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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Different progestin-primed ovarian stimulation protocols in infertile women undergoing in vitro fertilization/intracytoplasmic sperm injection: an analysis of 1188 cycles

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Abstract

Purpose To evaluate the efficacy in suppressing the premature LH surge, embryo quality and pregnancy outcomes of progestin-primed ovarian stimulation (PPOS) protocols using medroxyprogesterone acetate versus utrogestan in women of all ages undergoing in vitro fertilization or intracytoplasmic sperm injection.

Methods 1188 patients were enrolled in the retrospective study, of which 1002 patients were treated with medroxyprogesterone acetate (M group) and recombinant follicle-stimulating hormone (r-FSH)simultaneously from day 3 of the cycle until trigger day, while 186 patients were treated with utrogestan (U group) and r-FSH instead. Viable embryos were cryopreserved for later transfer in both groups. Differences in baseline characteristics, ovarian stimulation characteristics, endocrinological characteristics, embryo development and clinical outcome between two groups were assessed. Statistical analyses were performed stratified by age and number of oocytes retrieved.

Results No significant differences were observed in the baseline characteristics, ovarian stimulation characteristics and clinical outcome of patients between groups. However, blastulation rate in the U group was significantly higher than that in the M group (49.4% vs. 32.9%, $P<0.001$). During ovarian stimulation, LH levels remained steady in both groups. Higher percentage of premature LH surge was found in the U group (2.4% vs. 10.2%, $P<0.001$), especially for patients aged more than 35 years or who had three oocytes or less retrieved.

Conclusions Both the administration of medroxyprogesterone acetate and utrogestan in PPOS were sufficient to prevent an untimely LH rise, while for patients with poor ovarian response or aged above 35 years, MPA may result in a more satisfactory LH level. PPOS protocol using medroxyprogesterone acetate or utrogestan was comparable in terms of oocytes and pregnancy outcome, whereas the administration of utrogestan may result in an improved blastulation than medroxyprogesterone acetate, which needs further exploration.

Keywords Progestin-primed ovarian stimulation (PPOS) · Medroxyprogesterone acetate (MPA) · Utrogestan · Embryo quality · Luteinizing hormone surge

Introduction

Premature luteinizing hormone (LH) surge is a major issue during controlled ovarian hyperstimulation (COH), which may result in advanced ovulation and cycle cancellation. Though gonadotropin-releasing hormone agonist (GnRH-a) and GnRH antagonist had been used as effective suppressant of the premature LH surge, the disadvantages have attracted increasing attention, such as excessive pituitary downregulation, increased dosage of gonadotropin (Gn), increased risk of ovarian hyperstimulation syndrome (OHSS) and high cost. As freeze-all strategy arises, which may avoid

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the potential harmful effects of ovarian stimulation on endometrial receptivity, and thus improve IVF outcomes, it is possible to consider new ovarian stimulation regimens [8]. Progestin-primed ovarian stimulation (PPOS), using progestin for suppressing the premature LH surge during the follicular phase, has been promoted in infertile women of normal ovarian reserve and polycystic ovarian syndrome [1, 16]. In combination with freeze-all strategy and double trigger, the risk of OHSS decreased in the PPOS protocol [8]. Both medroxyprogesterone acetate (MPA) and utrogestan have been used as effective oral alternatives to GnRH analog in the PPOS protocol, with an advantage of being user friendly in comparison to the requirement of repeated injections of the GnRH analog. However, concerns remain regarding over pituitary suppression or spontaneous LH surge in women with different ovarian reserves. Whether both MPA and utrogestan can be applied in PPOS with a moderate pituitary suppression for all ages of women is unclear. Due to different progestins with different biological effects contained in MPA and utrogestan, the differences in embryo quality and pregnancy outcome, which have not been reported in previous studies, need to be assessed. A retrospective study was designed to evaluate the efficacy of suppressing the premature LH surge, embryo quality and pregnancy outcomes of progestin-primed ovarian stimulation protocols using medroxyprogesterone acetate versus utrogestan in women of all ages undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

Materials and methods

Patients

A retrospective study was conducted with clinic-based data in the Reproductive Medicine Centre of the Sixth Affiliated Hospital of Sun Yat-sen University from May 2016 to January 2018. The data were approved by the ethics committee and, owing to the retrospective nature, the requirement of informed consent was waived. The following inclusion criteria were used: undergoing IVF or ICSI treatments by administration of MPA + recombinant follicle-stimulating hormone (r-FSH)or utrogestan + r-FSH protocol; regular menstrual cycles over the previous 3-month period. Couples were excluded if female patients were diagnosed with adenomyosis, polycystic ovarian syndrome, uterine cavity abnormalities, untreated hydrosalpinx, immunologic disease, or had any contraindications to progesterone and Gn use. Male patients whose sperm was collected by surgery were not included in the study because of the possible association with embryo quality. Depending on doctor's customs, patients used either MPA + r-FSH protocol or utrogestan + r-FSH protocol for ovarian stimulation. For analysis, couples were further grouped as follows: patients using MPA + r-FSH protocol were grouped into the "M group", while patients using utrogestan + r-FSH protocol were grouped into the "U group". After excluding and grouping, 1188 cycles were analyzed, of which 1002 cycles were available for the M group and 186 cycles for the U group.

Controlled ovarian stimulation

MPA (Zhejiang Xianju Pharmaceutical Co., China) 10 mg or utrogestan (Laboratories Besins International, Paris, France) 0.2 g was administered from the 3rd day of menstrual cycle (day 3).

