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Progesterone-Primed Ovarian Stimulation might be a Safe and Effective Alternative to GnRH-antagonist Protocol for Controlled-Ovarian Stimulation of Infertile PCOS Women

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Abstract

Objectives : This study evaluated the safety and efficacy of the progesterone-primed ovarian stimulation (PPOS) coupled with gonadotropin (FSH) for infertile women with polycystic ovary syndrome (PCOS) who were assigned for ICSI and frozen blastocyst transfer.

Patients : 200 infertile women were divided randomly into Group C, which received Cetrorelix injection (0.25 mg daily) on day-6, and Group S, which received oral dydrogesterone (20 mg/day) on day-2 of the menstrual cycle till the trigger day. All patients received an FSH injection of 225 IU daily from day-2 till triggering day. ICSI was performed and day-5 blastocysts underwent vitrification ultra-rapid cryopreservation till being transferred. Outcomes included the ability of PPOS to suppress the premature luteinizing hormone (LH) surge and prevent the development of ovarian hyperstimulation syndrome (OHSS), the incidence of profound LH suppression, the number of retrieved M2 oocyte and fertilization, chemical and clinical pregnancy rates, and the miscarriage rate.

Results: No moderate-to-severe OHSS or premature LH surge was reported in all patients. Serum levels of estradiol and LH increased significantly in all patients with insignificant differences between both groups. The numbers of mature follicles on triggering day, retrieved M2 oocytes and D-5 good-quality blastocysts, pregnancy rates and the miscarriage rate showed non-significant differences between the studied groups.

Conclusion: PPOS protocol for OS is safe and effective for infertile PCOS women. PPOS protocol achieved appropriate LH suppression with nearly no profound suppression or moderate-to-severe OHSS. The outcomes of PPOS protocol were comparable to the GnRH-antagonist protocol, however, PPOS is more cost-effective.

Keywords: Progesterone-primed ovarian stimulation, Premature LH surge, ovarian hyperstimulation syndrome, ICSI outcomes

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects women of reproductive age and is one of the most common causes of infertility [1]. PCOS is a composite endocrinal disorder characterized by hyperandrogenism; ovulatory dysfunction and polycystic ovarian morphology [2]. PCOS women are more frequently confronted with multiple reproductive problems, especially poor oocyte quality and fertilization failure, thus assisted reproductive techniques (ART) might be the sanctuary for infertile PCOS women [3]. The key factor for successful ART is the controlled ovarian stimulation (COS), using either gonadotropin-releasing hormone (GnRH) agonist or an antagonist protocol [4].

However, no protocol is immune; the agonist protocol through pituitary desensitization facilitates antral follicle synchronization, but triggering using the human chorionic gonadotropin (hCG) increases the risk of ovarian hyperstimulation syndrome (OHSS) [5]. On the contrary, the antagonist protocol induces rapid and reversible suppression of luteinizing hormone (LH) secretion, but a varied percentage of patients would experience premature LH surge that was defined as a serum LH level of >10 IU/L before the trigger day [6] and leads to luteinisation of the immature follicles [7].

Infertile PCOS women are mostly at high risk of using any of the COS protocols because of their vulnerability to developing OHSS [2] and have high basal LH levels that were reported in about 30-50% of PCOS women [8].

The rise in the circulating estradiol (E2) level that occurs synchronously with pre-ovulatory ovarian follicle development results in the pre-ovulatory GnRH/LH surge that was prevented by high progesterone levels during the luteal phase [9]. Earlier animal studies assured the ability of progesterone to block LH surge, even if applied after activation of the surge-generating system by high estradiol levels, through inhibition of the transmission of the estradiol signal and/or prevention of the release of the GnRH/LH surge [10]. These findings were the basis for priming with progesterone through the progesterone-primed OS (PPOS) protocol [11].

Objectives

This study tried to evaluate the applicability of PPOS protocol coupled with gonadotropin (FSH) for infertile PCOS women and assigned for ICSI using frozen blastocyst transfer (FBT).

