European Review for Medical and Pharmacological Sciences 2016; 20: 1940-1946

Serum and follicular fluid irisin levels in poor and high responder women undergoing IVF/ICSI

M. ACET[1], N. CELIK[2], T. ACET[3], S. ILHAN[4], M. YARDIM[5], H.L. AKTUN[1], S. BASARANOGLU[6], A. DEREGOZU[7], S. AYDIN[5]

[1]Department of Obstetrics and Gynecology, Medipol University School of Medicine, Istanbul, Turkey
[2]Department of Biochemistry, Behçet Uz Children's Hospital, Izmir, Turkey
[3]Department of Obstetrics and Gynecology, Medicine Hospital, Istanbul, Turkey
[4]Department of Medical Pharmacology, Firat University School of Medicine, Elazig, Turkey
[5]Department of Medical Biochemistry, Firat University School of Medicine, Elazig, Turkey
[6]Department of Obstetrics and Gynecology, Fatih University School of Medicine, Istanbul, Turkey
[7]Department of Obstetrics and Gynecology, Bahçelievler State Hospital, Istanbul, Turkey

Abstract. – OBJECTIVE: We examined the follicular fluid (FF) and serum levels of irisin in high and poor responders undergoing IVF/ICSI to test whether irisin has a role in the metabolic regulation of energy homeostasis in growing follicle.

PATIENTS AND METHODS: Twenty infertile women with PCOS and 20 poor responder participants undergoing controlled ovarian stimulation (COS) with GnRH antagonist protocol for IVF/ICSI treatment were allocated. Blood was obtained at the time of oocyte retrieval. The follicular fluid content of mature follicles was collected from both high and poor responder women. Irisin levels were measured by using EIA.

RESULTS: There was no significant difference between serum and FF-irisin levels in women with PCOS. (11.18 ± 5.14 µg/mL *vs*. 11.06 ± 4.93 µg/mL, $p < 0.96$). In contrast, serum levels of irisin in poor responders were significantly higher than in the FF-irisin levels (13.13 ± 4.27 µg/mL *vs*. 10.09 ± 4.14 µg/mL, $p < 0.01$). FF-irisin levels of PCOS subjects were positively and significantly correlated with serum levels of irisin (r: 0.81, $p < 0.00$). Serum irisin was positively associated with serum levels of total testosterone but was negatively associated with HOMA-IR in the overall patient population. FF-irisin levels were also noted to be negatively correlated with HOMA-IR. Although there is no correlation between serum irisin and AMH levels, FF irisin levels were negatively correlated with serum AMH levels in PCOS subjects. Contrary to PCOS group there were no significant correlation between serum and FF-irisin levels in poor responder group (r: 0.21; $p < 0.35$).

CONCLUSIONS: The present study is the first attempt to explore the role of irisin in oocyte development by measuring FF and serum levels of this molecules in patients with poor and high responders undergoing IVF/ICSI.

Key Words:
PCOS, Poor responder, Serum, Follicular fluid, Irisin.

Introduction

Interference between muscle to other tissues comes true via some messengers. Irisin is a newly discovered muscle-derived brown adipose differentiation factor which regulates energy expenditure in several tissues[1]. This molecule is encoded by FNDC5 gene which encodes a type I membrane protein that is processed to form of irisin peptide[1]. Irisin is present in biological fluids of human and increases with exercise[2,3]. Regular exercise leads to muscle cells to release irisin which induces a thermogenic program and energy homeostasis in distant tissues including ovary[2,3]. A recent comprehensive study conducted by Aydin et al[3] demonstrated that irisin is locally produced by many human tissues and regulate metabolic energy regulation. It has also been reported that irisin mediate the beneficial impacts of exercise on metabolism through the activation of uncoupling protein 1, PPAR coactivator-1 α, and insulin in brown fat-tissue[4,5]. Studies demonstrated that irisin metabolism is abnormal in patients with PCOS, type 2 diabetes, and gestational diabetes mellitus[6-8]. Although many studies have investigated the levels of irisin in the biological fluids its level in the follicular fluid (FF) of infertile women has not been reported yet[3,9]. To investigate whether irisin secretion is altered in the biological fluids of infertile women under-

Corresponding Author: Mustafa Acet, MD; e-mail: acetmustafa@hotmail.com.tr

going IVF/ICSI, we analyzed follicular fluid and serum levels of irisin in high and poor responder participants. Subsequently to establish a possible relationship between measured irisin levels and biochemical and anthropometric factors we compared to all parameters with others.

