Progestin-primed ovarian stimulation improves the outcomes of IVF/ICSI cycles in infertile women with diminished ovarian reserve

Chun-Mei Yu, Xiu-Liang Dai, Yu-Feng Wang, Ting-Ting Gao, Fang Cao, Xi-Yang Xia, Li Chen*

Department of Reproductive Medicine Center, the Affiliated Changzhou Maternal and Child Health Care Hospital of Nanjing Medical University, Changzhou, Jiangsu, China

Abstract

Background: Ovarian stimulation with clomiphene (CC) or progestin has been applied for patients with diminished ovarian reserve (DOR). However, it remains unclear which treatment confers greater benefits. This study aimed to compare the outcomes of progestin-primed ovarian stimulation (PPOS) protocol vs CC-primed ovarian stimulation (CPOS) in infertile women with DOR.

Methods: A before-and-after self-controlled study was conducted to retrospectively investigate the data from 50 infertile women with DOR, who failed to conceive in their first in vitro fertilization/intracytoplasmic sperm injection-frozen embryo transfer cycle when stimulated with CPOS, and switched to PPOS, in the Reproductive Medicine Center of Changzhou Maternal and Child Health Care Hospital.

Results: Our results showed that PPOS significantly suppressed the luteinizing hormone (LH) surge and yielded more satisfactory results in patients with DOR, including increased number of retrieved oocytes, MII mature oocytes, normal fertilized oocytes, cleaved embryos, high-grade embryos, cryopreserved embryos, pregnancy rate, live-birth rate, and decreased miscarriage rates.

Conclusion: Our study demonstrated that compared with CPOS protocol, PPOS protocol could not only suppress the LH surge but also improved the quantity, particularly the quality of oocytes in patients with DOR, suggesting that PPOS treatment is more effective than CPOS for patients with DOR.

Keywords: Clinical outcomes; Clomiphene-primed ovarian stimulation; Diminished ovarian reserve; In vitro fertilization; Progestin-primed ovarian stimulation

1. INTRODUCTION

The development of controlled ovarian hyperstimulation (COH), which allows multiple oocytes to grow and mature simultaneously, has paved the way for in vitro fertilization (IVF) –embryo transfer.[1] After decades of clinical practice and investigation, several COH protocols have been developed for IVF, and can be classified into two types, conventional pituitary downregulation protocol and others.[2–5] Women who receive IVF treatment can be classified as having poor, normal, or high ovarian responsiveness to gonadotropin (Gn). Patients with poor ovarian responses (PORs) are least likely to get pregnant via IVF treatment.[6] One of the main causes resulting in the poor response is diminished ovarian reserve (DOR).[7,8] DOR is characterized by increased follicle-stimulating hormone (FSH), decreased estrogen secretion, low anti-Müllerian hormone (AMH), and antral follicle count (AFC), which can be age dependent or independent.[9] Clinicians and researchers worldwide are seeking ways to improve the number and quality of retrieved oocytes, particular for patients with DOR.

COH protocols play a critical role in determining the quantity and quality of retrieved oocytes; therefore, a proper COH protocol for patients with DOR could promote the success of IVF treatment.[10] Although pituitary downregulation is the standard protocol, and yields satisfactory results for most patients, it has been demonstrated as ineffective and expensive for patients with DOR.[11]This is due to lengthy treatment, increased Gn use, elevating the risk of ovarian hyperstimulation syndrome (OHSS), and decreased efficacy.[12,13] Recently, ovarian stimulation with clomiphene (CC), first developed by Fauser et al, has been tested in several types of patients.[14–17] Commonly, CC was used to block negative feedback trigged by estrogen, thereby resulting in elevated FSH and luteinizing hormone (LH) release from the hypothalamus. Due to the short treatment-period, decreased Gn use, decreased cost, and physiological comfort, CC-primed ovarian stimulation (CPOS) is regarded as patient friendly.[18,19] Furthermore, studies have demonstrated that CPOS achieves equal pregnancy outcomes on POR patients compared with the conventional COH protocol.[20] Therefore, CPOS has been widely used in clinics as an ideal protocol for POR patients despite the risk of early LH peak and menstrual cycle cessation.

