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Original Article

Treatment outcomes using different progesterones for external fertilization-embryo transfer with progestin primed ovarian stimulation (PPOS) in patients with poor ovarian response

Xiaoyu Zhang[1], Hong Zhang[2], Zhaowen Ma[1], Bingqing Jiang[3], Chonglan Wang[1], Chen Ding[1]

Departments of [1]Reproductive Center, [2]Obstetrics and Gynecology, [3]Postpartum Healthcare Department, Zaozhuang Maternal and Child Healthcare Hospital, Zaozhuang 277000, Shandong, China

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Abstract: Objective: This study analyzed the outcomes obtained using different progesterone treatments with external fertilization-embryo transfer and progestin primed ovarian stimulation (PPOS) in patients with poor ovarian response. This study also explored the value of dydrogesterone in the PPOS protocol for patients with a poor ovarian response. Methods: The clinical data obtained from patients with poor ovarian responses who underwent *in vitro* fertilization-embryo transfer IVF-ET in the Zaozhuang Maternal and Child Healthcare Hospital from 2017 to 2019 were retrospectively analyzed. Ovulation was induced using PPOS. The hormone levels, medication status, and clinical outcomes were compared between patients who received medroxyprogesterone acetate as the progesterone treatment (MPA group) and patients who received dydrogesterone as the progesterone treatment (DYG group). Results: There were no significant differences in the basic characteristics between the MPA group and DYG group (P>0.05). The Gn concentrations, E2 levels on the day of HCG administration, and LH levels showed trends that were higher in the DYG group than the MPA group, but the differences did not reach statistical significance (P>0.05). The DYG group displayed a higher progesterone (P) level on the day of HCG administration, and LH levels that were greater than 10 IU/L and less than 20 IU/L during the stimulation period, both of which were statistically significant (P<0.05). There were no significant differences between the two groups in the number of retrieved oocytes, 2PN fertilization rate, cleavage rate, transplantable embryo rate, quality embryo rate, freezing cycle rate, early ovulation rate, and non-transplantable embryo cycle rate (P>0.05). With respect to the thawing cycle, the DYG group exhibited a trend towards higher clinical pregnancy and implantation rates, but the differences did not reach statistical significance (P>0.05). There were no significant differences in early spontaneous abortion and multiple pregnancy rates between the two groups (P>0.05). Also, no triplet or ectopic pregnancies were observed in either group. Conclusion: Dydrogesterone can effectively suppress LH peaks and prevent premature ovulation when used in the PPOS protocol, and it is a safer drug than MPA. Thus, dydrogesterone could be used in place of medroxyprogesterone acetate in PPOS for patients with poor ovarian response.

Keywords: Ovulation stimulation, PPOS protocol, IVF-ET, poor ovarian response

Introduction

Kuang et al. [1] first proposed the progestin primed ovarian stimulation (PPOS) protocol in 2015, which uses high progesterone treatment to suppress LH peaks during stimulation of ovulation. The obtained embryos are frozen and transplanted at a later time. The PPOS protocol exhibits simplicity, low cost, and effective control of LH peaks [2, 3]. Also, adequate numbers of oocytes are retrieved, the embryos are of high-quality, and the subsequent pregnancy rate for the frozen embryos that are transferred is similar to that of traditional protocols [4, 5]. Thus, the PPOS protocol has gradually become a new option for ovulation stimulation in patients with poor ovarian response [6, 7].