Patients and Methods

Patients Selection

A total of 40 participants at the IVF center of Istanbul Medipol University Hospital were recruited for the present study. Among the patients, 20 were diagnosed with PCOS based on the revised Rotterdam criteria which require two of the following three manifestations: (1) oligo- and/or anovulation, (2) clinical and/or biochemical hyperandrogenism, and (3) polycystic ovaries (10). The ultrasound criteria used for diagnosis of polycystic ovary were: presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or increased ovarian volume (> 10 mL). The remaining 20 women met the following inclusion criteria and were grouped as poor responder according to ESHRE consensus criteria. A diagnosis of poor responder was established when at least two of the following three features were present: (1) Advanced maternal age (≥ 40 years) or any other risk factor for POR; (2) A previous POR (≤ 3 oocytes with a conventional stimulation protocol); (3) An abnormal ovarian reserve test (i.e. AFC, 5-7 follicles or AMH, 0.5-1.1 ng/ml).

Participants who consumed alcohol or took medicines affecting glucose and lipid metabolism during the 6 months before enrollment were excluded in order to avoid the interference of any medication and irisin synthesis and secretion. Informed consents were obtained from all candidates after the approval of the study by the local investigation and ethics committee. A blood test was performed on the 2^{nd}-5^{th} day of the progesterone withdrawal bleeding of PCOS participants before the ovulation induction treatment. Blood samples were also obtained from poor responders on the day 3 of their menstrual cycle. Fasting blood samples were subsequently assessed for plasma glucose, insulin, estradiol, testosterone, LH, and FSH using routine laboratory methods, undertaken in the biochemistry laboratory at Istanbul Medipol University Hospital.

Patient demographic data were documented, including age and BMI. BMI (kg/m^2) was calculated as the ratio of the weight (kg) to the square of the height (m^2). For each participant, height and weight were evaluated by standard methods. We have used WHO guidelines to define normal and overweight subjects based on their BMI. Insulin resistance was calculated using the homeostasis model assessment insulin resistance index (HOMAIR)[11] formula; HOMA-IR = Fasting serum insulin (mU/mL) × Fasting plasma glucose (mg/dL)/405.

Induction Protocol

Antagonist protocol was main treatment option used in both groups of participants. All high and poor responder participants underwent antagonist protocol as previously described[12,13]. Shortly, PCOS women undergoing COS with GnRH antagonist were daily injected with Gonal-F (Merck-Serono, Modugno, BA, Italy), a recombinant human FSH (rhFSH), starting from the 2^{nd}-3^{th} day of the menstrual cycle. The initial rhFSH dose was individualized for each patient according to basal FSH levels, antral follicle count, body mass index, and previous response to ovarian stimulation. Dose adjustments were performed according to ovarian response, which was monitored by transvaginal scans and serum estradiol levels. GnRH antagonist, cetrotide (Merck-Serono, Halle, Germany) was added daily subsequent to the leading follicle reached a diameter of 14 mm and carried on until and including the day of HCG injection. Sequential transvaginal ultrasonography and serum estradiol concentrations were measured to monitor ovarian response. Oocyte maturation was triggered 36 h before transvaginal oocyte pick-up. Once steady rise in serum estradiol levels was related with the lead follicle achieved 18 mm in diameter or the lead two were 17 mm or the lead three were 16 mm, patients were subcutaneously injected with recombinant human chorionic gonadotropin (r-hCG, Ovitrelle, Merck-Serono, 250 mg, Modugno, BA, Italy). Ovarian follicles were aspirated using a single-lumen, 17-gauge needle (Cook Medical, Bloomington, IN, USA) guided by trans-vaginal ultrasonography. ICSI was used for fertilization. Two to five days after oocyte retrieval, the embryos were transferred into uterine cavity under ultrasound guidance. The luteal phase was supported progesterone vaginally initiated on the day of oocyte pick-up and continued until the 12^{th} week of gestation in cases where a pregnancy was achieved.