Design

Prospective two-phase randomized comparative study.

Setting

Gynecology & Obstetrics Department, Faculty of Medicine, Zagazig University and multiple private centers.

Patients

All women attending the Infertility clinic were evaluated for selection of infertile PCOS women, who are the study target. PCOS was diagnosed according to the Rotterdam criteria for PCOS diagnosis, which include the presence of oligomenorrhea, anovulation, hyperandrogenism, ovaries containing ≥ 12 follicles that measure 2-9 mm in diameter per ovary, ovarian volume of more than 10 ml (12). PCOS was diagnosed in the presence of at least two of the Rotterdam criteria (13). PCOS women underwent full history taking including age, and menstrual, obstetric and medical history. Clinical examinations for determination of body mass index (BMI) calculated as weight divided by the square of the height in meters and gynecological examination including transvaginal ultrasonography (TVU) for assurance of the presence of PCO and/or large ovary, presence of genital tract congenital anomalies and

other causes for infertility. Then, blood samples were withdrawn for estimation of serum levels of the anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), E2, LH, prolactin and thyroid-stimulating hormone in hospital lab.

Exclusion criteria

Women who were younger than 20 or older than 35 years, obese of grade II or III, had poor OR, other causes of infertility, had previous attempts of IVF, had a history of repeated pregnancy loss, endocrinopathies other than PCOS, congenital anomalies of the genital system, concomitant other cause for infertility, maintenance on hormonal therapy for any indication were excluded from the study.

Inclusion criteria

Infertile PCOS women, aged 20-35 years, had BMI <35 kg/m2 and free of exclusion criteria

Ethical Consideration

After the departmental approval of the study protocol, it was discussed with enrolled women and their husbands for acceptance to participate in the study. At the end of the study duration from June 2021 till Jan 2023, the final approval of the study protocol and outcomes was obtained by the University Ethical Committee on 25-6-2023 by approval number: ZU-IRB#10874-25/6-2023 and was registered by Clinical Trial.com by number NCT05939284. The enrolled couples signed a written fully informed consent to participate in the study and accept the result of randomization.

Randomization and grouping

The enrolled women were randomly allocated into Group-C and Group-S groups according to the OS protocol. Randomization was performed using computer software (Excel 2010, Microsoft, Redmond, WA, USA) using a 1:1 sequence with the irregular dropping of numbers to allow proper randomization. The generated sequences were transformed into letters; C and S that were printed on cards given to patients by an assistant who was blind about the significance of the letters. The cards were provided to the gynecologist in charge to carry on the OS protocol assigned for each group.

Study Protocol

The study was divided into two phases: the enrolled women during phase-I received OS according to the protocol assigned for each group and on oocyte retrieval, ICSI was performed. On day-5, the resultant blastocysts were frozen using the vitrification ultra-rapid cryopreservation as previously described by Balaban et al. (14). During phase II, the frozen blastocysts were graded according to the ASEBIR classification system, which entails grading the internal cell mass as A-to-C grades and the degree of expansion of the blastocele expansion on a 2-6 scale; good quality blastocysts must be graded as AA6 on day-5 (15). All women received the same preparation protocol, and good quality blastocysts of grade AA6 were selected for transfer and all women received single FBT.

Phase-I

All patients received FSH (Fostimon, IBSA, Switzerland) intramuscular injections of a daily dose of 225 IU, starting from day-2 of the menstrual cycle till the trigger day. Group-C patients received Cetrorelix subcutaneous injection (Cetrotide, MerckSerono, Germany) on the day-6 in a dose of 0.25 daily till the trigger day. Group-S patients received dydrogesterone (DYG; Duphaston, Abbott Biologicals B.V., Netherlands) in an oral dose of 20 mg/day in parallel with gonadotropin injection from day-2 of the menstrual cycle till the trigger day using 2 amp triptorelin (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany; 0.1 mg amp) when the most follicles were 17-18 mm.

