A Modified Ultra-long Down-regulation Protocol Improved Endometrial Receptivity and Clinical Outcome for Infertile Patients with Poly-cystic Ovarian Syndrome

Fei Gong¹²³, Xihong Li², Shunji Zhang², Hainan Ma¹, Sufen Cai², Juan Li², Ge Lin¹²³, Guangxiu Lu¹²³

1. Institute of Reproductive & Stem Cell Engineering, Central South University. No. 932, Lushan South Road, Changsha 410083, China.
2. Department of Reproductive Medicine, Reproductive & Genetic Hospital of CITIC-XIANGYA. No. 84, Xiangya Road, Changsha 410078, China.
3. Key Laboratory of human stem cell & Reproductive Engineering, Ministry of Health. No. 84, Xiangya Road, Changsha, Hunan, 410008, China.

Correspondence: Guangxiu Lu

Institute of Reproductive & Stem Cell Engineering, Central South University. No. 932 Lushan South Road, Changsha 410083, China.
Tel: +86-731-82355100-8401
Fax: +86-731-82355109
E-mail: luguangxiuBM@163.com
The mail addresses for all authors:

Fei Gong: lj_0305@126.com

Xihong Li: 1065770150@qq.com

Shunji Zhang: zhangshunji1202@aliyun.com

Hainan Ma: mahailan123@126.com

Sufen Cai: ajiu0305@163.com

Juan Li: 4531630@qq.com

Ge Lin: linggf@hotmail.com

Guangxiu Lu: luguangxiuBM@163.com
Abstract

Objectives: There are various ovarian stimulation protocols for in vitro fertilization (IVF). Particularly for Poly-cystic Ovarian Syndrome (PCOS) patients an optimized protocol of pituitary FSH and LH down regulating gonadotropin-releasing hormone agonist (GnRHa) medication without complications is still a challenge. Methods: We retrospectively compared the primary endpoint of an ultra-long and a conventional long GnRHa protocol for intracytoplasmic sperm injection (ICSI)/IVF treatments of PCOS patients with several clinical indexes. Results: In our modified ultra-long protocol group, the endometrial thickness, morphology and blood flow were significantly optimized compared to the conventional long protocol. The serum progestogen (P) concentrations and P/E2 (P*1000/estrogen (E2)) ratio on the day of human chorionic gonadotrophin (HCG) administration were significantly decreased in the modified ultra-long down-regulation protocol, while the pregnancy and implantation rates were significantly higher. There were no significant differences in the average number of obtained oocytes, good quality embryo rates, cancel rates, fertilization rates, abortion rates, serious ovarian hyperstimulation syndrome (OHSS) incidences, ectopic pregnancy rates as well as gonadotrophin (Gn) dosages between two groups. Conclusion: The modified ultra-long protocol plus human menopausal gonadotropin (hMG) medication was superior to the long protocol and leading to better implantation as well as pregnancy outcomes for infertile PCOS patients.

Key Words: PCOS; ultra-long down-regulation protocol; endometrial receptivity
Introduction

Poly-cystic Ovarian Syndrome (PCOS) is common endocrine disorder in women of childbearing age. Characteristic clinical presentations are menstrual disorders, rare or anovulation, infertility, hirsutism, obesity, ovarian enlargement and irregular serum hormone concentrations [1]. PCOS patients account for about 5-10% of women in childbearing age, 70-80% of ovulation disorder patients and 50% of patients who are undergoing assisted reproductive technologies (ART)[2].

Because of their complex endocrine conditions in PCOS patients the controlled ovulation induction for IVF or ICSI treatments is challenging, while the response range to gonadotropin is very narrow and OHSS as well as endogenous Luteinizing Hormone (LH) surge may affect the quality of oocytes and reduce the clinical pregnancy rates. Up to now there is no exact optimization scheme for PCOS patients [3]. Currently, most of the reproductive centers use traditional long protocols to inhibit endogenous premature LH surge and prevent premature luteinized follicles in order to induce high quality oocytes and successful pregnancies [4, 5]. Unfortunately the pregnancy rates of PCOS patients who received the long pituitary down regulation protocol were not always ideal and some patients had poor results because of insufficient down regulation and premature endogenous LH surge even with a long duration of Gn medication in large doses. The Ultra-long protocol was in the beginning mainly used for infertile patients with endometriosis and has led to good results. Therefore we tried a modified ultra-long protocol for PCOS patients in our hospital. In this study, we retrospectively summarized data from Sept. 2011 to Apr.
2012 in our assisted reproductive technology center. Patients with PCOS were divided into modified ultra-long protocol as well as long protocol groups and we compared endogenous LH levels, endometrial receptivity and clinical outcomes of the two groups.