Assessment of Serum and FF Irisin by EIA

Follicular fluid samples were collected during oocyte retrieval from both high and poor responders women undergoing COS for IVF/ICSI with GnRH antagonist protocol. Antecubital vein blood was collected at the time of oocyte retrieval for irisin measurement. All participants should have normal blood pressure, be nonsmoker and not be taking any medication or involved in intensive exercise. Women having follicular fluid contaminated with blood during oocyte retrieval, any medical disease or medication were excluded. After collecting the oocytes, the remaining granulosa cells with fluid were transferred into sterile tubes and centrifuged at 3000 × g for 10 min. Afterwards, the supernatant was aspirated and collected as follicular fluid and cell pellets were washed with PBS and subsequently a red blood cell lysis buffer to eliminate red blood cells. After centrifugation, serum and FF samples were stored at -80°C before analyses. Both serum and follicular fluid irisin levels were quantitatively determined by using human enzyme immunoassay (EIA) according to the manufacturer's instructions (Eastbiopharm, katalog no: CK-E90905, Torrance, CA, USA). The minimum detection limit of irisin was 0.05 μg/mL, the intra and interassay coefficient of variation (CV) were < 10% and < 12%, respectively. Assay validation for FF and serum was performed according to the previously published method[14]. Irisin concentrations were measured with ChroMate, Microplate Reader P4300 (Awareness Technology Instruments, Palm City, FL, USA).

Statistical Analysis

The Statistical Package for Social Sciences, version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Individual group parameters were assessed with one-sample Kolmogorov-Smirnov Z test and were found to be abnormally distributed. Hence, statistical comparisons between groups were performed by nonparametric Paired *t*-test. Pearson correlation analysis was used to assess relations between serum and FF irisin levels, biochemical, hormonal, and demographic findings. Data are presented as mean±standard deviation (SD). For all comparisons, statistical significance was defined by $p < 0.05$.

Results

The demographic features, cycle characteristics, and hormones of PCOS and poor responder groups are presented in the Table I. The PCOS patients were about 4 years younger than the poor responder participants on average ($p <$

Table I. Demographic, biochemical, and clinical characteristics of high and poor responders.

	PCOS (n:20)	Poor responder (n:20)	*p-value
Age	31.0 ± 3.68	35.3 ± 3.10	0.001
Body mass index (kg/m^2)	27.3 ± 4.90	24.5 ± 4.96	0.078
Infertilityduration (yr)	6.80 ± 0.23	13.8 ± 1.4	0.000
Day 3 FSH (mU/ml)	5.82 ± 1.34	9.87 ± 5.17	0.045
Day 3 LH (mU/ml)	7.98 ± 5.47	6.00 ± 2.31	0.137
Day 3 E2 (pg/ml)	41.6 ± 29.9	51.8 ± 42.1	0.368
Total testosterone (ng/dl)	74.4 ±3.32	34.1 ±6.72	0.001
HOMA-IR	4.22 ± 3.40	2.12 ± 1.52	0.005
Fasting insulin (mU/ml)	19.1 ± 1.43	12.1 ± 1.13	0.006
Fasting glucose (mg/dl)	93.4 ± 3.41	87.9 ± 6.41	0.340
Initial dose of rhFSH (IU)	267.8 ± 63.3	351.4 ± 440.0	0.394
Total dose of rhFSH (IU)	2540.4 ± 848.2	2078.5 ± 932.8	0.101
Duration of rhFSH (day)	8.95 ± 1.11	8.38 ± 2.03	0.266
E2 on the day of HCG (pg/ml)	4153.9 ± 1835.7	332.2 ± 126.6	0.001
Total oocyte number	19.8 ± 1.90	3.43 ± 0.20	0.001
2 PN	10.9 ± 6.25	1.95 ± 0.80	0.001
Fertilization rate (%)	78	67	0.001
Embryo transfer number	1.90 ± 0.30	1.66 ± 0.48	0.062
Implantation rate (%)	40	22.8	0.001
Clinical pregnancy rate (%)	75	35	0.001

Data are presented as mean ± SD, *$p < 0.05$ is accepted statistically significant.

0.001). Infertility duration of groups was found to be significantly different. Although there was a trend towards increased BMI in PCOS subjects, BMI of both groups of patients was similar. Fasting insulin levels, total testosterone, and HOMA-IR were significantly higher in women with PCOS compared to poor responder group. Serum levels of estradiol, and circulating levels of LH were similar in both groups of women. Day 3 basal FSH levels of poor responder group were significantly higher than those in PCOS group ($p < 0.045$).

