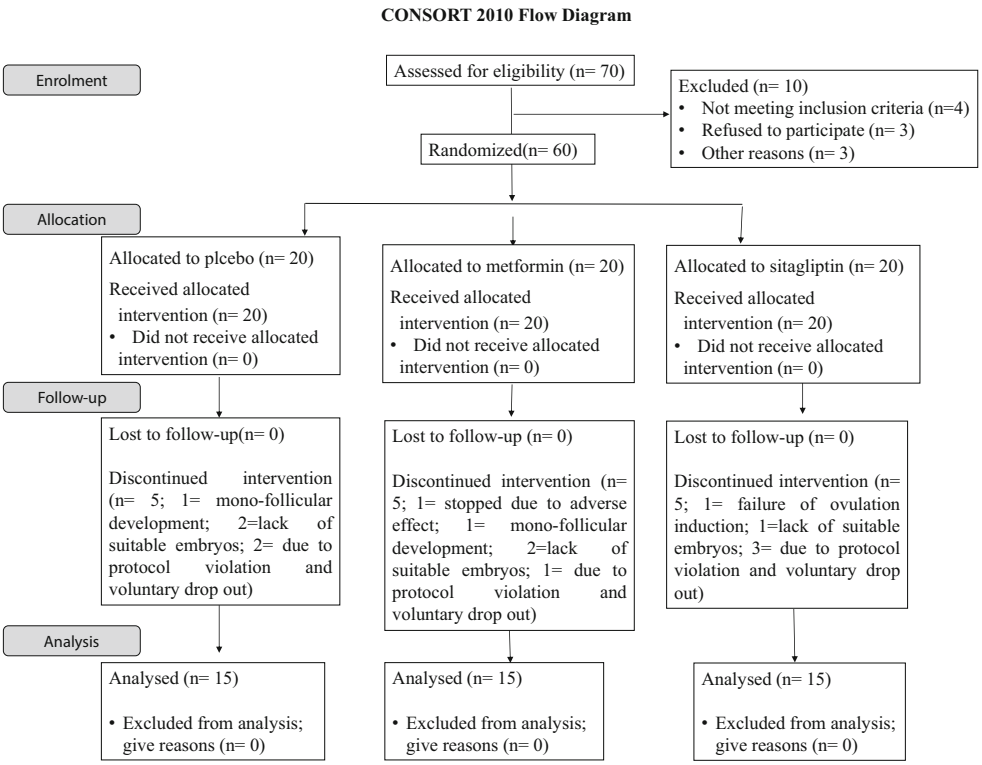

Fig. 1 Flow chart of the study participant

(Cetrotide, Merck Serono),a GnRH antagonist, was started and was continued until the day of HCG injection which was when the diameter of two or three follicles reached at least 16 mm on the ultrasound scan (Ovitrelle, Merck Serono). Using the transvaginal ultrasound guidance, the oocytes were retrieved approximately 36 h after HCG administration. Treatment in all groups was ceased on the day of oocyte aspiration.

Assessment of baseline and clinical features

The weight, height, and body mass index (BMI) were recorded for each patient once before the start of the treatment and once on the day of oocyte aspiration. Fasting blood samples were also collected from each individual after the treatment on the day of oocyte aspiration. After centrifuging at 1500 g for 10 min at room temperature (EBA20, Hettich, UK), the serum samples were stored at − 70 °C for further analysis. The serum levels of total testosterone, estradiol, and fasting insulin in all samples were measured using ELISA enzyme immunoassay (Demeditec Diagnostics GmbH, Germany) for hormonal profile.

Oocyte retrieval, ICSI, and embryo culture

Ultrasound-guided transvaginal aspiration was performed using a single-lumen needle (Reproline Medical, Rheinbach, Germany), and the retrieved oocytes had their cumulus cells removed by 30-s exposure to 20 $IUmL^{-1}$ hyaluronidase (ART-4007A; Sage BioPharma, Pasadena, CA, USA) in HEPES-based medium followed by thorough washing with HEPES-buffered human tubal fluid (HTF) containing 5 mg mL^{-1} human serum albumin (ART-3001; Sage BioPharma) and mechanical pipetting. The presence of the first polar body under a stereomicroscope (Olympus, Tokyo, Japan) indicated that the mature (MII) oocytes and the oocytes that had extruded the first polar body (MII oocyte) were selected for ICSI. The morphology of the MII oocytes was evaluated based on the MII oocyte morphological scoring system (MOMS) and the grading system described by Rienzi et al. [31] on the basis of coloration, granularity (large or small granules; homogeneous distribution or clustering of granules; and in the center or periphery of the oocyte), size of the perivitelline space, and the distribution of organelles (vacuoles and endoplasmic reticulum). According to these morphological criteria, oocytes were classified as follows: (1) normal oocytes, (2) oocytes with extracytoplasmic abnormalities (dark zona pellucida, large perivitelline space, and fragmented polar body), or (3) oocytes with intracytoplasmic abnormalities (dark or granular cytoplasm, vacuolated, structural deformities, and cytoplasmic fragments).