Phase-II

Gonadotropin-releasing hormone agonist (GnRH-a) therapy was started at 7-day before the expected day of the menstrual cycle in the form of triptorelin (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany) subcutaneous injection in a dose of 0.1 mg. Estradiol valerate (Progynova, 2 mg, Bayer Schering Pharma, UK) was started on day-2 of the menstrual cycle as a daily dose of 6 mg for four days and then the dose was adjusted according to the endometrial thickness (ET). Using TVU (Sonoline Prima 7.5 MHz, Siemens) ET was determined in the midsagittal plane as the distance between the outer edges of the endometrial/myometrial interface on days 10 to 12. At ET of 8 mm, progesterone vaginal supp. (Cyclogest; Actavis Co., USA) were received for the 5-day duration, and on the 6th day, the frozen blastocyst was rapidly thawed and transferred. Thereafter, progesterone therapy continued for 14 days whenever chemical testing for pregnancy was performed, and in case of a positive chemical pregnancy test, clinical pregnancy was assured by detecting viable embryos with pulsating heart using US examination.

Evaluated variate

1. Hormonal assay: serum levels of FSH, LH, E2 and prolactin were estimated at the time of enrolment as baseline levels. On day-7 of OS and at the time of triggering, serum E2 and LH were re-estimated.

2. The incidence of premature LH surge, profound LH suppression and moderate-to-severe OHSS. Profound LH suppression was defined as a serum LH level <1 IU/L during OS (6).

3. The numbers of mature follicles, oocytes retrieved M2 oocytes and number of AA6 blastocytes on day-5.

4. Positive chemical pregnancy was determined on detection of serum β-hCG level ≥5 IU/L at 2-wk after FBT. Positive clinical pregnancy was determined on detection of at least one gestational sac with fetal heart activity. The miscarriage rate calculated as the percent of spontaneous or therapeutic abortion during follow-up.

Study outcomes

1. The primary outcome is the success rate of PPOS as regards the suppression of premature LH surge and prevention of the development of OHSS.

2. The secondary outcomes include

- The incidence of profound LH suppression

- The number of retrieved M2 oocytes and fertilization rate

- The pregnancy rates and the incidence of miscarriage.

Results

Through 2-year study since June 2021, 238 PCOS infertile women were evaluated, 16 women were out of age range, 7 women had BMI>35 kg/m2, 4 women had previous failed ART trials, 3 women were maintained on hormonal therapy, and a woman had endocrinopathy. These 31 women and another 7 women, who were missed during follow-up, were excluded, and 200 women were enrolled in the study (Fig. 1). The enrolment data of women of both groups showed insignificant differences as shown in Table 1.

Table (1): Enrolment data

Variate		Group C	Group S	P-value
Age (years)		28.2±3.4	28±4.2	0.711
BMI (Kg/m2)		29.9±2.2	30.3±2.6	0.158
Duration of infertility (years)		2.98±1.1	2.66±1.2	0.056
Hormonal assay	AMH (ng/ml)	5.08±0.5	4.97±0.5	0.141
	FSH (IU/L)	5.96±1	6.19±0.7	0.057
	Prolactin (ng/ml)	18.6±4.1	18.3±3.2	0.568
TVU data	Ovarian volume (cc)	11.33±1.2	11.38±1.7	0.808
	Antral follicular count (follicles)	12.1±1.7	12.3±1.9	0.433