**Materials and Methods**

**Patients**

147 patients, who visited our hospital from Sept. 2011 to Apr. 2012 and underwent IVF/ICSI treatments, were divided into two groups: an ultra-long protocol group and a long protocol group. The exclusion criteria were related disorders, older than 38 years old, endometriosis, uterine malformation, untreated hydrosalpinx, intrauterine adhesions and endometriosis history. PCOS was diagnosed according to the Rotterdam criteria and patients were selected by at least two of the following three features: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) **polycystic ovaries with congenital adrenal hyperplasia**, **cushing syndrome and tumor related testosterone secretion as exclusion criteria**. All participating couples gave informed consent to the procedures and the use of their data for research purposes. The research protocol was approved by the Ethics Committee of Central South University and Reproductive & Genetic Hospital of CITIC-XIANGYA, and all participants provide their written informed consent to participate in this study.

**Treatments**

All GnRHa starting doses were chosen according to the woman’s age, history and
body weight and all patients received Dane-35 from the 3rd day on for one complete menstrual cycle.

**Routine long pituitary down-regulation protocol**

On day 20 of the cycle 1.5mg-1.875mg gonadotropin releasing hormone agonist (GnRHa) was intramuscularly injected. After 13-20 days, depending on confirmed pituitary–ovarian suppression, 75U-150 IU/d Recombinant Follicle Stimulating Hormone (rFSH) (Gonal-F or Puregon) was administered for four-five days. After adequate follicle stimulation was confirmed by ultrasound and hormone concentrations then, human chorionic gonadotropin (hCG) was injected.

**Modified ultra-long pituitary down-regulation protocol**

On day 20 of the first cycle 1.5mg-1.875mg GnRHa was intramuscularly injected, which was repeated on day 21 of the following second menstrual cycle. Then 13-20 days later after achieved pituitary–ovarian suppression, human menopausal gonadotropin (hMG, 75 U-375U/d, Menopur, Ferring, Germany) was injected for 4-5 days. After adequate follicle stimulation was confirmed then by ultrasound and hormone concentrations, human chorionic gonadotropin (hCG) was injected.

**Follicle stimulation evaluation criteria**

Ultrasonography combined with serum hormone concentration determinations were used for evaluation of the follicle developmental status. Controlled ovarian hyperstimulation (COH) was adapted by using a Gn step-up, step-down or withdrawal scheme according to E2, P, LH levels and to vaginal ultrasound results. Criteria for adequate pituitary–ovarian suppression were serum concentrations of estrogen (E2) <
40 pg/ml, progesteron (P) < 0.8ng/mL and luteinizing hormone (LH) < 3mIU/mL as well as endometrium (Em) < 5 mm and no follicle or corpus luteum cyst in the ovary was ≥10mm. rFSH or hMG was administered to achieve egg stimulation until the follicles ≥18mm accounted for 60-70% of the follicles bigger than 14mm or the follicles ≥20mm accounted for 40-50% of the follicles bigger than 14 mm and the patients were scheduled for oocyte retrieval 35 to 36 hours after the hCG injection. E₂ per every 14 mm follicle was 200-300pg/ml. Indications and techniques for oocyte aspiration, oocyte and embryo culture, insemination, intracytoplasmic sperm injection, assisted hatching and embryo transfer (ET) were based on the routine of the center.

**Endometrial change determinations**

The endometrial thickness, morphology and blood flow of the two groups were assessed by vaginal color ultrasound on the hCG injection day and the day ET. A GE V730-expert color Doppler ultrasonic diagnostic apparatus was used with an intra cavity probe frequency of 5~9MHz. All patients were measured by the same operator with the same settings and test indexes were longitudinal endometrial thickness as well as endometrial morphologic using the Gonen classification [6]. Type A: three linear or multilayer endometrium, the outer and central areas echogenic and the inner areas hypoechoic or dark, significant uterine cavity midline echo; Type B: the middle area shows an isolated echo like an uterine myometrium image and the uterine cavity midline echo is not obvious; Type C: the endometrium is homogeneous echogenic, without uterine cavity midline echo. The endometrial blood flow was categorized according to Chien et al. [7]: type A : blood flow could be monitored as endometrial
and subendometrial blood flow, type B: blood flow only could be monitored as subendocardial blood flow; type C: endometrium and endometrial blood flow could not be detected.

