Use of Utrogestan during controlled ovarian hyperstimulation in normally ovulating women undergoing in vitro fertilization or intracytoplasmic sperm injection treatments in combination with a "freeze all" strategy: a randomized controlled dose-finding study of 100 mg versus 200 mg

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Objective: To compare the clinical characteristics in a Utrogestan and hMG protocol with the use of different doses of Utrogestan in normally ovulating women undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments.
Design: Prospective controlled study.
Setting: Tertiary-care academic medical center.
Patient(s): A total of 150 infertile patients undergoing IVF/ICSI treatments.
Intervention(s): Utrogestan and hMG were administered simultaneously beginning on cycle day 3. The dose of Utrogestan was 100 mg/d in the study group and 200 mg/d in the control group. When the dominant follicles reached mature, 0.1 mg GnRH agonist was used for trigger. Viable embryos were cryopreserved in both protocols for later transfer.
Main Outcome Measure(s): The primary outcome measure was the incidence of premature LH surge. Secondary outcomes included the embryo results and clinical pregnancy outcomes.
Result(s): Consistent LH suppression was achieved during controlled ovarian hyperstimulation with Utrogestan at 100 mg, and the number of patients with profound LH suppression (LH <1.2 IU/L) in the low-dose group was significantly less than that in the high-dose group. The number of oocytes retrieved in the low-dose group was similar to that in the high-dose group (9.87 ± 5.77 vs. 10.25 ± 5.43). No significant differences were observed in the number of mature oocytes, viable embryos, clinical pregnancy rate, or implantation rate.
Conclusion(s): Utrogestan at 100 mg is as effective as Utrogestan at 200 mg in reducing premature LH surge during controlled ovarian hyperstimulation.
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Key Words: Utrogestan, controlled ovarian hyperstimulation, frozen-thawed embryo transfer, premature LH surge, dose

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Although the traditional down-regulation protocol has sharply reduced the occurrence of premature LH surges which are responsible for cancellation of 20% of cycles during controlled ovarian stimulation (COH) in women undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatments, it has failed to alleviate the risk of ovarian hyperstimulation syndrome (OHSS) from an hCG trigger, to simplify the complexity of achieving consistent pituitary suppression, and to lower the high cost, resulting in continuing interest in exploring surrogate regimens (1, 2).

Progesterone soft capsule (brand name Utrogestan) at 200 mg/d has recently been demonstrated to be an effective oral alternative for preventing premature LH surges during COH in normally ovulating women undergoing IVF/ICSI treatments, with optimal pregnant outcomes in frozen-thawed embryo transfer (FET) cycles. This novel protocol is promising with the advantages of being well tolerated, user convenience, and cost reduction, which will help to establish a convenient user regimen in combination with a "freeze all" strategy (3–5).

However, the LH value was found to be excessively inhibited, in which the nadir was 0.07 IU/L in our last study with Utrogestan at 200 mg/d, resulting in an increased hMG dose compared with the short protocol (3). It is well known that both FSH and LH are critical for adequate folliculogenesis and steroidogenesis, and studies have reported that LH should be neither too high nor too low (6, 7). However, the association between the extent of LH suppression and the dose of progesterone administration remains to be established. Therefore, the present prospective randomized controlled trial was designed to evaluate whether Utrogestan at 100 mg/d could suppress premature LH surges in normally ovulating women undergoing COH with the use of the Utrogestan and hMG protocol as well as to exhaustively analyze the embryo results and pregnancy outcomes to discriminate the differences between different doses of Utrogestan.

MATERIALS AND METHODS

Study Setting and Patients

A prospective randomized controlled study was performed at the Department of Assisted Reproduction of the Ninth People's Hospital of Shanghai Jiaotong University's School of Medicine from September 2014 to October 2015. The study protocol was approved by the Ethics Committee (Institutional Review Board) of the Ninth People's Hospital of Shanghai. The trial was registered with the Chinese Clinical Trial Registry (ChiCTR-OOC-14005277). It was performed according to the Declaration of Helsinki for Medical Research. All of the participants provided informed consents after counseling for infertility treatments and routine IVF procedures. Patients planning to undergo IVF/ICSI treatments were eligible to participate in the study.

To participate, patients had to meet the following criteria: 1) age $\leq$40 years; 2) regular menstrual cycles over the preceding 3-month period (25–35 days in duration); 3) antral follicle count (AFC) of more than four on menstrual cycle day 2–3; and 4) basal serum FSH concentration $\leq$10 IU/L.

The study exclusion criteria were: 1) documented ovarian failure, including basal FSH >10 IU/L or no antral follicles according to ultrasound examination; 2) endometriosis grade $\geq$3; 3) diagnosis of polycystic ovarian syndrome; 4) presence of a functional ovarian cyst with E_2 >100 pg/mL; 5) receipt of hormone treatments within the preceding 3-month period; and 6) any contraindications to ovarian stimulation treatment.