More recently, Kuang et al developed a new COH protocol called progestin-primed ovarian stimulation (PPOS).[21] Progestin is used to block the estrogen-induced LH surge without the

*Address correspondence: Dr. Li Chen, Department of Reproductive Medicine Center, the Affiliated Changzhou Maternal and Child Health Care Hospital of Nanjing Medical University, 16, Dingxiang Road, Changzhou 213000, Jiangsu, China. E-mail address: CZRCchenli@126.com (L. Chen).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2019) 82: 845-848.

Received November 29, 2018; accepted June 22, 2019.

doi: 10.1097/JCMA.0000000000000177.

occurrence of OHSS.[21] This protocol has been successfully used in patients with various ovarian conditions, including normal reserve and polycystic ovary syndrome.[5,22,23] Kuang et al used nature cycles as a control to demonstrate that PPOS could overcome premature ovulation for patients with DOR without affecting the quality of retrieved oocytes.[24] However, it is still unclear which protocol, CPOS or PPOS, is more appropriate for patients with DOR. In this study, we retrospectively analyzed data from patients with DOR who had a failed, mild ovarian stimulation and subsequently switched to the PPOS protocol. The clinical results including hormone level, oocytes retrieved, embryos fertilization and development, and clinical outcomes were compared between the two protocols that were used in the same patient.

2. METHODS

2.1. Patients

A retrospective study was conducted at the Changzhou Maternal and Child Health Care Hospital. From January 2016 to June 2017, a total of 50 women undergoing IVF/intracytoplasmic sperm injection (ICSI) cycles due to infertility were enrolled. The study protocol was approved by the Ethics Committee of the Changzhou Maternal and Child Health Care Hospital and conducted according to the Declaration of Helsinki for medical research. All participants provided informed consent after counseling for infertility treatments and routine IVF procedures. Patients with two or more of the following items were diagnosed with DOR: (a) 25IU ≥ FSH ≥ 10IU; (b) FSH/LH ≥ 2; (c) AFC ≤ 5; and (d) AMH ≤ 0.5-1.1. Patients had to meet the following standards: (1) DOR diagnosis and (2) failure of CPOS during the last IVF cycle, resulting in a switch to PPOS. Clinical results were compared between the two treatment protocols for each patient.

2.2. Controlled ovarian stimulation

CPOS protocol: CC (50 mg/d; Cyprus Goth Pharmaceutical Co., ltd.) and Gn (150-225 IU; Anhui Fengyuan Pharmaceutical Co., China) were administered from menstrual cycle day 3 (MC3) to human chorionic gonadotropin (hCG) day. The initial dose of Gn (150-225 IU) was based on patient age, basic FSH, AFC accounts, and previous promotion plan. Ultrasound examination and serum hormone level tests were performed regularly, and the dose of Gn was adjusted according to follicle development. When the dominant follicle diameter reached >18 mm, triptorelin (0.1 mg; Decapeptyl, Ferring Pharmaceuticals) and hCG (4000 IU; Lizhu Pharmaceutical Trading Co., German) were administered to trigger ovulation. Oocytes were retrieved 36 to 38 hours later.

PPOS protocol: Medroxyprogesterone acetate (MPA; 6 mg/d; Guang Zhou Xianling Pharmaceutical Co., China) and Gn (150-225 IU; Anhui Fengyuan Pharmaceutical Co., China) were administered from MC3 onward. The remainder of the procedure was identical to CPOS.

2.3. In vitro fertilization and embryo culture

All follicles >10 mm in diameter were retrieved, and standard insemination or ICSI were performed within 6 hours depending on semen parameters. Embryos were examined for cell number, homogeneity, and the degree of embryonic fragmentation on the third day following specifications of Cummins et al. Grade I-III embryos were frozen by vitrification technology on the third day. Remaining embryos were placed in extended culture, and only blastocysts with good morphology were frozen on day 5 or 6. The cryopreservation procedure has been described previously.[20] All patients in the present study had received frozen embryo transfer. Hormone replacement treatment was used for endometrial preparation. Briefly, ethinyl estrogen (25 μg tid) was administered for 14 days, and then shifted to oral progesterone (8 mg estradiol and 40 mg dydrogesterone) and soft vaginal progesterone capsules (200 mg bid; Merck Serono Co., England). Once pregnancy was achieved, exogenous estrogen and progesterone supplements were continued until 10 weeks gestation.