LH: Luteinizing hormone; *: indicates significant difference versus baseline level
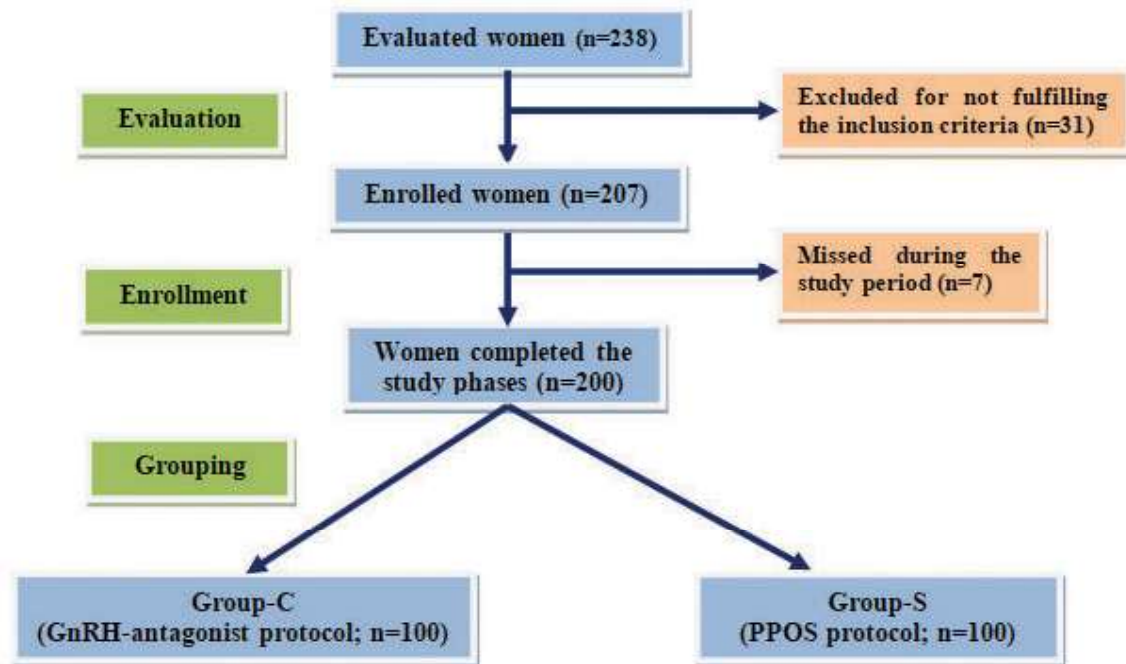
Figure 1: Study Flow Chart

All women had passed Phase-I concerning OS uneventfully and no patient developed moderate-to-severe OHSS. Estimated serum LH levels decreased significantly (P<0.001) at D-7 and time of triggering in patients of both groups in comparison to their respective baseline levels. However, both applied protocols provided perfect LH suppression during OS as manifested by the non-significant differences between mean LH levels estimated in samples of both groups at baseline, D-7 of OS and at triggering time (Fig. 2). Fortunately, no patient developed premature LH surge and on D-7, 53 women developed profound suppression and at triggering time 7 women had profound LH suppression with non-significantly lower incidence of profound LH suppression in Group-S patients (Table 2).

Table (2): LH dynamics during OS

Variate		Group C	Group S	P-value
Serum LH (IU/L)	Baseline	5.86±1.58	5.95±1.75	0.703
	D-7 of OS	4.85±2.91*	4.48±2.44*	0.332
	Triggering time	5.1±1.96*	4.79±1.65*	0.228
Incidence of premature surge (>10 IU/L)		0	0	
Incidence of profound suppression	D-7 of OS	30 (30%)	23 (23%)	0.262
	Triggering time	5 (5%)	2 (2%)	0.248