Allocation and Sample Size Estimate

This was a prospective noninferiority trial. The clinical characteristics of the Utrogestan and hMG protocol were unknown at the start of the study, so the sample size was estimated according to the relevant data in an antagonist protocol. The incidence of premature LH surge in an antagonist protocol was reported to be 1.56% (8). Assuming that the mean difference should be <0.08 between the two groups, the sample size required would be 60 for each group to obtain a significance of 0.05 and power of 0.8 (PS power and sample size calculations, version 2.1.30). Given the possibility of dropouts, we designed the study to include a total of 75 women in each group. Patients were recruited with the use of a random number table based on a computer-generated drawing of numbers.

Procedures

Controlled ovarian stimulation and allocation. The Utrogestan and hMG protocol used for all participants was performed as previously described method (3–5). Briefly, hMG (Maanshan Pharmaceutical Trading Co.) and Utrogestan (Laboratories Besins International) was administered from menstrual cycle day (MC) 3 until the trigger day. Follicular monitoring started at MC9 and was performed every 2–4 days. In addition, serum FSH, LH, E_2, and P concentrations were measured. The final stage of oocyte maturation was triggered with the use of triptorelin 0.1 mg (Decapeptyl; Ferring Pharmaceuticals). Utrogestan at 200 mg/d was used in the high-dose group, and Utrogestan at 100 mg/d was administered in the low-dose group.

Transvaginal ultrasound–guided oocyte retrieval was performed 34–36 hours after the trigger. All follicles with diameters >10 mm were retrieved. Fertilization of the aspirated

oocytes was performed with the use of either IVF or ICSI, depending on the semen parameters. According to the number and regularity of the blastomeres and the degree of embryonic fragmentation, good-quality embryos (including grade 1 and grade 2 8-cell embryos) were frozen by means of vitrification on the 3rd day after oocyte retrieval, and non–top-quality embryos were placed in extended culture, out of which good morphologic grade blastocysts were frozen on day 5 or day 6.

Hormone measurement. Hormone levels were measured with the use of chemiluminescence (Abbott Biologicals). The lower limits of sensitivity were: FSH 0.06 IU/L, LH 0.09 IU/L, E_2 10 pg/mL, and P 1 ng/mL. The upper limit of E_2 measurement was 5,000 pg/mL. The E_2 values were recorded as 5,000 pg/mL if the E_2 levels on the trigger day or day after trigger were higher than the upper limit.

Endometrium preparation and FET. In this study, endometrium preparation was performed similarly in both groups, as we described previously (3–5). Briefly, natural cycle was used for the women with regular menstrual cycles, letrozole was used for women with irregular menstrual cycles, and hormone replacement treatment was recommended for patients with a thin endometrium during either natural cycles or stimulation cycles. The transfer of day 3 embryos or blastocysts was scheduled based on the embryo and endometrium synchronization. When pregnancy was achieved, the P supplement was continued until 10 weeks of gestation.

Outcome Measures

The primary outcome measure was the incidence of premature LH surge. The secondary measures included the number of oocytes retrieved, mature oocytes, fertilized oocytes, cleaved embryos, viable embryos, viable embryo rate per oocyte, and clinical pregnancy outcomes from FET cycles. The cutoff level of premature LH surge was 10 IU/L (5). The viable embryo rate per oocyte was defined as the number of viable embryos divided by oocytes retrieved. Clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity during ultrasound examination 7 weeks after FET. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. The miscarriage rate was defined as the proportion of patients with spontaneous termination of pregnancy. Cycle cancellation referred to patients who completed oocyte retrieval without viable embryos.

Statistical Analysis

Data are presented as mean ± SD and analyzed with the use of the Student *t* test, Mann-Whitney *U* test, and chi-square test where appropriate. The Mann-Whitney *U* test was used for the variables of nonnormal distribution. $P<.05$ was considered to be statistically significant. All data were analyzed with the use of the Statistical Package for the Social Sciences for Windows (SPSS, v. 16.0).

RESULTS

Patient Characteristics

A total of 214 patients were assessed for eligibility; 150 patients were randomized into the two groups: high-dose group (n = 75) and low-dose group (n = 75). Supplemental Figure 1 shows the flowchart of the study (Supplemental Figs. 1 and 2 and Supplemental Tables 1 and 2 are available online at www.fertstert.org). A total of 150 women completed oocyte retrieval cycles, and 116 women completed FET cycles. The baseline characteristics and hormonal profile of the patients analyzed are presented in Table 1. No significant differences were observed between the two groups regarding baseline characteristics, indication for IVF, previous IVF failures, and basal hormonal profile.

Ovarian Stimulation, Follicle Development, and Oocyte Performance

As presented in Table 2, there was one woman in the low-dose group and two women in the high-dose group for whom fertilization failed. The high-dose group was characterized by a higher stimulation dose of hMG (1,747 ± 366.76 IU vs. 1,646 ± 223.95 IU; $P>.05$), without statistical difference. The numbers of follicles with diameters >10 mm (11.39 ± 4.95 vs. 9.99 ± 5.36; $P>.05$) or >14 mm (10.77 ± 4.97 vs. 8.09 ± 5.05; $P>.05$) were similar between the two groups. The number of oocytes retrieved in the high-dose group was slightly higher but did not reach a significant difference compared with the low-dose group (10.25 ± 5.43 vs. 9.87 ± 5.77; $P>.05$). The numbers of top-quality embryos (4.15 ± 3.17 vs. 3.73 ± 2.34; $P>.05$) showed no significant difference between the two groups. The fertilization rate was

TABLE 1

General patient information (mean ± SD).