The immature oocytes (MI + germinal vesicle (GV) stage) were also numbered. The processed sperm suspension was added to a 50-μL droplet of polyvinylpyrrolidone (PVP; ART-4006-A; Sage BioPharma) immediately before the injection. Four hours after oocyte retrieval, a single motile

spermatozoa with an apparently normal morphology was immobilized and used to inseminate the oocytes, which were then transferred to the fertilization medium (ART-1520; Sage BioPharma), covered with mineral oil (Reproline Medical). If two pronuclei (2PN) were present in the fertilized oocytes the following day, fertilization was confirmed, and the fertilized oocytes were transferred to the equilibrated cleavage medium (ART-1526; Sage BioPharma), and cleavage was evaluated 24–36 h after fertilization. Embryo quality was assessed on the third day of insemination and graded as follows: grade I, symmetric blastomeres and no fragmentation; grade II, unequal blastomeres and < 30% fragmentation; and Grade III, unequal blastomeres and > 30% fragmentation [4]. Embryos were by using an embryo transfer catheter (Labotect, Gottingen, Germany). A maximum of three embryos were transferred to each patient, and daily injections of 100-mg progesterone (Gestone, London, UK) were performed in order to support the Luteal phase, starting on the day of oocyte retrieval until the day of the pregnancy test. Pregnancy was confirmed by measuring the serum b-HCG on days 14–15 after embryo transfer and also by performing ultrasound scans 6 weeks after embryo transfer to detect the presence of a gestational sac and the heartbeat.

Statistical analysis

The normality of continuous variables was confirmed using the Kolmogorov-Smirnov test, and data are reported as mean ± SD. Data were analyzed by one-way ANOVA. Tukey's and Dunnett's T3 tests were also used for post hoc analysis. A Chi-square test was used for statistical analysis where appropriate. Mean values were considered significantly different at $P \leq 0.05$. All data were analyzed using SPSS (version 16.0) for Windows (SPSS Inc., Chicago, IL, USA).

Results

Clinical and demographic characteristics

There were no significant differences in age, duration of marriage, duration of infertility, weight, height, and BMI in baseline between the three groups (Table 1). The patients' BMI was also measured at the end of the treatment, and no significant difference was found in any of the groups ($P > 0.05$). Data have not been shown.

Biochemical characteristics

There was a significant reduction in the serum levels of Insulin and total testosterone in the treated groups compared with the placebo ($P < 0.05$); meanwhile, the level of estradiol was not significantly different in any of the treatment groups when compared with the placebo group (Table 2).

Evaluation of oocyte morphology and embryos

The total number of retrieved oocytes, abnormal MII oocytes, fertilized oocytes, and cleaved embryos did not differ significantly in the two treatment groups compared with the placebo ($P > 0.05$). The number of mature oocytes and normal MII oocytes in the metformin and sitagliptin groups increased significantly in comparison with the placebo group ($P < 0.05$). While the number of immature oocytes (MI + GV stage) decreased significantly in the sitagliptin group compared with the placebo group, it also revealed a significant reduction in the sitagliptin group compared with the metformin group ($P < 0.05$). The number of grade I embryos increased significantly in the sitagliptin group when compared with the metformin and placebo groups ($P < 0.05$), whereas the number of grades II and III embryos were not significantly different in any of the treatment groups ($P > 0.05$). There was no significant difference in duration of stimulation days, the number of embryos transferred (grades I + II), biochemical and clinical pregnancy rates, as well as the ovarian hyperstimulation syndrome (OHSS) in any of the treatment groups ($P > 0.05$) (Table 3). Investigating intra- and extra-cytoplasmic abnormalities in oocytes showed no significant difference between the groups ($P > 0.05$) (Table 4).