The PPOS protocol initially utilized medroxyprogesterone acetate (MPA) as the exogenous progesterone, and currently, the MAP+HMG method is still widely used in clinical practice for

ovarian stimulation. However, MPA is a synthetic progesterone, its safety has been a point of concern and thus it has been contraindicated for use during human pregnancy due to its controversial safety [8, 9]. On the other hand, dydrogesterone is a natural progesterone. Due to its higher level of safety, dydrogesterone is often used clinically for abortion prevention in pregnancies for which spontaneous abortion is a risk [10]. Based on drug safety considerations, the use of dydrogesterone in the PPOS protocol for patients with poor ovarian response should be considered as a replacement for MPA to control LH peaks effectively, and produce favorable pregnancy outcomes. In this study, we retrospectively analyzed the clinical data from patients with poor ovarian responses who elected to use the PPOS protocol for ovulation stimulation for the first time. We compared the hormone levels, medication status, and final clinical outcomes between patients who received medroxyprogesterone acetate as the exogenous progesterone (MPA group) and patients who received dydrogesterone as the exogenous progesterone (DYG group).

Data and methods

Subjects and inclusion criteria

Subjects: Patients who underwent IVF-ET/ICSI-ET at the Reproductive Medicine Center of Zaozhuang Maternal and Child Healthcare Hospital from January 2017 to June 2019 were included in this study. The study was approved by the Ethics Committee of Zaozhuang Maternal and Child Healthcare Hospital. Because this was a retrospective study, a signed informed consent form was not required from the patients.

Inclusion criteria: Patients were included in the study who were diagnosed with a poor ovarian response according to the Bologna criteria for poor ovarian responses. The patients received PPOS for ovulation stimulation, and patients chose MPA plus HMG or DYG plus HMG protocols for their IVF-ET or ICSI-ET therapy.

Exclusion criteria: Patients were excluded if they were diagnosed with adenomyosis, an abnormal uterine cavity, untreated hydrosalpinx, or immunological diseases. Patients also were excluded if the patient or their spouse exhibited any chromosomal abnormalities.

Group composition

Patients in the MPA group received medroxyprogesterone acetate plus HMG. Patients DYG group received dydrogesterone plus HMG.

Controlled ovarian hyperstimulation

Starting on the second or third day of the patient's menstrual cycle, 10 mg/d medroxyprogesterone acetate (Zhejiang Xianju Pharmaceutical, China), or 20 mg/d dydrogesterone (Abbott Biologicals B.V., Netherlands) was given orally until ten days after oocyte retrieval. Considering the patient's age, body mass index (BMI), anti-Müllerian hormone (AMH) level, and ovarian reactivity in previous ovulation stimulation cycles, an intramuscular injection of 150-225 U/d menotrophin (HMG, Zhuhai Lizhu Group, China) was given. Vaginal B ultrasound and blood FSH, LH, E2, and *P* values were monitored every two to three days to appropriately adjust the Gn dose until the day of HCG administration. When the diameter of one follicle reached 18 mm, or the diameters of two follicles reached 17 mm, or the diameters of three follicles reached 16 mm, 6,000 to 10,000 units of human chorionic gonadotropin (HCG, Zhuhai Lizhu Group, China) were administered intramuscularly. Thirty-four to Thirty-six hours after HCG administration transvaginal B ultrasound-guided puncture and suction of follicles with a diameter greater than 10 mm was carried out for oocyte retrieval.

Fertilization and embryo culture

The semen used for fertilization was subjected to standard analysis based on the WHO laboratory manual. Motile sperm were collected using density gradient centrifugation. External fertilization was performed 39 to 40 hours after HCG injection. After sperm preparation, ICSI was performed when the concentration of motile sperm was less than 5×10^6/mL; Otherwise, short-term IVF fertilization was used. The embryos were cultured in an external fertilization culture solution (Vitrolife Sweden AB), and the fertilization status was observed 16 to 20 hours after oocyte retrieval. The presence of double pronuclei visualized under a microscope indicated normal fertilization. Embryo cleavage was observed after 72 hours.

Evaluation of embryo quality [11]

The embryos were graded as follows. Grade I indicated that the blastomeres were uniform in size, exhibited transparent and uniform cytoplasm, and no debris was present. Grade II embryos exhibited uniform blastomeres with good symmetry, and the cytoplasmic debris was less than 20%. Grade III embryos consisted of blastomeres with an uneven appearance, and the cytoplasmic debris was approximately 20 to 50%. Grade IV embryos consisted of blastomeres with an uneven appearance, and the cytoplasmic debris was greater than 50%. Embryos graded I and II were considered to be high-quality embryos, while embryos graded I to III still were considered transplantable.