Characteristic	200 mg/d Utrogestan + hMG	100 mg/d Utrogestan + hMG	*P* value
Cycle (n)	75	75	
Age (y)	31.23 ± 3.16	30.13 ± 3.8	.095
Duration of infertility (y)	2.88 ± 2.7	3.29 ± 2.55	.133
Body mass index (kg/m^2)	21.03 ± 3.46	21.13 ± 3.75	.736
Antral follicle count (n)	9.2 ± 4.19	10.13 ± 4.42	.08
Basal FSH (IU/L)	5.8 ± 1.51	5.51 ± 1.16	.273
Basal LH (IU/L)	3.44 ± 1.31	3.55 ± 1.71	.718
Basal E_2 (pg/mL)	37.46 ± 15.65	37.28 ± 15.27	.918
Basal P (ng/mL)	0.31 ± 0.2	0.32 ± 0.23	.713
Indication, n			.372
Tubal factor	47	44	
Male factor	8	14	
Combination of factors	20	17	
Previous FET failures, n			.817
0	54	54	
1–2	14	12	
≥3	7	9	

Note: FET = frozen-thawed embryo transfer; MII = metaphase II.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.

TABLE 2

Stimulation and embryonic characteristics of the patients (mean ± SD).

Characteristic	200 mg/d Utrogestan + hMG	100 mg/d Utrogestan + hMG	*P* value
hMG dose, IU	1,747 ± 366.76	1,646 ± 223.95	.054
hMG duration, d	9.36 ± 1.65	8.89 ± 1.18	.069
>10-mm follicles on trigger day, n	11.39 ± 4.95	9.99 ± 5.36	.157
>14-mm follicles on trigger day, n	10.77 ± 4.97	8.09 ± 5.05	.372
Oocytes retrieved, n	10.25 ± 5.43	9.87 ± 5.77	.594
MII oocytes, n	9.01 ± 4.87	8.24 ± 4.88	.284
Fertilized oocytes, n	6.85 ± 4.19	6.73 ± 4.23	.783
Cleaved embryos, n	6.71 ± 4.09	6.53 ± 4.09	.734
Day 3 top-quality embryos, n	3.65 ± 3.25	3.16 ± 2.55	.631
Viable embryos, n	4.15 ± 3.17	3.73 ± 2.34	.687
Oocyte retrieval rate, % (n)	68.72 (769/1,119)	69.48 (740/1,065)	.700
Mature oocyte rate, % (n)	87.91 (676/769)	83.51 (618/740)	.015
Fertilization rate, % (n)	66.84 (514/769)	68.24 (505/740)	.561
Cleavage rate, % (n)	97.86 (503/514)	97.03 (490/505)	.401
Viable embryo rate per oocyte retrieved, % (n)	40.4 (311/769)	37.3 (276/740)	.21
Cancellation rate, % (n)	9.1 (8/75)	6.5 (3/75)	.117
Incidence of moderate or severe OHSS, %	0	0	
Incidence of premature LH surge, %	0	0	

Note: MII = metaphase II; OHSS = ovarian hyperstimulation syndrome.

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significantly lower in the high-dose group (69.77 ± 22.22% vs. 75.97 ± 20.62%; $P<.05$). No significant differences were found in the numbers of mature oocytes, fertilized oocytes, and cleaved embryos, the rates of oocytes retrieved, and the viable embryo rates per oocyte retrieved between the two groups ($P>.05$). The cycle cancellation rates due to lack of viable embryos were not different between the two groups (9.1% vs. 6.5%; $P>.05$). No patients experienced moderate or severe OHSS in the two groups.

Supplemental Figure 2 shows that the number of patients with LH <1.2 IU/L in the low-dose group was significantly different from that in the high-dose group, not only on MC9 (two vs. eight; $P<.05$) but also on the trigger day (11 vs. 21; $P<.05$). The embryonic characteristics of patients with LH <1.2 IU/L are presented in Supplemental Table 1. No statistical differences were found between the subgroups.

Hormone Profile during Treatment

The values of circulating concentrations of FSH, LH, E_2, and P in the two groups are shown in Figure 1. The hormone levels were not normally distributed.

FSH levels increased significantly after hMG administration and were steady during ovarian stimulation. After the trigger, the average FSH levels were similar between the two groups (21.63 ± 6.77 IU/L vs. 23.12 ± 7.07 IU/L; $P>.05$).

The LH values gradually decreased during ovarian stimulation, with no premature LH surges detected. The average LH levels on MC9 (3.56 ± 1.9 IU/L vs. 4.12 ± 2.98 IU/L; $P>.05$) and on the trigger day (2.47 ± 1.91 IU/L vs. 2.67 ± 1.73 IU/L; $P>.05$) showed no statistical significance between the two groups. Furthermore, the LH values on the day after the trigger were also similar (47.25 ± 24.86 IU/L vs. 54.88 ± 29.2 IU/L; $P>.05$).