*: indicates significant difference versus baseline level; P-value indicates the significance of difference between both groups; P>0.05 indicates insignificant difference
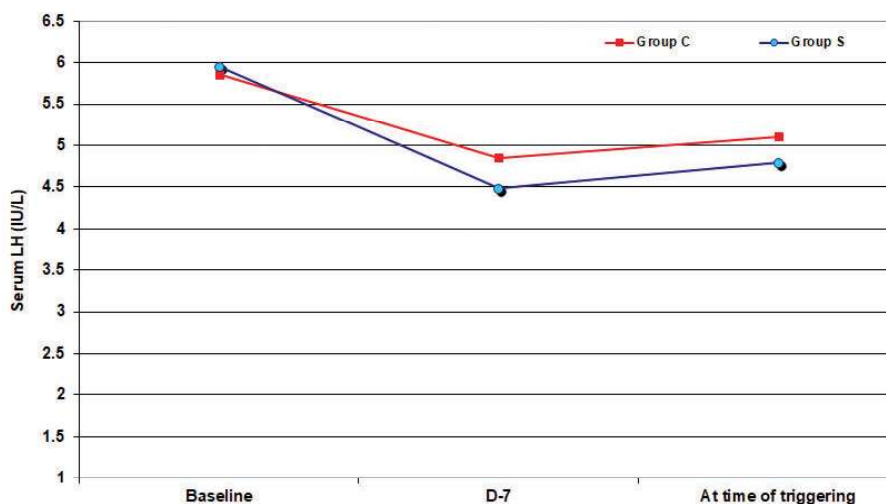

Fig. 2: Mean serum LH levels estimated in samples of women of the studied groups

The estimated serum E2 levels increased progressively and significantly in samples obtained at D-7 of OS and the triggering day in comparison to baseline levels in patients of both groups. However, the differences in estimated serum E2 levels in samples of both groups were insignificantly higher in favor of Group-S (Fig. 3). Correlation analysis showed a positive insignificant relation between the percentage of increase in serum levels of E2 and LH at the triggering day in relation to levels estimated in D-7 samples of Group-C (r=0.126, p=0.213) and Group-S (r=0.064, p=0.527); however, the relation in case of Group-C was nearer to be significant (Fig. 4). The numbers of mature follicles on triggering day, retrieved M2 oocytes and D-5 good quality blastocysts were non-significantly higher in Group-C than Group-S (Table 3).

Table (3): Phase-I outcomes

Variate		Group C	Group S	P-value
Serum E2	Baseline	39.92±16.6	44.2±20.3	0.104
	D-7 of OS	1298.5±466.6*	1347±604*	0.525
	Triggering time	2199.7±507.8*	2281.7±586.2*	0.292
Number of	Mature follicle on triggering day	21.9±4.4	20.5±5.7	0.054
	Retrieved M2 oocytes	18.9±4.2	17.8±5.4	0.109
	D-5 good quality embryos	14.7±3.5	13.6±4.7	0.061

*: indicates significant difference versus baseline level; P-value indicates the significance of difference between both groups; P>0.05 indicates insignificant difference
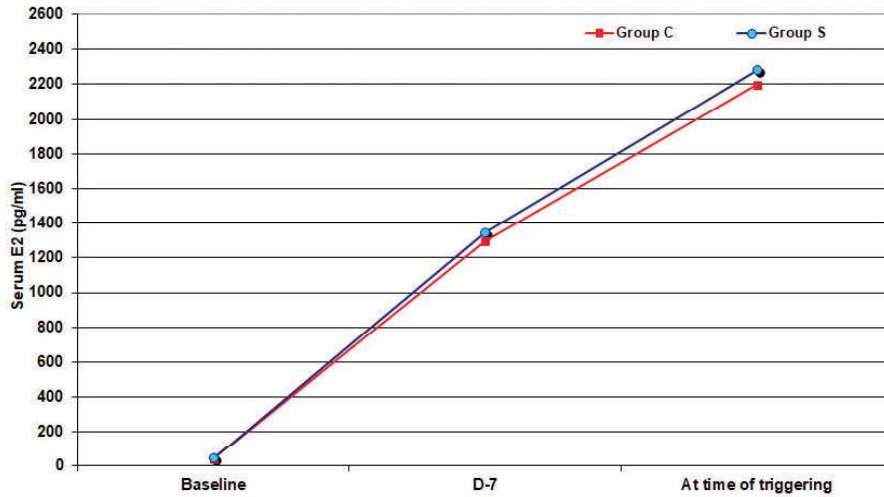
Fig. 3: Mean serum E2 levels estimated in samples of women of the studied groups
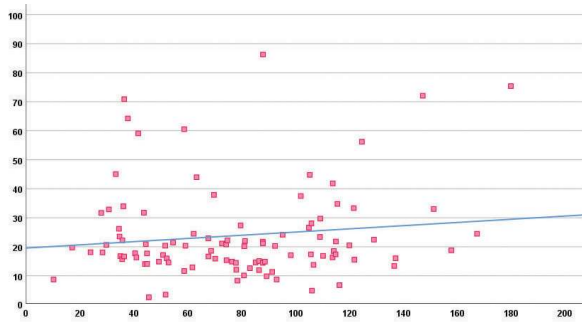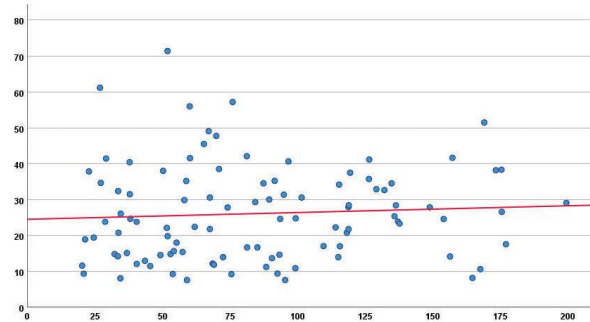