The blastocyst scoring criteria utilized six different grades. Grade 1 indicated that the blastocyst cavity occupied less than half of the total embryo volume, and was considered an early blastocyst. Grade 2 indicated that the blastocyst cavity occupied half of the embryo volume, and was considered an intermediate blastocyst. Grade 3 was designated if the blastocyst cavity occupied the entire embryo, which was defined as a complete blastocyst. Grade 4 was designated if the blastocyst cavity continued to enlarge, and the zonapellucida became thin, which allowed the blastocyst to expand. Grade 5 was indicated if the trophectoderm cells protruded from the zonapellucida, which indicated that the blastocyst was in the process of hatching. Grade 6 was designated if the blastocyst had escaped from the zonapellucida, indicating that the blastocyst had hatched.

The inner cell mass (ICM) from blastocysts graded 3 to 6 were further classified as ICM-A, tightly connected, abundant cells; ICM-B, a few, loosely grouped cells; and ICM-C, very few cells. The trophectoderm (TE) from blastocysts graded 3 to 6 was classified as TE-A, with multiple cells present that formed epithelial layers; TE-B, with a loose epithelial layer comprised of a few cells; TE-C, consisting of a layer of very few, large TE cells. Blastocysts classified as Grades of 3BB or higher, with the ICM and TE not graded as C, were considered high-quality embryos. All high-quality embryos (including 8-cell embryos graded I and II) were vitrified three days after oocyte retrieval, and lower-quality embryos were cultured further to form blastocysts. Blastocysts that achieved grade 3 or higher by five days after oocyte retrieval were frozen using vitrification.

Intimal preparation and luteal support for frozen-thawed embryo transfer (FET)

Intimal preparation for FET: A natural cycle program was used for patients who experienced regular menstruation. Nine to ten days after the initiation of menstruation, vaginal B-ultrasound was performed to monitor follicle number and size. When the follicles had developed to 16 mm or larger in diameter, the endometrium was 7 mm or greater in thickness, E_2 was 150 pg/ml or higher and P was 1.0 pg/ml or lower in sampled blood, 6,000 iu HCG was injected intramuscularly. Forty mg/d progesterone (Zhejiang Xianju Pharmaceutical, China) was injected intramuscularly, starting the following day. On the fourth and sixth day of progesterone injection, embryos were transplanted on days designated D3 and D5.

A hormone replacement protocol was used with patients who experienced irregular menstruation and ovulation disorders. Estradiol valerate (1 mg/tablet, Bayer Medical Care Co., Ltd.) was administered orally at 2 mg/dose, three times daily from the second to the fourth day of the menstrual cycle. Ten to 14 days after initiation of oral administration of estradiol valerate, if the endometrium was 7 mm or greater in thickness, and E_2 was 150 pg/ml or higher and P was 1.0 pg/ml or lower in the sampled blood, 40 mg/d progesterone (Zhejiang Xianjing Pharmaceutical, China) was injected intramuscularly. On the fourth and sixth days of progesterone injection, embryos were transplanted on days D3 and D5, respectively.

Luteal support: After transplantation, 40 mg/d progesterone was injected intramuscularly, accompanied by 20 mg/d dydrogesterone which was given orally. Alternatively, 400-600 mg/d progesterone was administered vaginally as a soft capsule (Utrogestan, Besins Manufacturing, France) accompanied by 20 mg/d dydrogesterone given orally. β-HCG levels that were 25 U/L or higher in blood samples assessed at 14 days after transplantation were considered indicative of a biochemical pregnancy. The presence of a gestational sac visualized with B ultrasound five weeks after transplantation indicated a clinical pregnancy.