Serum E_2 values showed a gradual increase accompanying the growth of follicles during ovarian stimulation. The E_2 levels were significantly higher in the high-dose group on the trigger day and the day after the trigger (3,453.55 ± 1,234.65 pg/mL vs. 2,923.33 ± 1,238.94 pg/mL; $P<.05$).

Serum P values increased after the administration of Utrogestan, with a range of 0.9–20.6 ng/mL in the low-dose group and 1–47.8 ng/mL in the high-dose group, and were maintained at stable concentrations. Serum P values in the high-dose group were higher than in the low-dose group on MC9 (6.81 ± 6.57 ng/mL vs. 3.68 ± 2.59 ng/mL; $P<.05$), the trigger day (6.55 ± 5.59 ng/mL vs. 4.19 ± 2.8 ng/mL; $P<.05$), and the day after the trigger (8.49 ± 4.32 ng/mL vs. 7.41 ± 4.39 ng/mL; $P<.05$), with statistical differences.

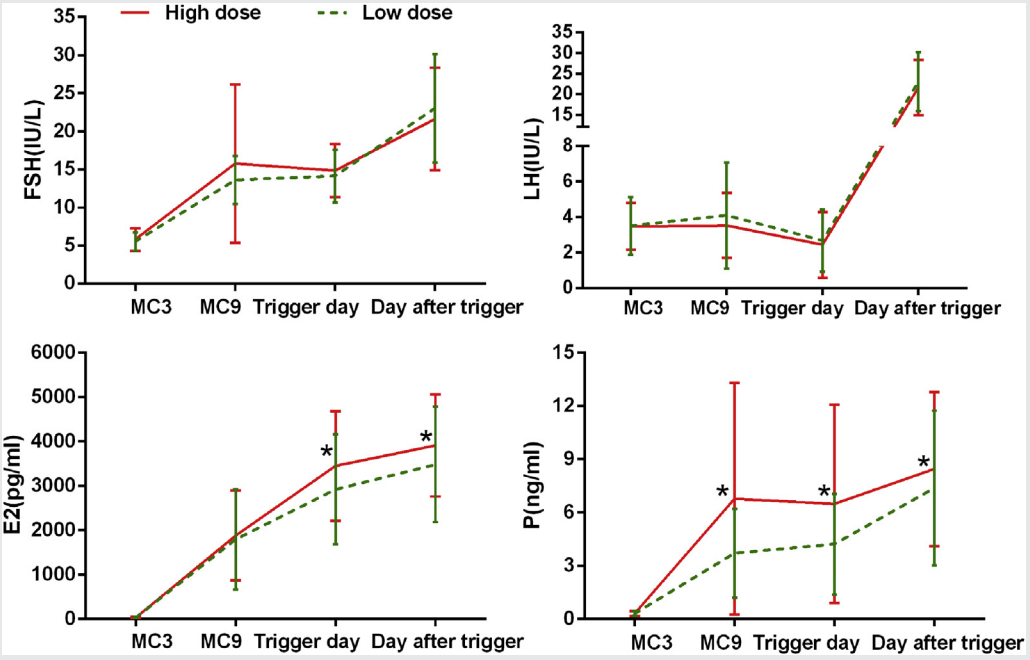
Pregnancy

In this study, 116 women completed a total of 156 FET cycles, including 79 women who underwent one FET each, 34 women who completed two FETs each, and three women who completed three FETs each. The remaining 34 women did not complete their FET cycles for personal reasons before the end of the study (Table 3).

In the low-dose group, 61 women completed 80 FET cycles, of which 39 women were pregnant, including one women who was pregnant twice owing to miscarriage. In the high-dose group, 55 women completed 76 FET cycles, of which 37 women were pregnant, including two women who were pregnant twice owing to miscarriage.

A total of 297 embryos were thawed, and the rate of viable frozen-thawed embryos was 100%. The clinical pregnancy rates per transfer (51.32% vs. 50%; $P>.05$) and implantation rates (36.05% vs. 38.67%; $P>.05$) were similar between the two groups, indicating that the embryos in the study group shared similar development potential. Note that 2.56% (1/39) of the patients in the high-dose group had a miscarriage before reaching the gestational age of 12 weeks,

FIGURE 1



Serum hormone profiles present during ovarian stimulation in the two regimens. The *green lines* represent the low-dose group (100 mg/d Utrogestan) and the r*ed lines* the high-dose group (200 mg/d Utrogestan). *$P<.05$ at the time point. MC = menstrual cycle day.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.

whereas 7.5% (3/40) of patients in the low-dose group miscarried.

Correlation between Endocrine Characteristics and Stimulation Characteristics

The correlation analysis results of LH and P, E_2, and the number of oocytes retrieved are presented in Supplemental Table 2. No correlations were observed between the LH level and P on MC9 or the trigger day in these two groups ($P>.05$). The serum E_2 concentration was significantly associated with the number of oocytes retrieved on MC9 and the trigger day ($P<.05$).