Discussion

This study is the first clinical trial to compare the effect of metformin and sitagliptin on the oocyte and embryo quality in patients with classic PCOS undergoing ICSI. The present study showed that treatment with metformin is effective in decreasing the serum level of insulin and total testosterone, as expected, and it relates to the mechanism of action of metformin. Moreover, metformin increases the number of mature and normal oocytes compared with the placebo group, and these findings are consistent with the findings of other studies [6, 10, 36]. Despite the desired effects achieved following treatment with metformin, there was no significant difference in the number of oocytes retrieved, rate of fertilization and cleavage, quality of embryos, pregnancy, and OHSS in the metformin group compared with the control group. Although the same results have been indicated by some studies [6, 21], controversy among the results reported by different studies remains; for example , Kalem et al. showed that although there was no significant difference in the pregnancy rate and OHSS in the metformin group compared with the control group, but the number of retrieved oocytes, MII oocytes, fertilized oocytes, and transferred embryos was significantly lower in the metformin group than the placebo [20]

Table 1 Baseline characteristics of patients in four groups

Parameters	Placebo	Metformin	Sitagliptin	*P* value
Mean age (25–35 years)	29.53 ± 2.97	28.93 ± 2.76	29.66 ± 3.53	0.790^{NS}
Mean duration of marriage (years)	7.73 ± 2.28	7.46 ± 2.35	7.60 ± 3.43	0.965^{NS}
Mean duration of infertility (years)	5.86 ± 1.99	5.93 ± 2.73	5.66 ± 2.38	0.951^{NS}
Weight (kg)	68.18 ± 2.84	69.88 ± 3.01	70.23 ± 2.56	0.115^{NS}
Height (cm)	160.86 ± 2.50	160.80 ± 2.62	161.66 ± 2.0	0.550^{NS}
BMI (kg/m^2)	26.36 ± 1.35	27.01 ± 0.73	26.87 ± 0.99	0.215^{NS}

Data are shown as mean ± SD. Analysis was performed by the one-way ANOVA followed by the Tukey's test for multiple comparisons. ^{NS} No significant differences were observed between the means within a row compared with the placebo

and does not support the prescribing of metformin as an adjunct to a GnRH antagonist treatment cycle. Genetic differences, treatment duration, and differences in the type of ovulation induction protocol are considered factors that can lead to conflicting results in metformin-treated patients [16, 18, 24]. Therefore, further studies on larger populations regarding the effect of metformin on the improvement of assisted reproductive technology (ART) outcomes in PCOS patients undergoing the GnRH antagonist protocol are suggested.

The present study showed a significant reduction in the level of insulin in the sitagliptin group compared with the placebo group, which is similar to the studies of others [1, 30]. Sitagliptin as a Dpp4 Inhibitor can decrease hyperglycemia and increase the levels of incretins, including GLP-1 and GIP (gastric inhibitory polypeptide), and improve the function of pancreatic beta cells, thereby controlling insulin secretion [30]. Incretin action facilitates the uptake of glucose by liver and muscle while simultaneously suppressing glucagon secretion by the islets cells, leading to reduced production of glucose from hepatic sources [39]. Incretins increase secretion of insulin in a glucose-dependent form by activation of specific receptors of β cell [30].The unique thing about incretins as a drug target is that incretins always require a permissive degree of hyperglycemia to exert their insulinotropic action. Thus, unlike metformin, sitagliptin regulates blood sugar and prevents hypoglycemia. Releasing of incretin is related to a meal and depends on rates of nutrient entry into the small intestine to reach K and L cells [33]. Therefore, it can be concluded that incretins regulate insulin secretion rather than increasing of insulin. In this respect it should be noted that sitagliptin has been observed to decrease plasma insulin concentrations perhaps by influencing better pancreatic beta cell function [30].

Our results also revealed a significant decrease in the serum level of total testosterone in the group treated with sitagliptin compared with the placebo. Ferjan et al. have reported similar results following sitagliptin treatment [15]. A study by Wang et al. showed that the expression of TGF-β1 (transforming growth factor beta1) is higher in the theca cells of PCOS rats than the control ones and that the high expression of TGF-β1 could be related to the occurrence of hyperandrogenism. Sitagliptin reduces the levels of excessive DPP4 which may reduce the levels of factors related to the TGF-β1/Smad2/3 signaling pathway and ultimately reduce androgen levels indirectly [42]. Meanwhile, Devin et al. reported no alterations in the serum testosterone levels after 1 month of treatment with sitagliptin [8]. Therefore, it is suggested that further studies are suggested to define the underlying mechanism of sitagliptin on androgens in PCOS patients.