Table 1. Patient characteristics from the MPA and DYG groups

	MPA Group (n = 330)	DYG Group (n = 216)	*P* value
Woman's age (years)	38.21 ± 5.62	36.94 ± 4.08	0.39
Man's age (years)	38.91 ± 6.75	38.50 ± 5.00	0.81
Infertility years (years)	4.49 ± 3.76	3.14 ± 3.05	0.19
Ratio of secondary infertility (%)	89.5	95.2	0.64
BMI (kg/m^2)	24.19 ± 3.17	24.87 ± 2.90	0.43
Antral follicle count (number)	4.69 ± 2.02	4.33 ± 2.06	0.55
AMH (ng/mL)	1.07 ± 0.84	0.98 ± 0.84	0.26
Basal FSH (IU/ml)	9.83 ± 3.76	11.64 ± 5.02	0.12
Basal LH (IU/ml)	4.26 ± 1.73	4.78 ± 2.26	0.14
Basal E2 (pg/ml)	110.27 ± 88.17	110.93 ± 75.71	0.83
Basal P (ng/ml)	0.75 ± 0.58	0.82 ± 0.60	0.66
IVF times	2.84 ± 1.29	2.48 ± 1.47	0.19

Statistics

Statistical analysis was performed using SPSS version 19.0. The analysis methods included t-tests and chi-square tests. Continuous data were presented as mean (SD) and assessed using t-tests for two independent groups. Count data were presented as numbers and percentages. The odds ratio (OR) and 95% CI were calculated using logistic regression. P<0.05 indicated statistical significance.

Results

Patient characteristics in the MPA and DYG groups

As shown in **Table 1**, there were no significant differences between the MPA group and the DYG group concerning the ages of men and women, the ratio of secondary infertility, IVF times, number of years of infertility, body mass index (BMI), antral follicle count (AFC), AMH levels, and the basal endocrine parameters (P>0.05).

Comparison of hormone levels between the MPA and DYG groups during ovulation stimulation

As shown in **Table 2**, there were no significant differences between the MPA and DYG groups in the basal LH levels and LH levels after five to seven days of stimulation (P>0.05). The DYG group exhibited a trend towards higher E_2 and LH levels on the day of HCG administration, but the differences did not reach statistical significance (P>0.05). The DYG group presented a higher P level on the day of HCG administration, which was statistically significant (P<0.05). During the stimulation process, the ratio of patients with a LH surge (LH≥10 IU/L) was significantly higher in the DYG group than the MPA group (P<0.05).

Comparison of clinical outcomes between the MPA and DYG groups

As shown in **Table 3**, the Gn levels and Gn days revealed trends to be higher in the DYG group than the MPA group, but the differences were not significant (P>0.05). There were no significant differences between the MPA and DYG groups concerning the number of retrieved oocytes, 2PN fertilization rate, cleavage rate, transplantable embryo rate, quality embryo rate, and cycle cancellation rate (P>0.05).

Pregnancy outcomes of freeze-thaw cycles in the MPA and DYG groups

At the study completion, 120 patients in the DYG group had thawed embryos in this cycle, and 168 patients in the MPA group had thawed embryos in this cycle.

As shown in **Table 4**, there was no statistical difference between the two groups in the percentage of embryos transferred (P>0.05). The clinical pregnancy rate and implantation rate showed a slightly higher trend in the DYG group than the MPA group, but the differences were not significant (P>0.05). Differences in the early pregnancy rate and multiple pregnancy

Table 2. Comparison of hormone levels between the MPA and DYG groups during ovulation stimulation

	MPA Group (n = 330)	DYG Group (n = 216)	*P* value
Basal LH (IU/L)	4.26 ± 1.73	4.78 ± 2.26	0.14
LH after 5-7 days' stimulation (IU/L)	6.78 ± 3.15	5.67 ± 3.52	0.32
LH on the day of HCG administration (IU/L)	6.03 ± 3.63	6.94 ± 5.03	0.49
E2 on the day of HCG administration (pg/mL)	1070.91 ± 700.52	1312.62 ± 1284.52	0.48
P on the day of HCG administration (ng/mL)	0.83 ± 0.76	1.92 ± 1.88	0.03*
Ratio of patients with LH surge (%)	4.1	22.2	0.02*

*indicated statistical significance.