DISCUSSION

This is the first prospective randomized controlled trial to evaluate the clinical outcomes between Utrogestan at 100 mg/d and 200 mg/d in normally ovulating women undergoing COH. The pituitary LH levels were suppressed after 6-day Utrogestan treatment at 100 mg/d, no premature LH surge was observed, and there were no significant differences between the two groups throughout the COH. Thus, Utrogestan at 100 mg/d could prevent premature LH surges.

Average LH levels were higher in the low-dose group than in the high-dose group during COH, but without statistical difference. Nevertheless, the number of patients with

TABLE 3

Pregnancy outcomes of frozen-thawed embryos originating from the two regimens.

Outcome	200 mg/d Utrogestan + hMG	100 mg/d Utrogestan + hMG	*P* value
Patients, n	55	61	
FET cycles, n	76	80	
Thawed embryos, n	147	150	
Viable embryos after thawed, n	147	150	
Transferred embryos, n	1.93 ± 0.25	1.88 ± 0.33	.211
Biochemical pregnancy rate per transfer, % (n)	56.58 (43/76)	60 (48/80)	.665
Clinical pregnancy rate per transfer, % (n)	51.32 (39/76)	50 (40/80)	.869
Implantation rate, % (n)	36.05 (53/147)	38.67 (58/150)	.642
Miscarriage rate, % (n)	2.56 (1/39)	7.5 (3/40)	.317
Ectopic pregnancy rate, % (n)	2.56 (1/39)	0 (/40)	.308

Note: FET = frozen-thawed embryo transfer.

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profound LH depression, defined as <1.2 IU/L (9), was less in the low-dose group than in the high-dose group, with statistical significance, demonstrating that Utrogestan at 100 mg/d could partly alleviate profound LH suppression. In addition, the duration of hMG administration and hMG dose were lower in the low-dose group than in the high-dose group, but failing to reach statistical difference. These findings were consistent with our previous findings, in which the extent of pituitary suppression was directly associated with the hMG dose (3, 4), indicating that the effect of Utrogestan for LH suppression was mitigated by the reduction of Utrogestan dose.

The optimal LH level still remains obscure with the use of the traditional down-regulation protocol, as well as with the use of the Utrogestan and hMG protocol. Some investigators concluded that a low LH concentration results in a suboptimal intrafollicular environment for oocyte maturation and subsequent embryo quality (10–12). One hypothesis was that LH plays a direct role in the cellular compartments linked to the maturing oocyte, which is supported by many studies involving LH receptor action on human cumulus cells (13, 14). The pregnancy loss rate was higher in patients with very low LH concentrations (<0.5 IU/L) compared with patients having normal LH concentrations (45% vs. 9%, respectively) with the use of the long protocol, as observed by Westergaad et al. (15). A higher incidence of grade 1 and grade 2 embryos was observed when supplementing the FSH stimulation with LH in women undergoing a long agonist protocol (16).

The profound LH suppression state in the present study did not impair the quality of the oocytes and embryos, which failed to distinguish the relationship between the LH value and clinical results because a urinary hMG preparation containing FSH combined with LH activity was used. The overall LH activity in hMG mainly consisted of hCG, a glycoprotein hormone with longer serum half-life (2.32 days for hCG vs. 1 hour for LH) and enhanced biologic activity (relative hCG:LH activity of 6:1), which was intentionally added or contaminated carelessly (17, 18). Therefore, whether there was a correlation between the LH levels and clinical outcomes was not elucidated in our trial and remains to be examined in future research studies.

Serum P values in the high-dose group were higher than in the low-dose group, with significant difference, which was consistent with earlier studies demonstrating that both absorption and elimination of Utrogestan were dose independent (19–24). Importantly, considerable interindividual variation was observed. Patients were informed to take Utrogestan once a day at bedtime in case of dizziness and sleepiness and to perform the blood determination in the morning instead of at a definite point. Therefore, the uncertain interval between the ingestion of the last dose and blood determination was a major factor because the absorption, further metabolism, and clearance of oral P are rapid (19–21). In addition, a previous review confirmed that absorption of Utrogestan could be enhanced twofold in the presence of food. Therefore, fasting might be another cause contributing to the variable P concentrations observed among the participants (22).

There was a trend that greater increase in P concentrations produced progressively greater decreases in circulating serum LH values, without direct statistical correlation. One explanation was the limited number of participants and the narrow ranges of P and LH. In addition, E_2 might enhance the ability of P to suppress LH secretion, a synergistic role of E_2 and P in the suppression of LH that has been confirmed by various studies, and may interfere with the analysis to a specific extent (25). Thus, the relationship between the extent of LH suppression and the dose of P administration was complicated in the Utrogestan and hMG protocol, unlike the dose-dependent effect in the traditional down-regulation protocol (26), owing to the auxiliary role of E_2.