2.4. Laboratory analysis

Serum was collected on MC3, the day prior to, and day ovulation was triggered. Levels of FSH, LH, E2, and progesterone were measured by chemiluminescence (Abbott Biologicals B.V., The Netherlands). The lower limits of sensitivity were as follows: FSH = 0.06 mIU/mL, LH = 0.09 mIU/mL, E2 = 10 pg/mL, and P = 0.1 ng/mL.

2.5. Statistical analyses

All the acquired data were analyzed using SPSS software (SPSS version 20.0; IBM/SPSS, Inc.). The χ^2 test was used to compare constituent ratio data. The paired student's t test was used for normally distributed data, and the Wilcoxon Signed-Rank test was used for data that failed the normality test. $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Basic characters

The average interval between unsuccessful IVF/ICSI with CPOS and initiation of PPOS was 117.9 ± 15.4 days, and the average infertility duration was 4.22 ± 0.61 years. The average patient age was 36.48 ± 0.83 years, BMI was 22.85 ± 0.39, basal FSH was 9.60 ± 0.71 mIU/mL, basal LH was 4.92 ± 0.58 mIU/mL, basal E2 was 26.78 ± 2.66 mIU/mL, and AFC was 3.88 ± 0.49 (Table 1).

3.2. Cycle characteristics in CPOS and PPOS

The total Gn dose was significantly lower in CPOS than PPOS treatment, while the Gn duration was similar. The LH levels were significantly higher in CPOS than PPOS treatment in the day ovulation was triggered. There were no statistical differences in FSH, E2, and P between the two treatments in the day ovulation was triggered (Table 2).

3.3. Follicle development, oocyte performance, and clinical outcomes

The number of dominant follicles (diameter >14 mm), oocytes retrieved, MII mature oocytes, normal fertilized oocytes, cleaved embryos, and high-grade embryos were significantly higher, while the miscarriage rate was significantly lower in PPOS than CPOS treatment. There were no statistical differences in cancellation rate, implantation rate, and clinical pregnancy rate between the two treatments. In addition, nine live-births were achieved after PPOS treatment, while no live-birth occurred after CPOS treatment (Table 3).

Table 1

The basic characteristics

No of patients	50
The interval between two protocols (days)	117.9 ± 15.4
Duration of infertility (year)	2.5 [5.25]
Ages (year)	36.48 ± 0.83
BMI (kg/m^2)	22.16 [3.87]
Basal FSH (IU/L)	7.850 [5.32]
Basal LH (IU/L)	4.070 [2.45]
Basal E2 (ng/L)	23.93 [26.3]
No of AFC	3 [3]

Data are represented as mean ± SEM or median [interquartile range].
AFC = antral follicle count; BMI = body mass index; FSH = follicle-stimulating hormone.

Table 2

The cycle characteristics in CPOS and PPOS treatments

	CPOS	PPOS	*p*
hMG doses (IU)	2134 ± 143.5	2684 ± 159.1	0.01
hMG duration (day)	9.34 ± 0.46	9.5 ± 0.44	0.81
Hormone level in the trigger day			
FSH (IU/L)	19.76 ± 1.01	21.64 ± 0.84	0.1586
LH (IU/L)	4.64 [3.975]	2.23 [3.12]	<0.0001
E2 (ng/L)	994.2 [1184.5]	826.6 [725.5]	0.5882
P (ng/L)	0.94 [0.6]	1.16 [0.85]	0.2217

Data are represented as mean ± SEM or median [interquartile range].
CPOS = clomiphene-primed ovarian stimulation; FSH = follicle-stimulating hormone; hMG = human menopausal gonadotropin; LH = luteinizing hormone; PPOS = progestin-primed ovarian stimulation protocol.

Table 3

Embryo development and clinical outcomes in CPOS and PPOS treatments

	CPOS	PPOS	*p*
No of dominant follicle	2.23 ± 0.17	3.17 ± 0.30	0.005
No of oocytes retrieved	2.39 ± 0.18	3.48 ± 0.30	0.002
No of MII oocytes	2.27 ± 0.17	3.33 ± 0.30	0.002
No of normal fertilized oocytes	1.17 ± 0.15	2.65 ± 0.24	0.001
No of cleavages	1.83 ± 0.15	2.74 ± 0.25	0.002
No of high-quality embryos	0.97 ± 0.12	1.52 ± 0.20	0.017
No of cryopreserved embryos	1 [1.25]	2 [2]	0.0003
No of embryos transfer	2 [1]	2 [1]	0.7533
FET cycles	39	40	
Cancellation rate (%)	22.0 (11/50)	12.0 (6/50)	0.183
Implantation rate (%)	10.8 (7/65)	17.7 (12/68)	0.257
Clinical pregnancy rate (%)	18.0 (7/39)	27.5 (11/40)	0.312
Miscarriage rate (%)	100.0 (7/7)	18.2 (2/11)	<0.000
Live-birth rate (%)	0.0 (0/39)	22.5 (9/40)	0.0016

Data are represented as mean ± SEM or median [interquartile range].
CPOS = clomiphene-primed ovarian stimulation; FET = frozen embryo transfer; PPOS = progestin-primed ovarian stimulation protocol.