Women with PCOS are at increased risk of OHSS due to the presence of a significant group of antral follicles capable of responding to exogenous gonadotropin administration. Previous studies have shown a correlation between DPP4 with AMH which can be used to predict the OHSS rates [3, 29], and since sitagliptin is considered a DPP4 inhibitor, it is expected to reduce the OHSS rate. In the present study, although the rate of OHSS in the sitagliptin group was lower than the

Table 2 Serum hormonal parameters of PCOS patients at the end of the treatment period

Parameters	Placebo	Metformin	Sitagliptin	*P* value
Fasting insulin (mIU/L)	17.75 ± 1.61^a	14.78 ± 2.16^b	15.15 ± 1.72^b	0.000
Estradiol (pg/ml)	72.90 ± 2.92^a	72.82 ± 2.03^a	73.52 ± 3.72^a	0.784^{NS}
Total testosterone (ng/ml)	1.53 ± 0.40^a	1.02 ± 0.41^b	1.01 ± 0.29^b	0.000

Data are shown as mean ± SD. Analysis was performed by the one-way ANOVA followed by the Tukey's test for multiple comparisons. Means with different letter codes have significant difference, and means with similar letter codes have no significant difference with each other. ^{NS} No differences were observed between the mean values of variables in the experimental groups compared with placebo ($P > 0.05$)

Table 3 Distribution of oocytes retrieved, quality of oocytes and embryos, and pregnancy outcome in polycystic ovary syndrome patients after the treatment

Parameters	Placebo	Metformin	Sitagliptin	*P* value
Duration of stimulation (days)	10.13 ± 0.91^{a}	9.93 ± 0.88^{a}	10.06 ± 0.88^{a}	0.824^{NS}
No. of oocytes retrieved	9.86 ± 4.05^{a}	12.46 ± 3.62^{a}	11.53 ± 5.12^{a}	0.258^{NS}
No. of immature oocytes (GV + MI)	3.66 ± 0.97^{a}	2.60 ± 1.50^{a}	1.46 ± 1.18^{b}	0.000
No. of mature oocytes (MII)	6.20 ± 3.83^{a}	9.86 ± 3.11^{b}	10.06 ± 4.86^{b}	0.018
No. of normal MII oocytes	3.60 ± 1.68^{a}	6.66 ± 2.25^{b}	7.00 ± 4.47^{b}	0.007
No. of abnormal MII oocytes	2.60 ± 2.64^{a}	3.20 ± 1.69^{a}	3.06 ± 1.94^{a}	0.723^{NS}
No. of fertilized oocytes (2PN)	5.80 ± 3.76^{a}	6.86 ± 2.66^{a}	6.40 ± 2.38^{a}	0.627^{NS}
No. of cleaved embryos	5.33 ± 3.24^{a}	6.13 ± 2.85^{a}	5.73 ± 2.21^{a}	0.738^{NS}
No. of grade I embryos^{A}	1.93 ± 1.03^{a}	2.06 ± 0.70^{a}	3.13 ± 1.35^{b}	0.006
No. of grade II embryos^{A}	1.60 ± 1.05^{a}	2.20 ± 1.47^{a}	1.53 ± 1.18^{a}	0.285^{NS}
No. of grade III embryos^{A}	1.80 ± 1.85^{a}	1.86 ± 1.68^{a}	1.06 ± 1.09^{a}	0.317^{NS}
No. of embryos transferred (grades I + II)	2.13 ± 0.35^{a}	2.20 ± 0.41^{a}	2.13 ± 0.51^{a}	0.888^{NS}
No. of OHSS (%)^{B}	$3(42.9)^{a}$	$2(28.6)^{a}$	$2(28.6)^{a}$	0.844^{NS}
No. of biochemical pregnancy(%)^{B}	$3(20)^{a}$	$2(13.3)^{a}$	$1(6.7)^{a}$	0.562^{NS}
No. of clinical pregnancy (%)^{B}	$2(13.3)^{a}$	$4(26.7)^{a}$	$3(20)^{a}$	0.659^{NS}

Data are the mean ± SD or numbers with percentages in parentheses. Statistical analyses were performed by the one-way ANOVA followed by Tukey's test for multiple comparisons. Means with different letter codes have significant difference, and means with similar letter codes have no significant difference with each other. ^{NS} No differences were observed between the mean values of variables in the experimental groups compared with placebo ($P > 0.05$). ^{A} Statistical analyses performed by Dunnett's T3 test for multiple comparisons. ^{B} Statistical analyses performed by the Chi-square test for multiple comparisons

Placebo group, this decrease was not significant. Therefore, further studies are needed to evaluate the effect of sitagliptin on OHSS rates.