Serum E_2 values were significantly higher in the high-dose group than in the low-dose group. It is well known that granulosa cells produce E_2 stimulated by FSH and LH. The FSH levels showed no significant difference between the two groups. However, the serum LH levels in the high-dose group were lower than in the low-dose group, but failing to reach statistical significance, resulting in a reduction instead of the theoretic increase in E_2 secretion that has been described in earlier studies (27). The number of oocytes retrieved in the high-dose group was higher than in the low-dose group, which may explain, at least in part, why serum E_2 levels in the high-dose group were observed to increase proportionally to the levels in the low-dose group with follicle development. One explanation confirmed by correlation analysis showed that the number of oocytes retrieved was statistically related to the E_2 values.

The number of oocytes retrieved, mature oocytes, and viable embryos in the study group were similar to those in the control group, which indicated that 100 mg Utrogestan per day was similar to 200 mg Utrogestan per day in terms of embryo characteristics. However, the mature oocyte rate in the low-dose group was lower than in the high-dose group. The serum P value was lower in the low-dose group than in the high-dose group, which may be associated with a decreased mature oocyte rate due to the effect of P on oocyte maturation. In addition, embryonic development may be dependent on its concentration and the mammalian species, as concluded by Salehnia and Zavareh (28). However, the dose-dependent correlation between the P value and the rate of mature oocytes has not yet been established.

It has been reported that the levels of P in follicular fluid and its ratio to estrogen levels are strongly associated with oocyte maturity (29). Studies performed by Aparicio et al. investigated the role of P on bovine oocyte developmental competence by inhibiting the P production of cumulus cells, which supported a positive role for P in oocyte quality (30). Furthermore, it was observed that the mature oocyte rate increased via supplementation of canine oocyte culture media with P and E_2 (31, 32). However, Hewitt and England observed no significant differences in canine oocyte maturation among four groups with distinct hormonal environment (33). An investigation performed by Salehnia and Zavareh in mice tested the effect of different P concentrations on in vitro oocyte maturation, reporting that the maturation rate decreased in a dose-dependent manner when P increased

from 10 to 100 μmol/L (28). Fukui et al. demonstrated the negative role of P on bovine oocyte maturation (34, 35). Thus, further studies are needed on a larger scale to determine the optimal P concentration to warrant follicle development in an appropriate intrafollicular steroidogenic milieu.

A major limitation of the present study is the limited number of participants enrolled. Furthermore, the calculation of sample size was according to the incidence of premature LH surge in an antagonist protocol, because the efficacy of the combination of Utrogestan and hMG in the IVF cycle had not been reported at the start of the study, which may decrease the power of this study. In addition, the steroid levels in the follicular fluid, which are directly associated with the follicular microenvironment were not determined, which is another limitation of this study. Third, some of the participants had not finished their FET cycle by the time of submission owing to reasons such as being ill, busy, or divorced, poor uterine environment, and so on, which may contribute to bias.

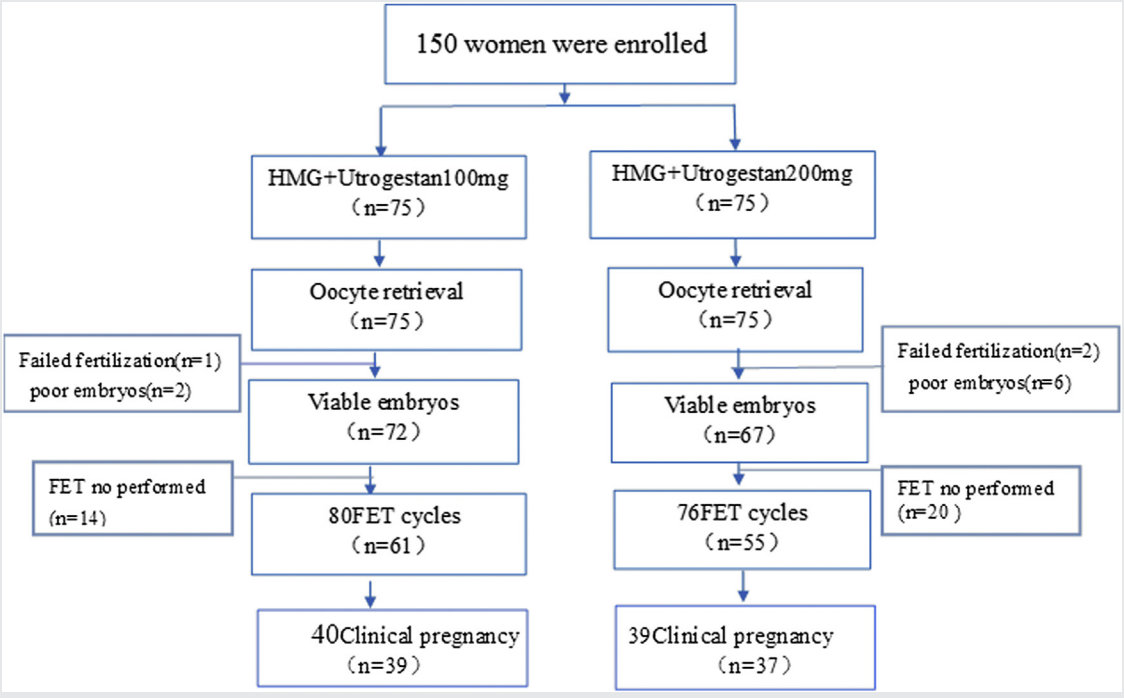
In conclusion, this study shows that Utrogestan at 100 mg/d can effectively block premature LH surges as well as alleviate profound LH suppression compared with Utrogestan at 200 mg/d in normally ovulating women undergoing IVF/ICSI treatments. Nevertheless, whether similar clinical outcomes could be obtained still needs further research, because the data of pregnant results were incomplete at the time of this report. Additional trials should be implemented on larger sample sizes, and basic research studies are indispensable to determine the alterations in the follicular microenvironment that may help to determine the optimal range of LH and P, elucidate the mechanism underlying how P affects oocyte quality, and provide evidence for the individual use of Utrogestan.