4. DISCUSSION

In the present study, we retrospectively compared the clinical outcomes of two COH protocols (CPOS with CC vs PPOS with MPA), which were successively used in patients with DOR. Our results showed that PPOS could significantly suppress the LH surge and yield more satisfactory results, including an increased number of dominant follicles, oocytes retrieved, MII mature oocytes, normal fertilized oocytes, cleaved embryos, high-grade embryos, number of embryos eligible for cryopreservation, live-births rate, and decreased miscarriage rate. These results are similar with a recent study comparing the outcomes from the classic long protocol and PPOS, which demonstrated the superiority of PPOS in improving oocyte utilization rate and number of high-quality embryos in aged infertile women.[25]

The first study reporting the use of PPOS in patients with low Gn responsiveness showed that the number of dominant follicles, oocytes retrieved, MII mature oocytes, normal fertilized oocytes, viable embryos, clinical pregnancy rate, implantation rate, and live-birth rate were higher, while miscarriage rate was lower compared to patients in a natural cycle. However, the difference of clinical pregnancy rate, implantation rate, live-birth rate, and miscarriage rate have no statistical significance.[24] Although the differences in clinical pregnancy rate and implantation rate between the two treatments were not significant in our study, we found that miscarriage and live-birth rates were significantly different, indicating that the improved oocyte quality achieved following PPOS contribute to a live-birth in patients with DOR. A self-controlled study with fewer variables may draw a confirmative conclusion with limited samples. Therefore, our results highlight the importance of PPOS in improving folliculogenesis or the quality of oocytes.

The possible mechanisms underlying the inhibitory effect of MPA on the early LH surge were explained by Kuang et al.[21] Clinical trials have demonstrated that, in the presence of a low level of E2, P administration could significantly inhibit LH surge.[21] It is known that the progestin receptor (PR) is crucial for the E2-induced LH surge.[26,27] E2 can induce PR expression in the hypothalamus, while progestin downregulates its own receptor.[28] High levels of progestin in the absence of adequate E2 will maintain a relatively low level of PR, thereby abolishing the E2 positive feedback pathway, resulting in low LH release.

However, the mechanisms by which MPA improves the number and quality of oocytes remain uncharacterized. Kuang et al proposed that administration of human menopausal gonadotropin (hMG) during the late follicular phase of the MPA treatment may contribute to the higher number of retrieved oocytes and embryos.[24] In the present study, hMG was used in both treatments and we noted that a higher dose was used in PPOS than CPOS treatment. However, the FSH level in the trigger day between the two treatments was similar. Several studies have demonstrated that increased Gn use did not benefit patients with DOR, especially those with low AFC.[11,29] Thus, it was not likely that the improved number and quality of oocytes retrieved can be attributed to increased Gn use in PPOS treatment.

LH plays a critical role in synthesizing and secreting androgens, which are required for further production of E2. It has been demonstrated that low androgen levels promote folliculogenesis, while high levels inhibit folliculogenesis.[30,31]This suggests that LH levels should be maintained in a suitable range. Extremely low or high LH levels will have negative influences on the outcomes of IVF/ICSI.[32,33] In the present study, no premature LH surge occurred in either treatment. In addition, E2 levels were similar at the time of ovulation, indicating that a high level of LH in CPOS treatment did not further promote E2 synthesis. A recently published article showed that CC increased LH levels during PPOS treatment without affecting IVF/ICSI outcomes, indicating that oocyte improvements associated with PPOS are likely unrelated to LH levels.[34] A recent publication showed differentially elevated lipids in follicular fluid between PPOS and a short treatment protocol, and that difference may be associated with improved IVF/ICSI outcomes.[35] Future studies are needed to investigate the exact mechanisms regarding how MPA improves folliculogenesis.