The results of this study also indicated a significant increase in the number of MII oocytes, normal MII oocytes, and grade I embryos and a significant decrease in the number of immature oocytes following treatment with sitagliptin. Sitagliptin can decrease AGE (advanced glycation end) products which are considered inducers of oxidative stress as well as activators of inflammatory pathways in vascular endothelial cells by binding to RAGE (receptor for advanced glycation end products) [19]. Studies have shown a decrease in inflammatory factors and oxidative stress in diabetic patients following sitagliptin consumption [23] and also recuperate oxidative stress and inflammatory cytokine expression in the ovary of PCOS rats [5] which could explain the improved quality of the oocytes and embryos in the sitagliptin group. In addition, previous studies have shown the effect of GLP-1 on the activation of anti-apoptotic pathways [25, 41]; therefore, sitagliptin may decrease apoptosis in granulosa cells through increasing GLP-1 which leads to improved quality of oocytes. Moreover, studies have identified the presence of GLP-1 and its receptors on

Table 4 Distribution of oocyte abnormalities in polycystic ovary syndrome patients

Parameters	Placebo	Metformin	Sitagliptin	*P* value
No. of extracytoplasmic abnormalities (%)				
Dark zona	9 (60)	8 (53.3)	6 (40)	0.771^{NS}
Large PVS	13 (86.7)	12 (80)	8 (53.3)	0.222^{NS}
Fragmented polar body	10 (66.7)	7 (41.5)	7 (49.9(	0.643^{NS}
No. of intracytoplasmic abnormalities (%)				
Granulated	13 (86.7)	11 (73.3)	6 (40)	0.186^{NS}
Vacuolated	12 (80)	4 (26.7)	5 (33.3)	0.128^{NS}
Subzonal fragmentation	10 (66.7)	10 (66.7)	8 (53.3)	0.897^{NS}
Morphological abnormalities	13 (86.7)	6 (40)	6 (40)	0.102^{NS}

Statistical analyses were performed by Dunnett's T3 test for multiple comparisons. ^{NS} No significant differences were observed between mean values in the experimental groups compared with the placebo group ($P > 0.05$). *PVS* perivitelline space

the hypothalamic-pituitary-ovarian axis [32] which may indicate the effective role of GLP-1 in reproduction. The patients who received sitagliptin also showed a significant decrease in the number of immature oocytes and also a significant increase in the number of grade I embryos compared with the placebo- and metformin-treated individuals. Therefore, it can be concluded that sitagliptin may be more effective than metformin in improving the quality of embryos and maturation of oocytes in PCOS patients undergoing ICSI.

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Data availability The data will be made available for researchers working in academic and scientific institutions upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Iran (IR.SBMU.RETECH.REC.1396.424).

Consent to participate A written informed consent was obtained from patients.

Consent for publication The authors, including Delbar Daneshjou, Shahrzad Zadeh Modarres, Malek Soleimani Mehranjani, and Mohammad Ali Shariatzadeh, agree to publish this article.