ART Results

Although PCOS patients were treated with the rhFSH at a lower prime dose duration of rhFSH was similar to poor responder group. Likewise, total amounts of rhFSH were given to the PCOS and poor responder participants were found to be similar ($p < 0.101$). Both the number of 2 PN embryo and total oocyte were collected from the PCOS group was significantly higher than those in poor responder group ($p < 0.001$). The fertilization, implantation, and clinical pregnancy rates of PCOS group were significantly higher than those in the poor responder group ($p < 0.001$). The E2 levels of PCOS subjects on the day of HCG were significantly higher than those in poor responder group ($p < 0.001$).

Serum and FF Irisin Levels in PCOS and Poor responder Participants

We were able to collect a sufficient amount of follicular fluid from both PCOS and poor responders participants. There were no significant difference between serum and FF-irisin levels in women with PCOS (11.18 ± 5.14 µg/mL *vs.* 11.06 ± 4.93 µg/mL, $p < 0.96$). Serum levels of irisin in PCOS subjects were similar to serum levels of poor responders (11.18 ± 5.14 µg/mL *vs.* 13.13 ± 4.27, $p < 0.19$). Likewise, there were no significant difference between PCOS and poor responder groups in terms of FF irisin levels (11.06 ± 4.93 µg/mL *vs.* 10.09 ± 4.14, $p < 0.50$). Pearson correlation analysis showed that FF-irisin levels of PCOS subjects were positively and significantly correlated with serum levels of irisin (r: 0.81, $p < 0.00$). While serum levels of irisin was positively associated with the levels of total testosterone, negatively associated with HOMA-IR in PCOS patients. Both serum and FF-irisin levels of PCOS women did not correlate with BMI. The levels of FF-irisin were also noted to be negatively correlated with HOMA-IR. The correlation was not detected between FF-irisin and serum androgen levels. Although there is no correlation between serum irisin and AMH levels, FF irisin levels were negatively correlated with serum levels of AMH in PCOS subjects. Significant negative correlation was also noted between Day 3 LH and serum irisin levels (Table II). Serum levels of irisin in poor responders were significantly higher than in the FF-irisin levels (13.13 ± 4.27 µg/mL *vs.* 10.09 ± 4.14 µg/mL, $p < 0.01$). In contrast to PCOS group there were no significant correlation between serum and FF-irisin levels in poor responder group (r: 0.21; $p < 0.35$). Likewise, neither positive nor negative correlation were detected between irisin levels and hormonal and demographic parameters in poor responder group.

Discussion

When reviewing the literature controversial results are obtained regarding irisin levels because of the heterogeneity of women suffering from metabolic disturbances. In terms PCOS, conflicting results from various studies can be attributed to the unsuitable patient selection and lack of

Table II. Pearson correlation coefficients (r) between irisin levels and measured parameters in PCOS participants.

Independent variables	Serum irisin		FF irisin	
	r	p	r	p
BMI	-0.13	0.55	-0.43	0.056
AMH	-0.03	0.89	-0.55	0.010
Day 3 LH	-0.38	0.01	-0.18	0.254
HOMA-IR	-0.41	0.01	-0.41	0.002
Testosterone	0.37	0.01	0.28	0.132
Serum irisin	Not applicable	Not applicable	0.81	0.001

precision in the description of the PCOS. In most studies, PCOS patients with different BMI often being included in the same study group. Additional controversial conclusions come from the irisin detection method used in the studies since the circulating irisin concentrations exhibits a wide range[2,3,14]. The differences in the circulating levels of irisin in different studies vary depend on the kit used. Therefore, to evaluate the possible effect of irisin on non-adipose tissues such as follicular fluid is much more difficult than we expect. To avoid these handicaps, the inclusion of patients in our study was restricted to infertile patients with PCOS. To prove possible relations between serum and FF-irisin appropriate controls without disease is required. This was realized in the current study where poor responder women were used.

The present work is the first attempt to explore the role of irisin on follicle development by measuring FF and serum levels of this peptide in patients with PCOS and poor responders. We analyzed potential correlations between FF-irisin and various demographic and laboratory parameters. We have observed that irisin is present in detectable levels in the FF of women having PCOS. Irisin was also detectable by EIA method in both serum and FF of poor responder women.