Fig. 4a   Fig. 4b

Correlation between the percentage of change in serum levels of LH & E2 estimated in D-7 and triggering day samples of patients of groups C (Fig. 4a) & S (Fig. 4b)

Regarding the Phase-II outcomes, the total chemical pregnancy rate was 71.5% with a non-significant (P=0.434) difference between both groups. Totally, 90 women showed viable gestational sac on US examination for clinical pregnancy rate of 45%; 49% and 41% in groups C and S, respectively. Unfortunately, 12 women had miscarriages that account for 13.3% of women who had viable gestational sacs, and Group-S women had a higher miscarriage rate (17.1%) than Group-C women (10.2%), but the difference is insignificant (P=0.339) as shown in Table 4

Table (3): Phase-I outcomes

Variate		Group C	Group S	P-value
Pregnancy rates	Chemical	74 (74%)	69 (69%)	0.434
	Clinical	49 (49%)	41 (41%)	0.256
Miscarriage rate		5 (10.2%)	7 (17.1%)	0.339

The P-value indicates the significance of the difference between both groups; P>0.05 indicates the insignificant difference

Discussion

The main target for the OS protocols is to be safe and successful in the provision of the required M2 oocyte without complications that mainly for PCOS women include premature LH surge [8] and/or development of OHSS [2]. Fortunately, the studied PPOS protocol using dydrogesterone (DYG) could achieve this target with results comparable to that of the settled GnRH-antagonist protocol. These findings supported the document by Zhu et al. [16] that oral utrogestan achieved LH suppression during controlled OS (COS) with no premature LH surge. Thereafter, Guan et al. [17] reported a lower rate of OHSS with PPOS versus GnRH-antagonist protocol and Khurana et al. [18] reported no cases of severe OHSS and insignificantly lower incidence of mild-to-moderate OHSS with PPOS than antagonist protocol. Recently, Xu et al. [19] retrospectively documented the clinical efficacy of PPOS and GnRH-a long protocol applied for patients with normal OR and found PPOS could reduce the incidence of OHSS, especially in risky women. Further, Tandulwadkar et al. [20] prospectively documented the ability of PPOS using medroxyprogesterone acetate (MPA) and Gn-RH-antagonist protocols to prevent the premature LH surge in hyper-responders undergoing COS.

Regarding the progesterone formula, the used oral DYG resulted in outcomes comparable to that of the GnRH-antagonist protocol as regards abolishment of OHSS and LH suppression. In line with the efficacy of DYG, Huang et al. [21] reported consistent LH suppression with no premature surge with DYG and MPA, but DYG induced lower LH levels with lower consumption of hMG than MPA.