At the same time, r-FSH(Gonal-F, Serono, Sweden)was administered in both group individually, until the trigger day. Follicle growth was monitored by means of serum follicle-stimulating hormone (FSH), estradiol (E_2), progesterone and LH measurements and vaginal ultrasound investigation every 2–4 days. When one follicle reached a diameter of 18 mm or two follicles reached 17 mm, triptorelin (Decapeptyl, Ferring pharmaceuticals, Germany) 0.1 mg and hCG (Lizhu Pharmaceutical Trading Co., China) 1000 IU were administered. Oocyte retrieval was performed 36 h later. To examine the vitality of semen and sperm, standard analysis was performed according to the World Health Organization laboratory manual, after which mobile sperm was collected by means of density gradient centrifugation and the sperm swim-up technique. At the same time, oocytes were retrieved through ultrasound-guided puncture and then cultured in human tubal fluid (HTF) medium (Quinn's Advantage fertilization HTF medium; Quinn's, SAGE, USA), in 5–6% CO_2 at 37 °C. IVF was carried out 39–40 h after hCG injection. ICSI was performed if the concentration of motile sperm was $< 1 \times 10^6$/mL after sperm preparation; otherwise, a conventional IVF method was used.

Hormonal measurement

Serum FSH, LH, E_2 and progesterone levels were measured on day 3, day 8–10 and day 10–12 of the menstrual cycle, as well as the day of trigger and oocyte retrieval. Hormonal levels were measured by immunoassay (cobas e 601, Riche, Sweden). The lower limits of sensitivity were as follows: FSH, 0.100 IU/L; LH, 0.100 IU/L; E_2, 5 pg/ml; progesterone, 0.1 ng/ml. Since the upper limit of E_2 measurement was 3000 pg/ml, in the case of a serum E_2 level higher than that, the serum was diluted for a second measurement. Intra- and inter-assay coefficients of variation were 5.6% and 7.8% for FSH, 5.8% and 9.1% for LH, 8.2% and 9.3% for E_2 and 7.0% and 9.6% for progesterone.

Embryo culture and assessment

On day 1 after oocyte retrieval, the granulosa cells were stripped off, and the single fertilized egg was transferred into a 20-mL droplet of balanced and oil-covered cleavage medium (Quinn's, SAGE). Normal fertilization was verified by the observation of the pronucleus under microscopy. The presence of two pronuclei indicated normal fertilization. Embryos were cultured in commercial sequential IVF medium (Quinn's Advantage Cleavage Medium; SAGE, Pasadena, CA, USA) as described previously [4]. On day 2, day 3 and day 5 after oocyte retrieval, embryos were observed, and the morphological scores were assessed under microscopy. D3 embryos were assessed for cell number, fragmentation and asymmetry of blastomere, according to grading criteria modified from the criteria described by Racowsky et al. [9]. Grade 1: the size of the blastomeres was uniform, with no fragmentation; Grade 2: the blastomere size was slightly uneven with fragmentation < 20%; Grade 3: the blastomere size was heterogeneous or with fragmentation 20–50%; and Grade 4: fragmentation > 50%. Embryos with more than four cells and graded 1–2, or four-cell-embryo with a grade of 1 were defined as available D3 embryo. High-scoring D3 embryos were defined as embryos with six to nine cells and their grade of fragmentation and asymmetry of blastomere were at least 2. According to the strategy of embryo freezing conducted in our center, two of the good-quality embryos in D3 were frozen and others had culture extended into blastocyst. Blastocysts were graded from 1 to 6 depending on the degree of expansion and hatching status [12]: grade 1: blastocoele takes up less than half of total embryo volume, referring to an early blastocyst; grade 2: blastocoele occupies half of total embryo volume, developing into an intermediate blastocyst; grade 3: blastocoele takes up the whole embryo, defined as a full blastocyst; grade 4: blastocoele continues growing and the zona pellucida demonstrates thinning, becoming an expanded blastocyst; grade 5: herniation occurs of trophectoderm cells from the zona pellucida, described as a hatching blastocyst; and grade 6: blastocyst escapes from the zona pellucida, developing into a hatched blastocyst. The inner cell mass (ICM) and trophectoderm (TE) of blastocysts of Grades 3–6 were furthermore graded as follows: ICM: A (tight connection, many cells); B (grouped loosely, several cells); and C (very few cells); and TE: A (multiple epithelial layers, many cells); B (loose epithelium layers, few cells); and C (very few large TE cells). Blastocysts which reached grade 2 or more at D5 were transferred or frozen. Blastocysts of grade ≥ 3BB (neither C grades in the evaluations of ICM nor TE) were defined as high scoring.

Thawed embryo transfer (TET)

For patients with regular ovulation, TET was carried out with a natural cycle or modified natural cycle. Follicle development and serum LH were monitored from the 10th day of the natural menstrual cycle. When the dominant follicle reached a diameter of 18 mm, blood test was performed. If the LH peak was not present, 10,000 IU hCG (Lizhu Pharmaceutical Trading Co., China) was administered. Vaginal ultrasound was performed 24–48 h after the LH peak or 36–48 h after hCG injection, and the absence of dominant follicle was defined as ovulation. Embryos were transferred 3 or 5 days after ovulation. TET was carried out with hormone replacement cycle for patients with irregular menstrual cycle. As described previously [3], luteal support was given with the administration of 20–40 mg of progesterone in oil until 14 days after embryo transfer and was administered until 9–12 weeks of gestation in pregnant patients.

Results determination

Serum β-hCG level was measured to perform a biochemical pregnancy test 12 days after embryo transfer. Fetal heart rate, fetal sac number and embryo development were evaluated with the administration of B ultrasound examination 3 weeks after the blood test, in which the fetal heart beat in an intrauterine gestational sac was defined as clinical pregnancy. The presence of two or more fetal sacs per pregnancy was defined as multiple gestation. The implantation rate was defined as the number of gestational sacs seen on the ultrasound divided by the total number of embryos transferred. In China, miscarriage is defined as the loss of fetal heartbeat before 28 weeks of gestation age.