Poor responder patients had overtly decreased FF irisin levels compared to serum levels of irisin. In contrast, serum and FF levels of irisin in PCOS women were similar. Positive correlation was detected between FF-irisin and serum irisin levels in PCOS women. Conversely, there was no significant correlation between serum and FF-irisin levels in poor responder women. Insignificant differences between serum and FF-irisin levels in PCOS subjects could represent novel insight into the follicular development. Balanced levels of serum and FF irisin could suggest a compensatory mechanism in PCOS subjects, with a metabolically compromised state, as proposed previously for serum irisin[8,15]. Furthermore, the presence of positive correlation between serum and FF irisin levels suggests that main source of irisin in FF is periphery. We do not exactly know that whether the existence of follicle-blood-barrier (FBB) regulating the transport of irisin between the circulation and follicle. It is most likely that due to increased vascularity in the follicles of PCOS subjects FBB is well established and might maintain local thermoregulation of follicle cells and oocyte during physiological or stimulated cycles. Similar levels of serum irisin in PCOS and poor responder participants suggest that irisin synthesis and secretion from peripheral tissues have not changed in high and poor responder participants. Together, circulating irisin may contribute to follicle development by regulating follicle temperature during folliculogenesis irrespective of causes underlying infertility.

We do not know the physiological importance of similar serum and FF-irisin levels in PCOS subjects. One may speculate that unlike to the insulin resistance transport of irisin from blood to FF may not be dysfunctional in PCOS women. Since the development of PCOS is often associated with the insulin resistance[16], we have suggested that irisin may play an important role in follicle development independent of insulin resistance. This may provide a novel therapeutic target for the treatment of PCOS-related metabolic disturbance and subfertility. However, to demonstrate the possible functional role of irisin in follicle cells and oocyte, it would be important to identify whether irisin receptors were present in cumulus oocyte complex.

In contrast to the PCOS cases, significant differences in serum and FF-irisin levels in poor responders lead us to think that the transfer of irisin from the blood into the FF could not be simple diffusion. If irisin was transporting from the blood into the FF by the receptor-mediated system, entry of irisin into the follicle should be facilitated at the high serum irisin levels in poor responders. Lack of significant correlation between serum and FF irisin levels in poor responders supports our idea. Weak vascular supply of follicle cells in poor responders might prevent the transport of irisin molecules from circulation into the follicle.

Irisin secretion has been related with BMI and muscle mass in humans[15]. In the current study, poor responders and PCOS subjects had similar BMI. On the other hand, lack of significant correlation between serum and FF irisin levels in poor responders led us to the suggest that regulation of irisin transport between serum and FF are irrespective of BMI status. Therefore, the differences detected in the serum and FF-irisin levels in poor responders cannot be attributed to differences in muscle mass or BMI.

To determine the contribution of both metabolic and demographic parameters upon follicle development, we correlated each parameter with the serum and FF-irisin. Our data clearly show that balanced levels of serum and FF irisin may

occur secondary to metabolic sequelae of PCOS. In line with, Chang et al[8] reported that serum irisin levels of PCOS women are negatively associated with both insulin sensitivity and insulin resistance index. In good agreement, in the current study we have detected a negative correlation between HOMA-IR, serum, and follicular fluid irisin levels. Moreover, in this study positive correlation between serum irisin and testosterone levels were detected in the PCOS subjects. Therefore, we can suggest that insulin resistance and hyperandrogenism may be involved in the regulation of irisin transport between serum and follicle cells. It has been reported that androgens are closely linked to low-grade inflammation in PCOS subjects[17]. Moreover, serum irisin levels of PCOS women was reported to be positively associated with hyperandrogenemia[8]. However, we have noted an insignificant association between FF-irisin and androgen levels in PCOS subjects. We, therefore, may propose that the balance between serum and FF-irisin occur regardless of serum levels of androgens.

Energy storage reservoir of the female is adipose tissue which is essential for the initiation and the maintenance of reproductive functions. Female fertility is negatively affected by perturbation in the adipose mass. Irisin induces some reactions in the adipose tissue by stimulating browning and UCP1 expression which lead to an increase in total body energy expenditure[2]. By regulating temperature change within the follicle cells, irisin may contribute to the follicle development. Thus, improper levels of FF-irisin may disturb energy consumption by both oocyte and follicle cells in poor responders. Unbalanced serum and FF-irisin levels may, therefore, contribute to the unsuitable follicle development in poor responders. Negative correlation between FF-irisin and serum AMH levels in poor responders supports our idea. In addition to the poor vascular supply of follicle cells, it is most likely that irisin resistance is evident in the follicles of poor responders. This resistance in follicle cells may deregulate the expression of several gene and gene-products that are potentially involved in folliculogenesis. Moreover, irisin resistance in follicle cells may disturb mitochondrial biogenesis and angiogenesis during follicular development. Concordantly, it has been reported that irisin controls mitochondrial biogenesis and oxidative metabolism in many cell types[18].