**Outcome parameters**

Serum hormone concentrations of the two groups were evaluated before GnRHa stimulation and at the day of hCG application. The mean number of retrieved oocytes, fertilization rate, good quality embryo rate, cycle cancellation rate, the number of transferred embryos, implantation rate, pregnancy rate, miscarriage rate after transplantation, severe OHSS incidence, the incidence of ectopic pregnancy, the GnRHa dosage and the days of drug administration were compared between the two groups.

**Statistical analyses**

Sigmastat software was used to carry out statistical analyses. Values are expressed as mean ± SD or number and percentage. The comparison of quantitative variables between groups was done by one way analysis of variance (ANOVA). Homogeneity of variance and normality of data were estimated using Levene and Kolmogorov-Smirnov tests respectively. Qualitative variables were analyzed by Chi-square test. P values <0.05 were considered statistically significant.

**Results**

We compared the patients’s age, infertility duration (years), BMI, basal FSH and basal LH values between the two groups in Table 1. There were no significant differences of these parameters. Table 2 showed the comparison of clinical outcomes between the
two different protocols. LH level on the day before Gn, P level (ng/ml) and P/E2 (P (ng/ml) × 1000/E2 (pg/ml)) ratio on the hCG day of the ultra-long protocol group were statistically lower than that of the long protocol group, P < 0.05; The implantation and clinical pregnancy rates of the ultra-long protocol were significantly higher than in the long protocol, (P<0.05). Other parameters such as dosage of Gn, duration of Gn, E2 and LH levels on the hCG day, the mean number of oocytes retrieved, fertilization rates, good quality embryo rates, cycle cancellation rates, the number of transferred embryos, implantation rates, pregnancy rates, miscarriage rates of transplantations, severe OHSS incidences, the incidence of ectopic pregnancies showed no significantly differences. The ultra-long protocol increase of the clinical pregnancy rate compared to the long protocol was caused by the optimal P level and P/E2 ratio on the hCG day which resulted in good endometrial receptivity. Endometrial morphology and blood flows are shown in Figure 1 and Figure 2, while Table 3 shows the differences of endometrial thickness, morphology and blood flow in the two protocols. The proportions of type A and type B endometrial morphology on the day of hCG application in the ultra-long protocol were 32%, and 57.3% and higher than that in the long protocol group (23.6% and 43.1% respectively), but without significant difference. The proportion of type C endometrial morphology on the hCG application day in the ultra-long protocol (10.7%) was significantly lower than that in long protocol group (33.3%)(p=0.001) and the endometrial morphology were type C in both protocols on the ET day. The proportions of type A and type B blood flow of endometrial and sub-endometrial tissue on the day of hCG medication
and on the day of ET in the ultra-long protocol were higher than in long protocol group, but showed no significant difference. The proportions of type C endometrial morphologies on the hCG and ET days in the ultra-long protocol were significantly lower than in long protocol group.