REFERENCES

1. Eibschitz I, Belaisch-Allart JC, Frydman R. In vitro fertilization management and results in stimulated cycles with spontaneous luteinizing hormone discharge. Fertil Steril 1986;45:231–6.
2. Xiao JS, Su CM, Zeng XT. Comparisons of GnRH antagonist versus GnRH agonist protocol in supposed normal ovarian responders undergoing IVF: a systematic review and meta-analysis. PLoS One 2014;9:e106854.
3. Zhu XX, Zhang XL, Fu YL. Utrogestan as an effective oral alternative for preventing premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation for in vitro fertilization. Medicine 2015;94:e909.
4. Zhu XX, Zhang XL, Fu YL. Effect of progesterone used to prevent LH surges in controlled ovarian stimulation. Reprod Contracept 2015;35:384–8.
5. Zhu XX, Ye HJ, Fu YL. The Utrogestan and hMG protocol in patients with polycystic ovarian syndrome undergoing controlled ovarian hyperstimulation during IVF/ICSI treatments. Medicine 2016;95:28.
6. Barri PN, Balasch J. LH neither too high nor too low. Gynecol Endocrinol 2007;23:309.
7. Wong PC, Qiao J, Ho C, Ramaraju GA, Wiweko B, Takehara Y, et al. Current opinion on use of luteinizing hormone supplementation in assisted reproduction therapy: an Asian perspective. Reprod Biomed Online 2011;23:81–90.
8. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 2002;17:874–85.
9. Lahoud R, Al-Jefout M, Tyler J, Ryan J, Driscoll G. A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates. Hum Reprod 2006;21:2645–9.
10. Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. Fertil Steril 2001;75:519–24.
11. O'Dea L, O'Brien F, Currie K, Hemsey G. Follicular development induced by recombinant luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in anovulatory women with LH and FSH deficiency: evidence of a threshold effect. Curr Med Res Opin 2008;24:2785–93.
12. Humaidan P, Bungum L, Bungum M, Andersen CY. Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod 2002;17:2016–21.
13. McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, et al. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum Reprod 2004;19:2869–74.
14. Assou S, Anahory T, Pantesco V, le Carrour T, Pellestor F, Klein B, et al. The human cumulus-oocyte complex gene-expression profile. Hum Reprod 2006;21:1705–19.
15. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod 2000;15:1003–8.
16. Lisi F, Rinaldi L, Fishel S, Caserta D, Lisi R, Campbell A. Evaluation of two doses of recombinant luteinizing hormone supplementation in an unselected group of women undergoing follicular stimulation for in vitro fertilization. Fertil Steril 2005;83:309–15.
17. Yen SS, Llerena O, Little B, Pearson OH. Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in man. J Clin Endocrinol Metab 1968;28:1763–7.
18. Rao CV. Differential properties of human chorionic gonadotrophin and human luteinizing hormone binding to plasma membranes of bovine corpora lutea. Acta Endocrinol (Copenh) 1979;90:696–710.
19. Whitehead MI, Townsend PT, Gill DK, Collins WP, Campbell S. Absorption and metabolism of oral progesterone. Br Med J 1980;280:825–7.
20. Fitzpatrick LA, Good A. Micronized progesterone: clinical indications and comparison with current treatments. Fertil Steril 1999;72:389–97.
21. Norman TR, Morse CA, Dennerstein L. Comparative bioavailability of orally and vaginally administered progesterone. Fertil Steril 1991;56:1034–9.
22. Simon JA, Robinson DE, Andrews MC, Hildebrand JR 3rd, Rocci ML Jr, Blake RE, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 1993;60:26–33.
23. Nahoul K, Dehennin L, Scholler R. Radioimmunoassay of plasma progesterone after oral administration of micronized progesterone. J Steroid Biochem 1987;26:241–9.
24. de Lignières B. Oral micronized progesterone. Clin Ther 1999;21:41–60.
25. Goodman RL, Bittman EL, Foster DL, Karsch FJ. The endocrine basis of the synergistic suppression of luteinizing hormone by estradiol and progesterone. Endocrinology 1981;109:1414–7.
26. Ganirelix Dose-Finding Study Group. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). Hum Reprod 1998;13:3023–31.
27. Smitz J, Andersen AN, Devroey P, Arce JC, MERIT Group. Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. Hum Reprod 2007;22:676–87.
28. Salehnia M, Zavareh S. The effects of progesterone on oocyte maturation and embryo development. Int J Fertil Steril 2013;7:74–81.
29. Moor RM, Polge C, Willadsen SM. Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. J Embryol Exp Morphol 1980;56:319–35.