Table 3. Comparison of clinical outcomes between the MPA and DYG groups

	MPA Group (n = 330)	DYG Group (n = 216)	*P* value
Gn amount	1643.30 ± 489.26	2012.50 ± 338.60	0.68
Gn days	8.04 ± 1.68	9.22 ± 1.48	0.75
Number of retrieved oocytes	3.93 ± 2.41	3.71 ± 2.75	0.75
2PN fertilization count (IVF)	2.77 ± 1.74	2.54 ± 1.76	0.68
2PN fertilization count (ICSI)	3.36 ± 2.94	2.75 ± 0.96	0.11
Normal cleavage count (IVF)	2.73 ± 1.55	2.38 ± 1.71	0.49
Normal cleavage count (ICSI)	3.36 ± 2.94	2.75 ± 0.96	0.11
Number of transferable embryos	1.33 ± 1.30	1.40 ± 1.12	0.86
High quality embryos	1.02 ± 1.02	1.00 ± 0.82	0.95
MII oocyte count	3.35 ± 2.12	2.43 ± 1.51	0.13
2PN fertilization rate (IVF)	74.1%	76.5%	0.74
2PN fertilization rate (ICSI)	88.9%	80.0%	0.20
2PN cleavage rate	99.0%	96.1%	0.21
Transplantable embryo rate (per oocyte)	34.4%	40.2%	0.06
Quality embryo rate (per oocyte)	26.6%	27.1%	0.16
Cycle cancellation rate	17.3%	16.6%	0.25

Table 4. Pregnancy outcomes of freeze-thaw cycles in the MPA and DYG groups

	MPA Group (n = 168)	DYG Group (n = 120)	OR (95% CI)	*P* value
Average number of embryos transferred	2.00	1.80		0.16
Single embryo transplantation (%)	13.1	20.0	0.75 (0.39-1.45)	0.40
Number of hormone replacement cycles (%)	78.6	80.0	1.09 (0.61-1.95)	0.66
FET clinical pregnancy rate (%)	42.9	50.0		0.62
Implantation rate (%)	36.2	44.4	0.71 (0.50-1.00)	0.052
Early abortion rate (%)	25.0	20.0	1.13 (0.50-2.56)	0.78
Multiple pregnancy rate (%)	33.3	40.0	0.75 (0.367-1.53)	0.75
Ectopic pregnancy rate (%)	0	0		

rate between the two groups were not statistically significant (P>0.05). Neither group presented a triplet pregnancy or ectopic pregnancy.

Discussion

The conventional protocol used in the IVF-ET process might cause excessive ovarian suppression, thereby leading to high gonadotropin consumption, a higher number of ovulation stimulation days, and an increased risk of ovarian hyperstimulation syndrome (OHSS). More reproductive physicians now prefer the use of gentle stimulation or micro-stimulation protocols for ovulation stimulation. A micro-stimulation protocol is easy to use, inexpensive, and,

most importantly, dramatically reduces the incidence of ovulation stimulation complications. However, due to the early high LH level in follicles, a micro-stimulation protocol is prone to induce premature ovulation due to endogenous LH peaks, resulting in cycle cancellation. Excessive LH levels also might affect oocyte quality [12]. Numerous studies have reported that LH peaks cannot be induced under high progesterone levels, despite the administration of large doses of estrogen.