Subgroup statistical analysis

Differences between groups in baseline characteristics of patients, ovarian stimulation characteristics, endocrinological characteristics, embryo development and clinical outcome were assessed. All the statistical analyses were performed with SPSS statistics software, version 20.0. Patient characteristics, ovarian stimulation characteristics and endocrinological characteristics are presented as *n* or the means ± standard deviations. The rates of premature LH surge, embryo development and pregnancy are presented as n/n. Hormone profile during ovarian stimulation is presented with a broken line graph, created by Excel. Statistical analyses were performed with the Chi square test, Yates' correction or Fisher's exact probabilities (after comparing frequencies/proportions) and the independent *t* test (after comparing means). Stepwise multiple linear regression analysis was conducted to assess the potential factors related to the value of LH on the trigger day. Since the data included

infertile women in all age groups and with different ovarian reserves, which may result in different ovarian response to stimulation, statistical analyses were performed stratified by age and number of oocytes retrieved, including baseline characteristics of patients, ovarian stimulation characteristics and clinical outcome. $P<0.05$ was considered as statistically significant.

Results

Patient characteristics

Tables 1 and 2 show the comparison of basic patient characteristics between ovarian stimulation groups. No statistically significant differences were found between groups with respect to age, duration of infertility, body mass index (BMI), anti-Müllerian hormone (AMH), basal FSH and antral follicle count ($P>0.05$). No difference was observed in the proportion of primary infertility ($P>0.05$).

Ovarian stimulation characteristics

The ovarian stimulation characteristics and embryological outcomes of both groups are presented in Tables 3 and 4. For patients aged above 35 years, those in the U group were administered a greater amount of Gn than those in the M group (1912.03 ± 670.28 vs. 1747.69 ± 727.11 IU, $P=0.016$). In total, there was no significant difference in the dosage of Gn between groups when all ages of patients were taken into account. The mean number of oocytes retrieved was 5.13 in the M group and 5.10 in the U group ($P>0.05$), respectively. The dosage of Gn per MII oocyte was comparable in the two groups ($P>0.05$). About 33.4% (335/1002) of cycles and 30.2% (1552/5137) of oocytes in the M group underwent ISCI for fertilization, and about 30.1% (56/186) of cycles and 28.9% (274/949) of oocytes in the U group underwent ISCI ($P>0.05$).

Embryological outcomes

There was no statistical difference in the MII rate, 2PN rate, 2PN cleavage rate and available embryo rate between groups. For patients aged below 35 years, the rate of good-quality embryo at cleavage stage in the M group was slightly higher than that in the U group (63.8% vs. 55.3%, $P=0.024$), while no difference was found in the other subgroups or in the overall statistics. Compared with the M group, a significantly higher percentage of blastulation was noted in the U group, with the overall statistics (32.9% vs. 49.4%, $P<0.001$) and stratified analyses showing that this association was found in all subgroups. No difference was found in

Table 1 Comparison of patient demographics between two groups stratified by age

Characteristics	ALL			< 35 or 35 years			> 35 years		
	M group ($n=1002$)	U group ($n=186$)	*P*	M group ($n=308$)	U group ($n=53$)	*P*	M group ($n=694$)	U group ($n=133$)	*P*
Female age (year)	37.89 ± 5.43	38.07 ± 5.17	0.678	31.18 ± 3.01	31.28 ± 3.03	0.810	40.87 ± 3.09	40.77 ± 2.83	0.736
Male age (year)	39.84 ± 6.54	40.50 ± 6.53	0.205	33.32 ± 4.59	33.45 ± 3.74	0.84	42.73 ± 5.01	43.31 ± 5.14	0.227
Female infertility (year)	5.27 ± 4.20	5.28 ± 4.08	0.964	4.45 ± 2.60	4.22 ± 2.45	0.548	5.64 ± 4.70	5.72 ± 4.51	0.864
Male infertility (year)	4.87 ± 4.21	4.74 ± 4.05	0.691	4.07 ± 2.59	3.85 ± 2.47	0.563	5.23 ± 4.71	5.10 ± 4.48	0.762
Secondary infertility, % (*n*)	67.0 (671/1001)	66.1 (123/186)	0.810	40.4 (124/307)	39.6 (21/53)	0.916	78.8 (547/694)	76.7 (102/133)	0.585
Failed cycles	2.45 ± 1.72	2.51 ± 1.79	0.681	2.41 ± 1.54	2.23 ± 1.37	0.418	2.47 ± 1.79	2.62 ± 1.93	0.382
BMI (kg/m^2)	22.70 ± 2.87	22.63 ± 2.78	0.761	21.86 ± 3.11	21.76 ± 3.06	0.831	23.08 ± 2.68	22.99 ± 2.58	0.726
AMH (ng/ml)	1.50 ± 1.67	1. 50 ± 1.89	0.956	2.08 ± 2.25	1.89 ± 2.53	0.575	1.23 ± 1.24	1.35 ± 1.55	0.324
Basal FSH (IU/ml)	9.13 ± 4.51	9.58 ± 4.82	0.241	8.51 ± 4.09	9.80 ± 5.29	0.051	9.42 ± 4.66	9.49 ± 4.62	0.869
Antral follicle count	7.05 ± 5.38	6.88 ± 5.04	0.773	8.03 ± 6.18	7.69 ± 5.70	0.709	4.91 ± 3.56	4.88 ± 3.63	0.922

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

BMI body mass index, *AMH* anti-Müllerian hormone

*P values < 0.05

Table 2 Comparison of patient demographics between two groups stratified by number of oocytes retrieved