In conclusion, our before-and-after self-controlled study demonstrated that PPOS could not only suppress the LH surge but also improve the quantity and quality of oocytes through improved folliculogenesis in patients with DOR, suggesting that PPOS treatment is an ideal ovarian stimulation protocol for patients with DOR.

ACKNOWLEDGMENTS

This study was supported by a basic and applied research project from Changzhou (CJ20179049) and a special fund for clinical research from the Chinese Medical Association (17020490718).

REFERENCES

1. Navot D, Sandler B. Controlled ovarian hyperstimulation for the new reproductive technologies. *Acta Eur Fertil* 1989;**20**:217–21.
2. Wildt L, Diedrich K, van der Ven H, al Hasani S, Hübner H, Klasen R. Ovarian hyperstimulation for in-vitro fertilization controlled by gnrh agonist administered in combination with human menopausal gonadotrophins. *Hum Reprod* 1986;**1**:15–9.

3. Olivennes F, Fanchin R, Bouchard P, de Ziegler D, Taieb J, Selva J, et al. The single or dual administration of the gonadotropin-releasing hormone antagonist cetrorelix in an in vitro fertilization-embryo transfer program. *Fertil Steril* 1994;**62**:468–76.
4. Barroso G, Menocal G, Felix H, Rojas-Ruiz JC, Arslan M, Oehninger S. Comparison of the efficacy of the aromatase inhibitor letrozole and clomiphene citrate as adjuvants to recombinant follicle-stimulating hormone in controlled ovarian hyperstimulation: a prospective, randomized, blinded clinical trial. *Fertil Steril* 2006;**86**:1428–31.
5. Wang Y, Chen Q, Wang N, Chen H, Lyu Q, Kuang Y. Controlled ovarian stimulation using medroxyprogesterone acetate and hMG in patients with polycystic ovary syndrome treated for IVF: a double-blind randomized crossover clinical trial. *Medicine (Baltimore)* 2016;**95**:e2939.
6. Karande VC. Managing and predicting low response to standard in vitro fertilization therapy: a review of the options. *Treat Endocrinol* 2003;**2**:257–72.
7. Tal R, Seifer DB. Ovarian reserve testing: a user's guide. *Am J Obstet Gynecol* 2017;**217**:129–40.
8. Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder–a plea for universal definitions. *J Assist Reprod Genet* 2015;**32**:1709–12.
9. Coccia ME, Rizzello F. Ovarian reserve. *Ann N Y Acad Sci* 2008;**1127**:27–30.
10. Shrestha D, La X, Feng HL. Comparison of different stimulation protocols used in in vitro fertilization: a review. *Ann Transl Med* 2015;**3**:137.
11. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the bologna criteria. *Hum Reprod* 2011;**26**:1616–24.
12. Siristatidis CS, Hamilton MP. What should be the maximum FSH dose in IVF/ICSI in poor responders? *J Obstet Gynaecol* 2007;**27**:401–5.
13. Lekamge DN, Lane M, Gilchrist RB, Tremellen KP. Increased gonadotrophin stimulation does not improve IVF outcomes in patients with predicted poor ovarian reserve. *J Assist Reprod Genet* 2008;**25**:515–21.
14. Ferraretti AP, Gianaroli L, Magli MC, Devroey P. Mild ovarian stimulation with clomiphene citrate launch is a realistic option for in vitro fertilization. *Fertil Steril* 2015;**104**:333–8.
15. Fauser BC, Devroey P, Yen SS, Gosden R, Crowley WF Jr, Baird DT, et al. Minimal ovarian stimulation for IVF: appraisal of potential benefits and drawbacks. *Hum Reprod* 1999;**14**:2681–6.
16. Jones T, Ho JR, Gualtieri M, Bruno-Gaston J, Chung K, Paulson RJ, et al. Clomiphene stair-step protocol for women with polycystic ovary syndrome. *Obstet Gynecol* 2018;**131**:91–5.
17. Rinaldi L, Lisi F, Selman H. Mild/minimal stimulation protocol for ovarian stimulation of patients at high risk of developing ovarian hyperstimulation syndrome. *J Endocrinol Invest* 2014;**37**:65–70.
18. Oride A, Kanasaki H, Miyazaki K. Comparison of human menopausal gonadotropin stimulation with and without clomiphene for in-vitro fertilisation in poor-responders. *J Obstet Gynaecol* 2015;**35**:163–7.