In 1984 [13], progesterone was used to suppress the pre-ovulation LH peak caused by positive estrogen feedback. Based on a study in sheep, Richter et al. [14] proposed that progesterone might directly or indirectly regulate GnRH neuron activity by regulating related nuclei controlled by the hypothalamic arcuate nucleus, which played a role in canceling the positive feedback of estrogen. Studies have shown that the application of progesterone during the follicular phase could reduce the LH pulse frequency, increase the LH pulse amplitude, and reduce the average level of plasma LH. Considering previous research on clinical micro-stimulation protocols and luteal phase applications, in 2015, KuangYanping first proposed the continuous use of a specific dose of exogenous progesterone coupled with a Gn ovulation stimulation protocol. This PPOS protocol suppressed endogenous LH peaks during the early follicular phase. Subsequently, the PPOS protocol has received increasing attention from researchers worldwide, especially in treating patients with ovarian dysfunction and poor ovarian responses. In this study, dydrogesterone was used for the first time in a PPOS protocol for patients with poor ovarian response to investigate whether the use of dydrogesterone among patients with poor ovarian response could effectively control LH peaks, inhibit premature ovulation, and achieve a favorable pregnancy outcome.

In current clinical practice, the most commonly used progesterone in PPOS protocols for patients with poor ovarian response is medroxyprogesterone acetate (MPA). However, MPA is a synthetic progesterone contraindicated for use during human pregnancy due to its controversial safety. Thus, the safety of MPA has been the focus of attention. MPA exhibits dose-related teratogenicity and toxicity in animal tests. Even though previous studies have shown that the use of MPA within therapeutic doses does not increase the risk of fetal malformations in humans, nor does MPA harm oocyte quality, fertilization, or embryonic developmental potential [15, 16], MPA is contraindicated for use during human pregnancy. Dydrogesterone is a synthetic stereoisomer of progesterone that exhibits high oral bioavailability. Its unique molecular structure provides high binding specificity and agonistic activity at progesterone receptors [17]. However, it exhibits zero or negligible activity associated with androgens, adrenocortical hormones, and glucocorticoid receptors, so undesirable side effects are minimized. Thus, dydrogesterone is widely used as a safer option than MPA to prevent spontaneous abortions in at-risk patients.

There was no difference between the two groups concerning the basal LH levels and LH levels at mid-ovulation stimulation in this study. However, the P levels on the day of HCG administration and the incidence of LH elevation>10 IU/L were significantly higher in the DYG group than the MPA group, which indicated that MPA could more effectively inhibit the hypothalamus-pituitary-ovarian axis than dydrogesterone. This result might be related to the time of action for the different progesterones and the regulation of different GnRH secretion patterns in the hypothalamus [18, 19]. However, the LH levels of the two groups did not increase above 20 IU/L on average, and no patient experienced a canceled cycle due to premature ovulation. Therefore, dydrogesterone appeared to inhibit LH peaks in patients with poor ovarian responses effectively.

There were no significant differences between the two groups in the number of retrieved oocytes, 2PN fertilization rate, cleavage rate, transplantable embryo rate, quality embryo rate, and freezing cycle rate. These results are consistent with a prospective randomized controlled study published by Sha Yu et al. [9] in 2018. In that study, 516 patients with normal ovarian reserve function who underwent IVF-ET/ICSI-ET for the first time were treated with dydrogesterone plus HMG or medroxyprogesterone acetate plus HMG to stimulate ovulation. There were no significant differences in the clinical pregnancy rate, implantation rate, early abortion rate, and multiple pregnancy

rate between the two groups of embryos after FET, indicating that the embryos of both groups had similar developmental potential.

To conclude, we believe that dydrogesterone is safe to use in PPOS protocols for patients with poor ovarian response in place of medroxyprogesterone acetate for patients with poor ovarian responses. However, due to the limited sample size of this study, we need to carry out prospective randomized controlled studies with larger sample sizes and multi-regional populations to confirm whether dydrogesterone is advantageous.

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Disclosure of conflict of interest

None.

Address correspondence to: Chen Ding, Zaozhuang Maternal and Child Healthcare Hospital, 25 Wenhua East Road, Shizhong District, Zaozhuang, Shandong, China. E-mail: 124400170@qq.com

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