Characteristics	< 3 or 3 oocytes			> 3 oocytes		
	M group ($n=473$)	U group ($n=91$)	P	M group ($n=529$)	U group ($n=95$)	P
Female age (year)	39.40 ± 5.10	38.97 ± 4.57	0.447	36.54 ± 5.36	37.21 ± 5.58	0.264
Male age (year)	41.04 ± 6.5	41.15 ± 6.04	0.873	38.77 ± 6.39	39.87 ± 6.95	0.126
Female infertility (year)	5.19 ± 4.26	4.91 ± 3.88	0.571	5.35 ± 4.15	5.65 ± 4.26	0.523
Male infertility (year)	4.75 ± 4.24	4.33 ± 3.80	0.371	4.98 ± 4.18	5.14 ± 4.25	0.736
Secondary infertility, % (n)	70.6 (334/473)	64.8 (59/91)	0.272	65.6 (347/529)	64.2 (61/95)	0.794
Failed cycles	2.74 ± 1.99	2.86 ± 2.07	0.597	2.20 ± 1.38	2.18 ± 1.40	0.880
BMI (kg/m^2)	22.91 ± 2.75	22.70 ± 2.56	0.505	22.52 ± 2.97	22.57 ± 2.98	0.881
AMH (ng/ml)	0.73 ± 0.73	0.70 ± 0.46	0.643	2.18 ± 1.95	2.28 ± 2.36	0.641
Basal FSH (IU/ml)	10.60 ± 5.32	11.17 ± 5.38	0.379	7.83 ± 3.12	8.09 ± 3.66	0.487
Antral follicle count	3.30 ± 2.10	3.31 ± 2.26	0.976	7.96 ± 5.29	7.71 ± 4.91	0.659

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

BMI body mass index, *AMH* anti-Müllerian hormone

*P values < 0.05

the percentage of cycles in which at least one cryopreserved embryo was available (68.9% vs. 66.1%, $P=0.461$). Besides, more patients aged below 35 years in the M group chose to receive embryo transfer before the end of this study than those in the U group ($P<0.05$).

Pregnancy outcomes

In total, 990 embryos were transferred in the M group, and 168 embryos were transferred in the U group. 190 out of 585 cycles in the M group and 32 out of 102 cycles in the U group resulted in clinical pregnancy, which included 23 and 6 twin pregnancies, respectively. As Tables 5 and 6 show, biochemical pregnancy rate, clinical pregnancy rate per transfer, implantation rate, miscarriage rate and ongoing pregnancy rate were comparable between groups. Although it took nearly 1 year to collect the pregnancy outcome after completing oocyte retrieval, the live birth rate was still conservative, as there was ongoing pregnancy until the end of the study. Therefore, ongoing pregnancy rate was assessed to provide a more credible marker for comparison.

Hormone profile and multiple linear regression analysis

Figure 1 presents the hormone profiles of both groups, which includes the values of serum FSH, LH, E_2 and P. In both groups, the FSH levels increased after Gn administration. Compared with the M group, the LH levels on day 10–12 and day of trigger in the U group were significantly higher (6.14 vs. 4.92 IU/L, $P<0.05$, 5.41 vs. 4.26 IU/L, $P<0.05$, respectively). In general, the LH levels remained steady during stimulation in both groups, below 10 IU/L. The E_2 values increased gradually in both groups after Gn administration and decreased rapidly on the OPU day. The E_2 level showed significant difference between groups in day 10–12, the trigger day and the OPU day (M group versus U group, 881.47 versus 1132.89 pg/ml, $P<0.05$, 1326.31 versus 1858.25 pg/ml, $P<0.05$, 639.83 versus 842.26 pg/ml, $P<0.01$, respectively). In the U group, the serum P values increased after the administration of utrogestan and remained steady at a range of 13.51–19.88 ng/ml. However, the P value in the M group did not increase until the OPU day. Except the basal P value, the serum P value in the U group was significantly higher than that in the M group ($P<0.01$).

In the M group, the LH level slightly decreased from day 8 to 10 ($P<0.001$). However, in the U group, the LH level remained steady from the beginning of COS to the trigger day. There was no significant difference in the value of basal LH between groups ($P>0.05$), whereas the LH level on the trigger day in the U group was significantly higher than that in all subgroups of the M group ($P<0.05$), except patients aged below 35 years. As Tables 7 and 8 show, compared with the M group, the percentage of patients with LH levels above 10 IU/L on the trigger day was higher in the U group (5.5% vs. 12.9%, $P<0.001$), especially for patients aged above 35 years. To adjust the potential confounding effect of basal LH, the LH surge was assessed by the combination of LH levels on the trigger day and basal LH. For 24 out of 1002 (2.4%) patients in the M group, the LH level on the trigger day was above 10 IU/L and more than two times the basal LH, and the percentage in the U group was 19/186 (10.2%) ($P<0.001$). Furthermore, multiple linear regression analysis was performed to assess the potential factors related to the value of LH on the trigger day. The dose of Gn, basal LH, AMH,

Table 3 Comparison of ovarian stimulation characteristics and embryological outcomes between groups stratified by age