30. Aparicio IM, Garcia-Herreros M, O'Shea LC, Hensey C, Lonergan P, Fair T. Expression, regulation, and function of progesterone receptors in bovine cumulus oocyte complexes during in vitro maturation. Biol Reprod 2011; 84:910–21.
31. Willingham-Rocky LA, Hinrichs K, Westhusin ME, Kraemer DC. Effects of stage of oestrous cycle and progesterone supplementation during culture on maturation of canine oocytes in vitro. Reproduction 2003; 126:501–8.
32. Vannucchi CI, de Oliveira CM, Marques MG, Assumpção ME, Visintin JA. In vitro canine oocyte nuclear maturation in homologous oviductal cell co-culture with hormone-supplemented media. Theriogenology 2006;66: 1677–81.
33. Hewitt DA, England GC. Effect of preovulatory endocrine events upon maturation of oocytes of domestic bitches. J Reprod Fertil Suppl 1997;51:83–91.
34. Fukui Y, Fukushima M, Terawaki Y, Ono H. Effect of gonadotropins, steroids and culture media on bovine oocyte maturation in vitro. Theriogenology 1982;18:161–75.
35. Zavareh S, Saberivand A, Salehnia M. The effect of progesterone on the in vitro maturation and developmental competence of mouse germinal vesicle oocytes. Int J Fertil Steril 2009;3:21–8.

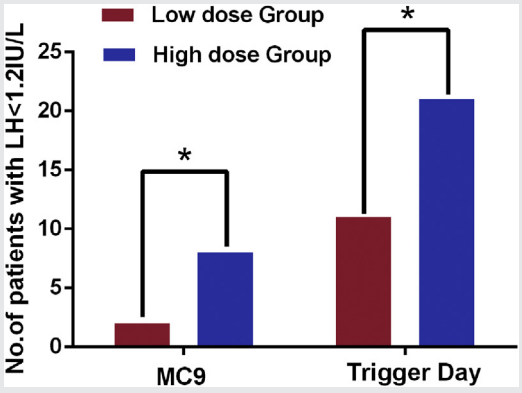
SUPPLEMENTAL FIGURE 1



Flowchart of the study. FET = frozen-thawed embryo transfer.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 2



The number of patients with profound LH suppression (LH <1.2 IU). The *red columns* represent the low-dose group (100 mg/d Utrogestan) and the *blue column* the high-dose group (200 mg/d Utrogestan). *$P<.05$.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.

SUPPLEMENTAL TABLE 1

Embryologic characteristics of the patients with LH ≤1.2 IU/L.

	Based on MC9 LH value		Based on trigger day LH value	
Index/group	**200 mg/d Utrogestan**	**100 mg/d Utrogestan**	**200 mg/d Utrogestan**	**100 mg/d Utrogestan**
Cycles, n	8	2	21	11
Oocytes retrieved, n	12 ± 5.5	14.5 ± 10.61	11.52 ± 5.75	12.82 ± 7.28
MII oocytes, n	11 ± 4.99	12.5 ± 7.78	10.62 ± 5.31	9.91 ± 5.47
Fertilized oocytes, n	8.63 ± 3.29	11 ± 5.66	8.29 ± 4.88	8.36 ± 4.06
Cleaved embryos, n	8.25 ± 3.37	11 ± 5.66	8 ± 4.8	8.27 ± 4.03
Day 3 top-quality embryos, n	4.5 ± 2.78	6 ± 2.83	4.19 ± 3.54	3.45 ± 2.62
Viable embryos, n	5.75 ± 3.01	6 ± 1.41	4.86 ± 3.54	4.82 ± 3.16

Note: MC9 = day 9 of menstrual cycle; MII = metaphase II.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.

SUPPLEMENTAL TABLE 2

Correlation analysis of serum LH, E_2, and P levels according to the dose of Utrogestan.

Protocol	Correlation of LH and P on MC9	Correlation of LH and P on trigger day	Correlation of MC9 E_2 and no. of oocytes retrieved	Correlation of trigger day E_2 and no. of oocytes retrieved
200 mg/d Utrogestan + hMG	$r = -0.132$; $P = .265$	$r = -0.138$; $P = .239$	$r = 0.441$; $P < .001$	$r = 0.655$; $P < .001$
100 mg/d Utrogestan + hMG	$r = -0.126$; $P = .303$	$r = -0.082$; $P = .492$	$r = 0.573$; $P < .001$	$r = 0.696$; $P < .001$

Note: MC9 = day 9 of menstrual cycle.

Zhu. Utrogestan dosage for COH. Fertil Steril 2016.