References

1. Aoki K, Masuda K, Miyazaki T, Togashi Y, Terauchi Y (2010) Effects of miglitol, sitagliptin or their combination on plasma glucose, insulin and incretin levels in non-diabetic men. Endocr J 57: 667–672
2. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, Stener-Victorin E, Fauser BCJM, Norman RJ, Teede H (2016) The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update 22(6):687–708
3. Blauschmidt S, Greither T, Lampe K, Köller S, Kaltwaßer P, Behre HM (2017) Dipeptidyl peptidase 4 serum activity and concentration are increased in women with polycystic ovary syndrome. Clin Endocrinol 87(6):741–747
4. Brezinova J, Oborna I, Svobodova M, Fingerova H (2009) Evaluation of day one embryo quality and IVF outcome–a comparison of two scoring systems. Reprod Biol Endocrinol 7(1):9
5. Carani AV, Dipti N (2019) Sitagliptin recuperates oxidative stress and inflammatory cytokine expression in ovary of PCOS rats. J Drug Deliv Ther 9(4-s):244–251
6. Cheraghi E, Mehranjani MS, Shariatzadeh MA, Esfahani MHN, Ebrahimi Z (2016) N-Acetylcysteine improves oocyte and embryo quality in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection: an alternative to metformin. Reprod Fertil Dev 28(6):723–731
7. Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HW, Behre HM et al (2010) World Health Organization reference values for human semen characteristics. Hum Reprod Update 16(3):231–245
8. Devin JK, Nian H, Celedonio JE, Wright P, Brown NJ (2020) Sitagliptin decreases visceral fat and blood glucose in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 105(1): dgz028
9. Dicker D (2011) DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. Diabetes Care 34(supplement 2):S276–S278
10. Doldi N, Persico P, Di Sebastiano F, Marsiglio E, Ferrari A (2006) Gonadotropin-releasing hormone antagonist and metformin for treatment of polycystic ovary syndrome patients undergoing in vitro fertilization–embryo transfer. Gynecol Endocrinol 22(5): 235–238
11. El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G (2016) Poly cystic ovarian syndrome: an updated overview. Front Physiol 7:124
12. El Omda F, Abd El Fattah A, Hashish M, Khalil M (2018) A randomized trial of combined letrozole with metformin versus clomiphene citrate with metformin in polycystic ovary (PCOS) patients. Egypt J Hosp Med 72(4):4382–4388
13. ESHRE/ASRM (2004) Rotterdam sponsored PCOS consensus workshop group revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81:19–25
14. ESHRE/ASRM (2008) Thessaloniki sponsored PCOS consensus workshop group consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod 23:462–477
15. Ferjan S, Janez A, Jensterle M (2018) DPP4 inhibitor sitagliptin as a potential treatment option in metformin-intolerant obese women with polycystic ovary syndrome: a pilot randomized study. Endocr Pract 24(1):69–77
16. Germeyer A, Jauckus J, Zorn M, Toth B, Capp E, Strowitzki T (2011) Metformin modulates IL-8, IL-1β, ICAM and IGFBP-1 expression in human endometrial stromal cells. Reprod BioMed Online 22(4):327–334
17. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR (2012) Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of diabetes (EASD). Diabetologia 55(6):1577–1596
18. Jacob, S. L., Brewer, C., Tang, T., Picton, H. M., Barth, J. H., & Balen, A. H. (2016). A short course of metformin does not reduce OHSS in a GnRH antagonist cycle for women with PCOS undergoing IVF: a randomised placebo-controlled trial. Human reproduction, 1-9
19. Jinno M, Takeuchi M, Watanabe A, Hirohama J, Hiura R, Suciu N (2012) A novel therapy with sitagliptin for metformin-ineffective aged ART repeaters: dramatical increase in pregnancy rate by decreasing postprandial glycemic levels and advanced glycation end-products. Fertil Steril 98(3):S174
20. Kalem MN, Kalem Z, Gurgan T (2017) Effect of metformin and oral contraceptives on polycystic ovary syndrome and IVF cycles. J Endocrinol Investig 40(7):745–752
21. Kjøtrød SB, Carlsen SM, Rasmussen PE, Holst-Larsen T, Mellembakken J, Thurin-Kjellberg A et al (2011) Use of metformin before and during assisted reproductive technology in non-obese young infertile women with polycystic ovary syndrome: a