Characteristics	ALL			< 35 or 35 years			> 35 years		
	M group ($n=1002$)	U group ($n=186$)	P	M group ($n=308$)	U group ($n=53$)	P	M group ($n=694$)	U group ($n=133$)	P
Starting dose of Gn (IU)	212.82 ± 56.84	216.47 ± 45.97	0.345	210.57 ± 56.25	204.75 ± 47.94	0.490	213.82 ± 57.12	220.87 ± 44.60	0.114
Total dosage of Gn (IU)	1762.19 ± 724.31	1838.79 ± 662.80	0.182	1794.83 ± 718.07	1647.79 ± 608.49	0.168	1747.69 ± 727.11	1912.03 ± 670.28	**0.016***
Gn duration (day)	8.20 ± 2.52	8.41 ± 2.11	0.275	8.45 ± 2.39	8.00 ± 2.14	0.204	8.08 ± 2.57	8.57 ± 2.09	**0.039***
Number of oocytes retrieved	5.13 ± 4.58	5.10 ± 4.89	0.947	6.90 ± 5.76	6.75 ± 7.00	0.867	4.34 ± 3.68	4.44 ± 3.56	0.762
ICSI cycles, % (n)	33.4 (335/1002)	30.1 (56/186)	0.375	40.3 (124/308)	37.7 (20/53)	0.729	30.4 (211/694)	27.1 (36/133)	0.441
ICSI oocytes, % (n)	30.2 (1552/5137)	28.9 (274/949)	0.408	35.7 (759/2126)	38.0 (136/358)	0.404	26.3 (793/3011)	23.4 (138/591)	0.129
MII rate, % (n)	69.6 (3574/5137)	70.4 (668/949)	0.615	68.0 (1446/2126)	69.6 (249/358)	0.563	70.7 (2128/3011)	70.9 (419/591)	0.913
Total dosage of Gn/number of MII oocytes (IU)	783.30 ± 630.18	829.26 ± 668.29	0.389	667.28 ± 619.22	662.69 ± 475.26	0.961	835.48 ± 628.58	893.96 ± 721.01	0.360
2PN rate of IVF oocytes, % (n)	62.3 (1876/3013)	64.9 (371/572)	0.239	59.3 (635/1071)	65.1 (108/166)	0.158	63.9 (1241/1942)	64.8 (263/406)	0.738
2PN rate of ICSI oocytes, % (n)	72.1 (1119/1552)	74.5 (204/274)	0.422	71.1(540/759)	72.1(98/136)	0.829	73.0 (579/793)	76.8 (106/138)	0.350
2PN cleavage rate, % (n)	97.3 (2914/2995)	96.2 (553/575)	0.141	97 (1140/1175)	95.6 (197/206)	0.295	97.5 (1774/1820)	96.5 (356/369)	0.282
Available embryo rate, % (n)	81.6 (2378/2914)	79.4 (439/553)	0.220	79.9 (911/1140)	74.6 (147/197)	0.091	82.7 (1467/1774)	82.0 (292/356)	0.760
Good quality embryo rate at cleavage-stage, % (n)	65.17 (1899/2914)	62.7 (349/553)	0.256	63.8 (727/1140)	55.3 (109/197)	**0.024***	66.1 (1172/1774)	67.4 (240/356)	0.623
Blastulation rate, % (n)	32.9 (579/1762)	49.4 (169/342)	**< 0.001***	33.9 (224/660)	53.6 (59/110)	**< 0.001***	32.2 (355/1102)	47.4 (110/232)	**< 0.001***
Good quality blastocysts rate, % (n)	3.4 (96/1762)	6.1 (21/342)	0.609	4.8 (32/660)	6.4 (7/110)	0.502	5.8 (64/1102)	6.0 (14/232)	0.894
Cryopreserved cycles rate, % (n)	68.9 (690/1002)	66.1 (123/186)	0.461	79.2 (244/308)	81.1 (43/53)	0.750	64.3 (446/694)	60.2 (80/133)	0.366
Transferred cycles rate, % (n)	78.3 (540/690)	78.0 (96/123)	0.958	81.6 (199/244)	67.4 (29/43)	**0.035***	76.5 (341/446)	83.8 (67/80)	0.150
Transferred embryos rate, % (n)	66.7 (990/1485)	65.6 (168/256)	0.744	62.2 (377/606)	50.5 (47/93)	**0.032***	69.7 (613/879)	74.2 (121/163)	0.248

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

ICSI intracytoplasmic sperm injection

*P values < 0.05

grouping and age of women were significantly associated with the value of LH on the trigger day, with standardized coefficients of − 0.336, 0.279, − 0.166, − 0.180 and 0.121, respectively ($P < 0.01$).

Discussion

As early as 1983 [7], data represented that an LH surge was achieved in mid-follicular phase when progesterone infusion was superimposed 48 h after the initiation of estradiol (E_2) infusion. However in 1984 [15], progestin was used to block the estradiol-induced positive feedback to preovulatory LH surge. Studies had been performed to assess the role of progesterone in regulating LH secretion patterns, which demonstrated that progesterone could either inhibit the LH surge by pretreatment before estradiol-induced LH secretion or increase the LH surge by treatment at the time of the surge-inducing estradiol increment [6, 7, 11]. In conclusion, the roles that progesterone performs in LH surge differ in line with the timing and concentration of administration. Besides, neuroendocrine experiments [2, 10, 11] indicated that the inhibition of the LH surge by progesterone was associated with preventing the activation of GnRH neurons that are activated by estradiol, while the increase in the LH surge had no relation to the increase in GnRH neurons. It has been reported that in contrast with the GnRH antagonist, PPOS suppresses pituitary LH secretion in an indirect and slow way, mediated by the classical P receptor of the hypothalamus, preventing both the activation and transmission phases of the E_2-induced surge [5]. In this study, the administration of medroxyprogesterone acetate or utrogestan in progestin-primed ovarian stimulation turned out to be sufficient to prevent an untimely LH rise in infertile women in both groups.