19. Revelli A, Chiadò A, Dalmasso P, Stabile V, Evangelista F, Basso G, et al. "Mild" vs. "long" protocol for controlled ovarian hyperstimulation in patients with expected poor ovarian responsiveness undergoing in vitro fertilization (IVF): a large prospective randomized trial. *J Assist Reprod Genet* 2014;**31**:809–15.
20. Song D, Shi Y, Zhong Y, Meng Q, Hou S, Li H. Efficiency of mild ovarian stimulation with clomiphene on poor ovarian responders during IVF\ ICSI procedures: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2016;**204**:36–43.
21. Kuang Y, Chen Q, Fu Y, Wang Y, Hong Q, Lyu Q, et al. Medroxyprogesterone acetate is an effective oral alternative for preventing premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation for in vitro fertilization. *Fertil Steril* 2015;**104**:62–70.e3.
22. Dong J, Wang Y, Chai WR, Hong QQ, Wang NL, Sun LH, et al. The pregnancy outcome of progestin-primed ovarian stimulation using 4 versus 10 mg of medroxyprogesterone acetate per day in infertile women undergoing in vitro fertilisation: a randomised controlled trial. *BJOG* 2017;**124**:1048–55.
23. Ye H, Tian H, He W, Lyu Q, Kuang Y, Chen Q, et al. Progestin-primed milder stimulation with clomiphene citrate yields fewer oocytes and suboptimal pregnancy outcomes compared with the standard progestin-primed ovarian stimulation in infertile women with polycystic ovarian syndrome. *Reprod Biol Endocrinol* 2018;**16**:53.
24. Chen Q, Wang Y, Sun L, Zhang S, Chai W, Hong Q, et al. Controlled ovulation of the dominant follicle using progestin in minimal stimulation in poor responders. *Reprod Biol Endocrinol* 2017;**15**:71.
25. Chen YM, Qi QR, Xie QZ, Yang YF, Xia Y, Zhou XD. Effect of progestin-primed ovarian stimulation protocol on outcomes of aged infertile women who failed to get pregnant in the first IVF/ICSI cycle: a self-controlled study. *Curr Med Sci* 2018;**38**:513–8.
26. Chappell PE, Lydon JP, Conneely OM, O'Malley BW, Levine JE. Endocrine defects in mice carrying a null mutation for the progesterone receptor gene. *Endocrinology* 1997;**138**:4147–52.
27. Chappell PE, Levine JE. Stimulation of gonadotropin-releasing hormone surges by estrogen. I. Role of hypothalamic progesterone receptors. *Endocrinology* 2000;**141**:1477–85.
28. Chabbert-Buffeta N, Skinner DC, Caraty A, Bouchard P. Neuroendocrine effects of progesterone. *Steroids* 2000;**65**:613–20.
29. Lainas TG, Sfontouris IA, Venetis CA, Lainas GT, Zorzovilis IZ, Tarlatzis BC, et al. Live birth rates after modified natural cycle compared with high-dose FSH stimulation using gnrh antagonists in poor responders. *Hum Reprod* 2015;**30**:2321–30.
30. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest* 1998;**101**:2622–9.
31. Carmina E, Guastella E, Longo RA. Advances in the diagnosis and treatment of PCOS. *Curr Pharm Des* 2016;**22**:5508–14.
32. Stanger JD, Yovich JL. Reduced in-vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. *Br J Obstet Gynaecol* 1985;**92**:385–93.
33. Franco JG Jr, Baruffi RL, Oliveira JB, Mauri AL, Petersen CG, Contart P, et al. Effects of recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the gnrh-agonist protocol: a matched case-control study. *Reprod Biol Endocrinol* 2009;**7**:58.
34. Liu Y, Chen Q, Yu S, Wang Y, He W, Chang HY, et al. Progestin-primed ovarian stimulation with or without clomiphene citrate supplementation in normal ovulatory women undergoing in vitro fertilization/intracytoplasmic sperm injection: a prospective randomized controlled trial. *Clin Endocrinol (Oxf)* 2018;**88**:442–52.
35. Wen X, Kuang Y, Zhou L, Yu B, Chen Q, Fu Y, et al. Lipidomic components alterations of human follicular fluid reveal the relevance of improving clinical outcomes in women using progestin-primed ovarian stimulation compared to short-term protocol. *Med Sci Monit* 2018;**24**:3357–65.