Conclusions

In the literature controlled study investigating the serum and follicular fluid irisin levels in women suffering from infertility are lacking. This is the first study showing the presence of irisin in human follicular fluid. FF irisin correlated with serum irisin in the PCOS women. In contrast, there was no significant correlation between serum and FF-irisin levels in the poor responders. As a result, these findings suggest that irisin may have an important physiological role during follicular development in poor and high responder subjects undergoing IVF/ICSI.

Acknowledgements

We would like to thank Prof. Dr. Onder Celik who read and edited this paper.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Pardo M, Crujeiras AB, Amil M, Aguera Z, Jiménez-Murcia S, Baños R, Botella C, De La Torre R, Estivill X, Fagundo AB, Fernández-Real Jm, Fernández-Garcia JC, Fruhbeck G, Gómez-Ambrosi J, Rodriguez R, Tinahones FJ, Fernández-Aranda F, Casanueva FF. Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. Int J Endocrinol 2014; 2014: 857270.

2) Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo Jc, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481: 463-468.

3) Aydin S, Kuloglu T, Aydin S, Kalayci M, Yilmaz M, Cakmak T, Albayrak S, Gungor S, Colakoglu N, Ozercan IH. A comprehensive immunohistochemical examination of the distribution of the fat-burning protein irisin in biological tissues. Peptides 2014; 61: 130-136.

4) Kurdiova T, Balaz M, Vician M, Maderova D, Vlcek M, Valkovic L, Srbecky M, Imrich R, Kyselovicova O, Belan V, Jelok I, Wolfrum C, Klimes I, Krssak M, Zemkova E, Gasperikova D, Ukropec J, Ukropcova B. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J Physiol 2014; 592: 1091-1107.

5) Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998; 92: 829-839.

6) Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK, Kim JG, Lee IK, Park KG. Serum irisin levelsin new-onset type 2 diabetes. Diabetes Res Clin Pract 2013; 100: 96-101.

7) Kuzmicki M, Telejko B, Lipinska D, Pliszka J, Szamatowicz M, Wilk J, Zbucka-Kretowska M, Laudanski P, Kretowski A, Gorska M, Szamatowicz J. Serum irisin concentration in women with gestational diabetes. Gynecol Endocrinol 2014; 30: 636-639.

8) Chang CL, Huang SY, Soong YK, Cheng PJ, Wang CJ, Liang IT. Circulating irisin and glucose-dependent insulinotropic peptide are associated with the development of polycystic ovary syndrome. J Clin Endocrinol Metab 2014; 99: E2539-E2548.

9) Piya MK, Harte AL, Sivakumar K, Tripathi G, Voyias PD, James S, Sabico S, Al-Daghri NM, Saravanan P, Barber TM, Kumar S, Vatish M, Mcternan PG. The identification of irisin in human cerebrospinal fluid: influence of adiposity, metabolic markers, and gestational diabetes. Am J Physiol Endocrinol Metab 2014; 306: E512-E518.

10) Rotterdam Eshre/Asrm-Sponsored Pcos Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81: 19-25.

11) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.

12) Albano C, Felberbaum RE, Smitz J, Riethmüller-Winzen H, Engel J, Diedrich K, Devroey P. Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH) antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. Hum Reprod 2000; 15: 526-531.

13) Celik O, Celik E, Yilmaz E, Celik N, Turkcuoglu I, Ulas M, Kumbak B, Aktan E, Ozerol I. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin-releasing hormone agonist and antagonists, on follicular fluid stem cell factor and serum urocortin 1 levels on the day of oocyte retrieval. Arch Gynecol Obstet 2013; 288: 1417-1422.

14) Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides 2015; 72: 4-15.

15) Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, Mantzoros CS. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 2012; 61: 1725-1738.

16) Celik O, Yesilada E, Hascalik S, Celik N, Sahin I, Keskin L, Ozerol E. Angiotensin-converting enzyme gene polymorphism and risk of insulin resistance in PCOS. Reprod Biomed Online 2010; 20: 492-498.

17) González F, Sia CL, Shepard MK, Rote NS, Minium J. Inflammation in response to glucose ingestion is independent of excess abdominal adiposity in normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2012; 97: 4071-4079.

18) Handschin C, Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 2008; 454: 463-469.