This is the first study to compare the efficacy of PPOS using MPA and utrogestan in infertile women in all age groups and with different ovarian reserve. On the basis that no difference was found in basal LH, AMH, age of women and the dose of Gn administered between groups, which were proved to be significantly associated with the value of LH on the trigger day with multiple linear regression analysis, the use of MPA or utrogestan was believed to be related

Table 4 Comparison of ovarian stimulation characteristics and embryological outcomes between groups stratified by number of oocytes retrieved

Characteristics	< 3 or 3 oocytes			> 3 oocytes		
	M group ($n = 473$)	U group ($n = 91$)	P	M group ($n = 529$)	U group ($n = 95$)	P
Starting dose of Gn (IU)	196.24 ± 58.61	203.33 ± 44.89	0.201	227.11 ± 51.19	228.64 ± 43.77	0.762
Total dosage of Gn (IU)	1535.32 ± 761.97	1641.29 ± 721.91	0.226	1963.33 ± 624.3	2023.82 ± 543.889	0.376
Gn duration (day)	7.69 ± 2.97	7.93 ± 2.46	0.474	8.64 ± 1.93	8.86 ± 1.61	0.299
Number of oocytes retrieved	1.86 ± 0.82	1.79 ± 0.74	0.466	8.05 ± 4.58	8.27 ± 5.08	0.666
ICSI cycles, % (n)	25.4 (120/473)	22.0 (20/91)	0.493	40.6 (215/529)	37.9 (36/95)	0.615
ICSI oocytes, % (n)	22.9 (201/879)	20.9 (34/163)	0.573	31.7 (1351/4258)	30.5 (240/786)	0.508
MII rate, % (n)	77.0 (677/879)	77.9 (127/163)	0.803	68.0 (2897/4258)	68.8 (541/786)	0.661
Total dosage of Gn/number of MII oocytes (IU)	1148.11 ± 702.01	1202.11 ± 680.65	0.537	503.76 ± 379.65	521.25 ± 475.14	0.728
2PN rate of IVF oocytes, % (n)	69.3 (445/642)	69.3 (88/127)	0.996	60.4 (1431/2371)	63.6 (283/445)	0.199
2PN rate of ICSI oocytes, % (n)	77.1 (155/201)	82.4 (28/34)	0.496	71.4 (964/1351)	73.3 (176/240)	0.531
2PN cleavage rate, % (n)	97 (582/600)	98.3 (114/116)	0.649	97.4 (2332/2395)	95.6 (439/459)	**0.044***
Available embryo rate, % (n)	86.4 (503/582)	78.9 (90/114)	**0.04***	80.4 (1875/2332)	79.5 (349/439)	0.662
Good quality embryo rate at cleavage-stage, % (n)	71.1 (414/582)	68.4 (78/114)	0.561	63.7 (1485/2332)	61.7 (271/439)	0.437
Blastulation rate, % (n)	27.0 (79/293)	48.1 (25/52)	**0.002***	34.0 (500/1469)	49.7 (144/290)	**< 0.001***
Good quality blastocysts rate, % (n)	2.7 (8/293)	7.7 (4/52)	0.072	6.0 (88/1469)	5.9 (17/290)	0.933
Cryopreserved cycles rate, % (n)	54.3 (257/473)	50.5 (46/91)	0.507	81.9 (433/529)	81.1 (77/95)	0.853
Transferred cycles rate, % (n)	73.2 (188/257)	73.9 (34/46)	0.914	81.3 (352/433)	80.5 (62/77)	0.873
Transferred embryos rate, % (n)	83.9 (292/348)	77.4 (48/62)	0.211	61.4 (698/1137)	61.9 (120/194)	0.902

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

ICSI: intracytoplasmic sperm injection

*P values < 0.05

Table 5 Comparison of pregnancy outcomes between groups stratified by age

Characteristics	All				<35 or 35 years				>35 years			
	M group ($n=1002$)	U group ($n=186$)	OR (95% CI)	*P*	M group ($n=308$)	U group ($n=53$)	OR (95% CI)	*P*	M group ($n=694$)	U group ($n=133$)	OR (95% CI)	*P*
Biochemical pregnancy rate, % (n)	36.4 (213/585)	34.3 (35/102)	1.096 (0.704–1.705)	0.684	44.9 (101/225)	35.7 (10/28)	1.466 (0.648–3.317)	0.356	31.1 (112/360)	33.8 (25/74)	0.885 (0.521–1.505)	0.652
Clinical pregnancy rate per transfer, % (n)	32.5 (190/585)	31.4 (32/102)	1.052 (0.669–1.655)	0.826	40.4 (91/225)	32.1 (9/28)	1.434 (0.621–3.310)	0.397	27.5 (99/360)	31.1 (23/74)	0.841 (0.488–1.449)	0.533
Implantation rate, % (n)	21.5 (213/990)	22.6 (38/168)	0.938 (0.634–1.388)	0.748	27.9 (105/377)	23.4 (11/47)	1.263 (0.620–2.574)	0.519	17.6 (108/613)	22.3 (27/121)	0.745 (0.463–1.198)	0.223
Miscarriage rate, % (n)	21.1 (40/190)	21.9 (7/32)	0.952 (0.384–2.361)	0.916	13.2 (12/91)	0 (0/9)	–	0.095	28.3 (28/99)	30.4 (7/23)	0.901 (0.335–2.426)	0.837
Ongoing pregnancy rate, % (n)	78.4 (149/190)	78.1 (25/32)	1.018 (0.411–2.519)	0.970	86.8 (79/91)	100 (9/9)	–	0.095	70.7 (70/99)	69.6 (16/23)	1.056 (0.393–2.836)	0.914

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

The ongoing pregnancy rate included the number of ongoing pregnant women as well as the number of live births

There was one case of ectopic pregnancy in M group (age above 35, retrieved more than 3 oocytes)

*P values <0.05

Table 6 Comparison of pregnancy outcomes between groups stratified by number of oocytes retrieved