prospective, randomized, double-blind, multi-Centre study. Hum Reprod 26(8):2045–2053
22. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R (2016) Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril 106(1):6–15
23. Makdissi A, Ghanim H, Vora M, Green K, Abuaysheh S, Chaudhuri A, Dhindsa S, Dandona P (2012) Sitagliptin exerts an antinflammatory action. J Clin Endocrinol Metab 97(9):3333–3341
24. Maruthini D, Harris SE, Barth JH, Balen AH, Campbell BK, Picton HM (2014) The effect of metformin treatment in vivo on acute and long-term energy metabolism and progesterone production in vitro by granulosa cells from women with polycystic ovary syndrome. Hum Reprod 29(10):2302–2316
25. Miao XY, Gu ZY, Liu P, Hu Y, Li L, Gong YP, Shu H, Liu Y, Li CL (2013) The human glucagonlike peptide-1 analogue liraglutide regulates pancreatic beta-cell proliferation and apoptosis via an AMPK/mTOR/P70S6K signaling pathway. Peptides 39:71–79
26. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, Tinkanen H, Bloigu R, Puukka K, Ruokonen A, Tapanainen JS (2012) Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol 97(5):1492–1500
27. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B (2009) Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy. Diabetologia 52(1):17–30
28. National Institutes of Health. Evidence-based methodology workshop onpolycystic ovary syndrome, December 3-5, 2012. Executive summary. Available at: https://prevention.nih.gov/docs/programs/pcos/FinalReport.pdf. Accessed March 1, 2016.
29. Öztürk B, Gürbüz AS, Durak ZE, Öztürk HS (2019) Dipeptidyl peptidase-4 and adenosine deaminase enzyme levels in polycystic ovary syndrome. Gynecol Endocrinol 35(2):138–141
30. Palma JP, Byhen EL, Ibánez L, Macedo LB, Palma CP, Velázquez CR (2018) Comparative treatment between sitagliptin vs. metformin, alone or in combination, in patients with polycystic ovary syndrome. A clinical entity at high risk for developing diabetes mellitus and gestational diabetes: a pilot study. Revista Médica del Hospital General de México 81(1):15–26
31. Rienzi L, Balaban B, Ebner T, Mandelbaum J (2012) The oocyte. Hum Reprod 27(Suppl. 1):i2–i21
32. Romaní-Pérez M, Outeiriño-Iglesias V, Moya CM, Santisteban P, González-Matías LC, Vigo E, Mallo F (2015) Activation of the GLP-1 receptor by liraglutide increases ACE2 expression, reversing right ventricle hypertrophy, and improving the production of SP-A and SP-B in the lungs of type 1 diabetes rats. Endocrinology 156(10):3559–3569
33. Salvatore, T., Nevola, R., Pafundi, P. C., Monaco, L., Ricozzi, C., Imbriani, S., ... & Sasso, F. C. (2019). Incretin Hormones: The Link between Glycemic Index and Cardiometabolic Diseases. Nutrients, 11(8), 1878
34. Scheen AJ (2008) New therapeutic approaches in type 2 diabetes. Int J Clin Lab Med 63(6):402–407
35. Singh B, Panda S, Nanda R, Pati S, Mangaraj M, Sahu PK, Mohapatra PC (2010) Effect of metformin on hormonal and biochemical profile in PCOS before and after therapy. Indian J Clin Biochem 25(4):367–370
36. Stadtmauer LA, Toma SK, Riehl RM, Talbert LM (2001) Metformin treatment of patients with polycystic ovary syndrome undergoing in vitro fertilization improves outcomes and is associated with modulation of the insulin-like growth factors. Fertil Steril 75(3):505–509
37. Tang, T., Glanville, J., Orsi, N., Barth, J. H., & Balen, A. H. (2006). The use of metformin for women with PCOS undergoing IVF treatment. Human Reproduction, 21(6), 1416–1425
38. Teede HJ, Misso ML, Deeks AA, Moran LJ, Stuckey BG, Wong JL, ... & Guideline Development Groups. (2011). Assessment and management of polycystic ovary syndrome: summary of an evidence-based guideline. Med J Aust, 195, S65-S112
39. Trümper A, Trümper K, Trusheim H, Arnold R, Göke B, Hörsch D. Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. Molecular endocrinology 2001; 15: 1559–1570.
40. Urman B, Tiras B, Yakin K (2004) Assisted reproduction in the treatment of polycystic ovarian syndrome. Reprod BioMed Online 8(4):419–430
41. Wang Q, Li L, Xu E, Wong V, Rhodes C, Brubaker P (2004) Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. Diabetologia 47:478–487
42. Wang F, Zhang ZF, He YR, Wu HY, Wei SS (2019) Effects of dipeptidyl peptidase-4 inhibitors on transforming growth factor-β1 signal transduction pathways in the ovarian fibrosis of polycystic ovary syndrome rats. J Obstet Gynaecol Res 45(3):600–608
43. Yaralı H, Yıldız BO, Demirol A, Zeyneloğlu HB, Yiğit N, Bükülmez O, Koray Z (2002) Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. Hum Reprod 17(2):289–294