Further, the outcomes of the fixed PPOS protocol using a fixed daily dose of DYG started on day-2 of the menstrual cycle were efficient, in line with these findings, Durdağ et al. [22] compared the outcomes of the fixed versus the flexible PPOS protocol that entails giving DYG 20 mg daily starting when the leading follicle was 12 mm or serum E2 level >200 pg/ml and reported insignificant differences between both protocols but in favor of the fixed one especially for preventing LH surge.

Evaluated dynamics of LH secretion showed progressively decreased incidence of profound suppression (<1 ng/ml) with insignificant difference between both protocols, despite being higher with Gn-RH-antagonist. Further, the reported non-significant difference between the numbers of retrieved oocytes indicated the absence of a relation between the extent of suppression and OS yield. This finding is coincident with Goh et al. [23] who found Gn-RH antagonist is associated with a risk of profound LH suppression, but without significant differences in IVF and pregnancy outcomes in comparison to women without significant LH suppression.

Interestingly, the dynamics of both LH and E2 showed a positive insignificant relation with both protocols, but the significance was more with Gn-RH-antagonist. This finding indicated the multiplicity of the mechanisms of LH suppression by progesterone other than desensitization of the pituitary to the raising levels of E2 and supports the results of earlier animal studies that progesterone inhibits the transmission of the estradiol signal and prevents the release of the GnRH/LH surge [10]. Recent animal studies found progesterone also suppresses LH surge at the hypothalamic levels through increased dynorphin and GABAA receptor signaling that act through kisspeptin neurons in the anteroventral periventricular hypothalamic nucleus [24, 25].

Regarding the outcomes of frozen blastocyte transfer (Phase II), there was an insignificant difference between the effect of applied OS protocols on pregnancy rates or the miscarriage rate, despite being in favor of the GnHR-antagonist protocol. These data

are in line with multiple studies documenting the non-significant differences between PPOS and antagonist protocols regarding pregnancy outcomes after frozen embryo transfer [17, 26-28].

Patients' selection based on normal OR was targeted to equalize the results for comparative purposes and such selection was in line with the findings of Zhou et al. [28] who retrospectively found the cumulative living birth rate (LBR) after PPOS was significantly lower with normal, while was higher with poor OR than with Gn-RH-antagonist protocol, but for PCOS women the fertilization and pregnancy rates and the LBR was comparable with insignificant differences between PPOS and antagonist protocols.

Further, the PPOS protocol is a cost-effective protocol, where the cost of DYG is meaningless compared to that of Cetrorelix (4 vs. 500 EP/day), considering the reported non-significant differences in outcomes, PPOS is a resource-sparing and safe protocol. In support of these suggestions, Guo et al. [29] found PPOS protocol applied for women with advanced ovarian endometriosis resulted in significantly lower hMG doses and shorter duration of therapy with no differences in outcomes, but with lower costs. Also, Filippi et al. [30] found PPOS protocol for OS in cancer women who wish to preserve their fertility is an easy and affordable protocol with similar efficacy, but is friendlier and more economical than the recombinant FSH and GnRH-antagonist protocol. Furthermore, Zhao & Wang [31] found combining the PPOS protocol with clomiphene citrate and gonadotropin for IVF-ET in older women with poor OR effectively blocked the premature LH surge with an increased number of mature oocytes and recommended this protocol for such a population of women. Also, Kao et al. [32] found flexible GnRH-antagonist protocol had a higher risk of premature LH surges without improved pregnancy rates in poor ovarian responders compared to PPOS.

Conclusion

PPOS protocol for COS is the apropos choice for infertile PCOS women as preparatory for ICSI. PPOS protocol achieved appropriate LH suppression with nearly no profound suppression and no moderate-to-severe OHSS. The outcomes of PPOS were comparable to the GnRH-antagonist protocol; however, PPOS is more cost-effective.

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