Characteristics	< 3 or 3 oocytes				> 3 oocytes			
	M group ($n=473$)	U group ($n=91$)	OR (95% CI)	*P*	M group ($n=529$)	U group ($n=95$)	OR (95% CI)	*P*
Biochemical pregnancy rate, % (n)	25.3 (44/174)	22.6 (7/31)	1.160 (0.468–2.879)	0.748	41.1 (169/411)	39.4 (28/71)	1.072 (0.641–1.795)	0.790
Clinical pregnancy rate per transfer, % (n)	20.1 (35/174)	16.1 (5/31)	1.309 (0.469–3.654)	0.606	37.7 (155/411)	38.0 (27/71)	0.987 (0.587–1.658)	0.960
Implantation rate, % (n)	12.3 (36/292)	12.5 (6/48)	0.984 (0.391–2.480)	0.973	25.4 (177/698)	26.7 (32/120)	0.934 (0.602–1.449)	0.761
Miscarriage rate, % (n)	25.7 (9/35)	20.0 (1/5)	1.385 (0.136–14.071)	1.000	20.0 (31/155)	22.2 (6/27)	0.875 (0.325–2.352)	0.791
Ongoing pregnancy rate, % (n)	74.3 (26/35)	80.0 (4/5)	0.722 (0.071–7.34)	1.000	79.4 (123/155)	77.8 (21/27)	1.098 (0.409–2.947)	0.852

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

The ongoing pregnancy rate included the number of ongoing pregnant women as well as the number of live births

There was one case of ectopic pregnancy in M group (age above 35, retrieved more than 3 oocytes)

*P values < 0.05

to the difference in the LH level on the trigger day. Furthermore, lower LH level on the trigger day decreased the trend of LH value and a lower percentage of premature LH surge was found in the M group, especially for patients aged above 35 years. In all, the use of MPA during ovarian stimulation may lead to stronger pituitary suppression than utrogestan for patients aged above 35 years. The possible explanation is that being a natural exogenous progesterone, utrogestan can interfere with serum progesterone measurement and result in the neglect of possible premature LH surge. Moreover, as one of the 17α-hydroxyprogesterone derivatives, MPA has similar progesterone-like actions to natural progesterone, next to dydrogesterone [14]. It has been indicated that 10 mg MPA per day was equal to 300 mg progesterone per day regarding progestogenic effectivity in ovulation inhibition [13]. Previous studies [17] on the relationship between the dose of utrogestan used in the PPOS protocols and the LH surge or the embryo quality had focused on patients with normal ovarian reserve, while the administration of utrogestan in poor responders still lacks attention. Based on the data of this study, we propose that 200 mg utrogestan per day used in the U group of this study may result in an inadequate pituitary suppression for patients with poor response and decreased sensitivity of progesterone receptor.

According to the strategy of embryo freezing conducted in our center, two of the good-quality embryos in D3 were frozen and others had culture extended into blastocyst. In this study, compared with the U group, higher available embryo rate for patients who had three oocytes or less retrieved and higher percentage of good-quality embryo at cleavage stage for patients aged below 35 years were found in the M group. Therefore, more good-quality embryos were available for extended culture in the M group. However, embryos in the U group resulted in higher blastulation rate and comparable pregnancy outcomes in FET cycles compared with embryos in the M group. Due to the retrospective nature and limited sample of this study, whether MPA or utrogestan in the PPOS protocol results in different oocyte and embryo quality remains unknown. Previous studies found that utrogestan resulted in an improved oocyte quality than short protocol for patients with polycystic ovarian syndrome [16], and a comparable oocyte quality compared with short protocol for patients with normal ovarian reserve [18]. A prospective controlled study represented that the MPA protocol was an effective alternative to short protocol for patients with normal ovarian response. However, data on the comparison of different progestin-primed ovarian stimulation protocols are limited. We hypothesized that the difference in the blastulation between groups in this study may be related to the different GnRH secretion patterns regulated by different progestins. As a natural exogenous progesterone, the connection of utrogestan with intracellular progesterone receptors may be stronger, which improved the mediation of biological effects of progesterone. Besides, MPA was contraindicated in human pregnancy, and the long-term safety for children stemmed from ovarian stimulation using MPA

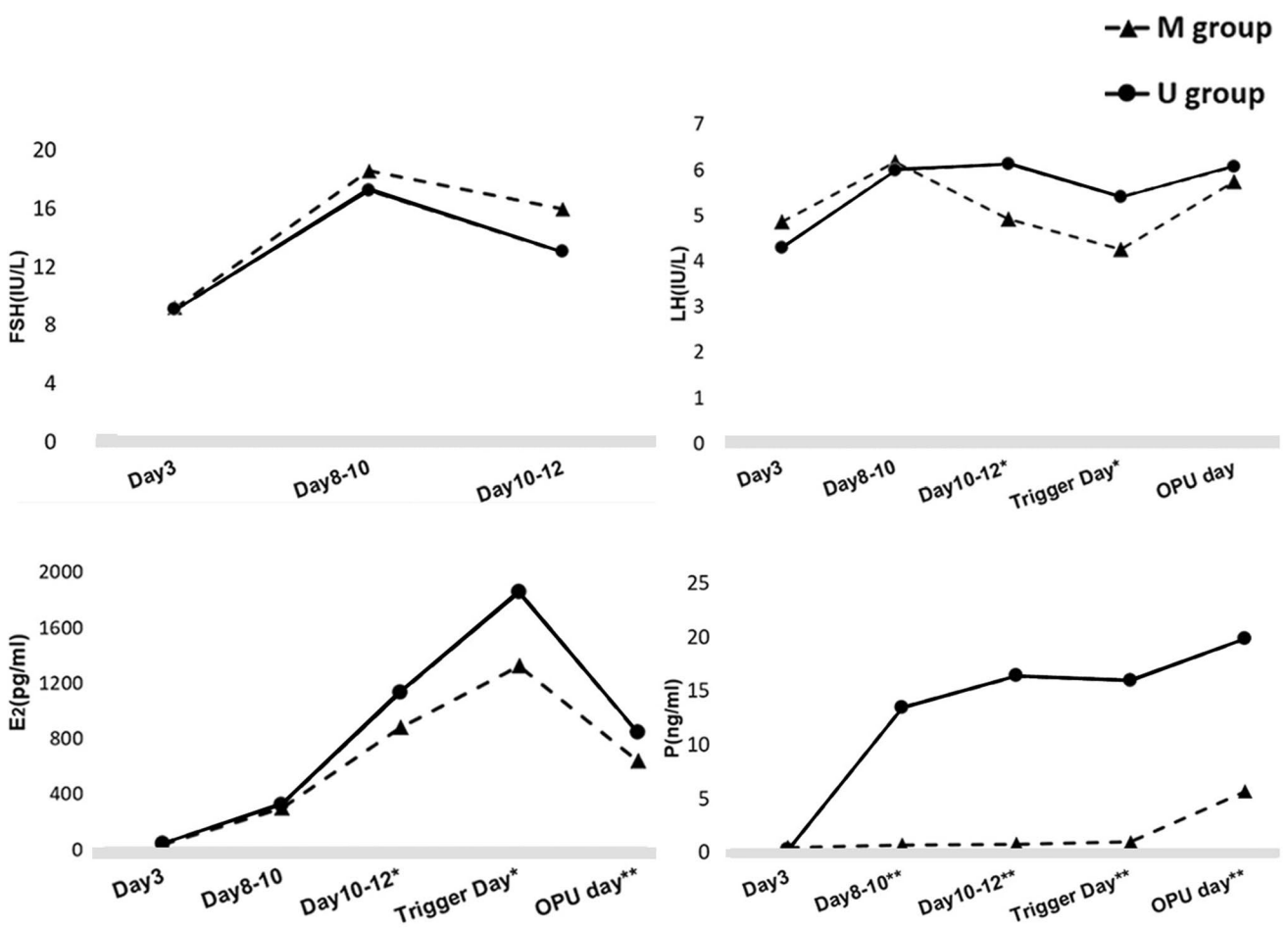

Fig. 1 Hormone profiles during ovarian stimulation of both groups

Table 7 Comparison of LH value in two groups stratified by age

Characteristics	ALL			< 35 or 35 years			> 35 years		
	M group ($n=1002$)	U group ($n=186$)	*P*	M group ($n=308$)	U group ($n=53$)	*P*	M group ($n=694$)	U group ($n=133$)	*P*
b-LH (IU/L)	4.64 ± 2.78	4.39 ± 2.03	0.275	4.42 ± 2.16	3.87 ± 1.50	0.03*	4.75 ± 2.94	4.57 ± 2.16	0.506
t-LH (IU/L)	4.44 ± 3.07	5.90 ± 4.71	< 0.001*	3.68 ± 2.69	4.67 ± 4.08	0.107	4.77 ± 3.10	6.11 ± 4.81	0.002*
t-LH ≥ 10 IU/L, % (n)	5.5 (55/1002)	12.9 (24/186)	< 0.001*	3.9 (12/308)	9.4 (5/53)	0.159	6.2 (43/694)	14.3 (19/133)	0.001*
t-LH ≥ 10 IU/L & t-LH/b-LH > 2, % (n)	2.4 (24/1002)	10.2 (19/186)	< 0.001*	2.3 (7/308)	7.5 (4/53)	0.103	2.4 (17/694)	11.3 (15/133)	< 0.001*

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

b-LH: basal LH, *t-LH*: LH on the trigger day

**P* values < 0.05

Table 8 Comparison of LH value in two groups stratified by number of oocytes retrieved

Characteristics	< 3 or 3 oocytes			> 3 oocytes		
	M group ($n=473$)	U group ($n=91$)	*P*	M group ($n=529$)	U group ($n=95$)	*P*
b-LH (IU/L)	4.98 ± 3.49	4.56 ± 2.03	0.296	4.34 ± 1.94	4.26 ± 2.03	0.707
t-LH (IU/L)	5.55 ± 3.46	7.67 ± 5.57	0.001*	3.48 ± 2.27	4.40 ± 3.16	0.008*
t-LH ≥ 10 IU/L, % (*n*)	9.1 (43/473)	20.9 (19/91)	0.001*	2.3 (12/529)	5.3 (5/95)	0.191
t-LH ≥ 10 IU/L & t-LH/b-LH > 2, % (*n*)	4.0 (19/473)	16.5 (15/91)	< 0.001*	0.9 (5/529)	4.2 (4/95)	0.047*

M group: cycles administered medroxy-progestrone acetate

U group: cycles administered utrogestan

b-LH: basal LH, *t-LH*: LH on the trigger day

*P values < 0.05

is still under investigation. Prospective controlled studies with remarkable sample and basic experimental researches on progestogenic effectivity in embryogenesis of different PPOS protocols are needed.

Author contribution YG: project development, data collection, data analysis, manuscript writing. PC: project development, data analysis, manuscript writing. TL: project development, data analysis, manuscript writing. LJ: project development, data analysis, manuscript writing. PS: project development, data analysis, manuscript writing. WZ: data collection, data analysis. CD:data collection, data analysis. CF: project development, manuscript writing tutor. XL: project development.

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Compliance with ethical standards

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Ethical approval This study was approved by the Institutional Reviewer Board of Sixth Affiliated Hospital of Sun Yat-sen University.

Research involving human participants and/or animals For this type of study formal consent is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Because of the retrospective nature of the study the requirement of informed patient consent was waived.

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