**Discussion**

In our study we found, that the clinical outcome of PCOS patients using an ultra-long protocol was better than that of a long protocol and may be associated with a P level reduction on the day of hCG administration and better endometrial receptivity. Otherwise, serum P levels and the ratio of P/E2 on the day of hCG administration were not considered to be related with IVF outcome of assisted reproduction [8]. In contrast, Bosch et al. showed, that serum P levels and the ratio of P/E2 on the day of hCG administration are indicators of an implantation window for the uterine endometrium, affecting the pregnancy rate through effecting endometrial receptivity [9]. The implantation window of IVF patients was forward shifted compared with women in natural menstruation cycles and lead to low implantation rates. Elgindy et al.[10] showed that clinical blastocyst pregnancy rates per transfer were reduced when the serum P levels and the ratio of P/E2 on the day of hCG administration were higher than 1.5ng/ml and 0.55, respectively. Other studies [11] displayed high P levels on the day of hCG administration and the results were lower clinical and complete pregnancy rates. In our study, serum P levels and the ratio of P/E2 on the day of hCG administration in the ultra-long protocol were significantly lower than those in the long protocol, thus inhibiting the forward shift of the uterine endometrium.
implantation window and improving endometrial receptivity, which also improved the success rate of IVF-ET. LH levels before the day of Gn stimulation reflect down regulation of pituitary suppression and there is no recognized threshold for this. In our research, the LH level before the day of Gn stimulation in the ultra-long protocol group was lower than in the long protocol group and was also lower than the ideal LH threshold range (0.5-1.5 IU/L) suggested in a previous publication [12]. We used hMG to start ovulation in the ultra long protocol, which was a supplement for low LH levels and obtained good pregnancy outcomes. Other studies reported that the LH level before the day of Gn stimulation does not influence ovarian response and pregnancy outcome [13], while others suggest that the clinical outcome of ART is not affected while LH<0.5 IU/L [14]. The sonographic detection of uterine endometrial thickness, morphology, blood flow distribution is the preferred assessment of endometrial receptivity, because it is non-invasive and became widely used by clinicians. Previous literature showed, that embryos were easier to implant and developed better on type A and type B endometrial morphologies than on type C [3, 15, 16]. Jarvela et al. suggested that in COH multilayer (three linear) endometrial morphology is more suitable for embryo implantation and homogeneous endometrial morphology indicates poor IVF outcomes. Non three linear endometrial morphology is a signal that the endometrium transformed to the secretory phase prematurely [17].

In our research, the proportions of type A and type B endometrial morphologies on the day of hCG in the ultra-long protocol were higher than in the long protocol group, but without significant difference, while the percentage of type C endometrial
morphology on the day of hCG medication in the ultra-long protocol was significantly lower than in long protocol group (P=0.001). The endometrial morphologies on ET day were C in both protocols. These findings illustrated, that the ultra-long protocol led to adequate pituitary down regulation and inhibited the premature endometrium transformation into the secretory phase, thus inhibiting the forward shift of the endometrial implantation window.

Other studies showed that good endometrial perfusion was important for successful embryo implantation and rich blood flows of endometrial and sub-endometrial tissues are favorable for embryo implantation [5, 7, 18]. In contrast, other studies indicated that reduced blood flow in endometrial and sub-endometrial tissues resulted in higher clinical pregnancy rates [19], whereas Jarvela et al. and Raine-Fenning et al. emphasized, that endometrial blood flows after oocyte retrieval of IVF/ICSI-ET pregnant patients and spontaneous pregnancies after ovulation were higher than in non-pregnant women [17, 20]. In our research, the proportions of type A and type B endometrial and sub-endometrial blood flows on the hCG and ET days in the ultra-long protocol were higher than in long protocol group, though without significant differences. Most of the current research noted, that appropriate thickness of the endometrium is essential for embryo implantation [16, 21-24]. The implantation and clinical pregnancy rates increased while the endometrial thickness raised to >14mm on the day of hCG application, while the implantation and pregnancy rates were almost zero with endometrial thickness <6-7mm [16, 22, 24]. Other studies suggested the opposite point of view in which the endometrial thickness
on the hCG day did not correlate with the pregnancy outcomes in IVF cycles, but endometrial thickness combined with sonographic endometrial morphology and blood flow distribution assessments of endometrial receptivity is more clinical significant [17, 21, 23, 25]. In our research, the endometrial thickness on the day of hCG medication in the ultra-long protocol group was thicker than in the long protocol group. Literature reported that obesity and a high body mass index (BMI) in patients have negative effects on the embryo quality and endometrial receptivity in the process of assisted reproduction [18, 26-28]. Lintsen et al. reported, that pregnancy rates of patients with BMI≥27kg/m² were significantly lower than that of patients with BMI≤25kg/m² and 25-27kg/m² [18]. The ideal BMI of Chinese women is 18-23kg/m². In this study, the patient’s BMI in the modified ultra-long protocol group was slightly higher than that in long protocol group. **However, the implantation and pregnancy rates were significantly higher than in the long protocol group and we propose, that PCOS patients with BMIs >23 kg/m2 and obesity are more suitable for the ultra long protocol.**

**Conclusion**

In conclusion, twice injection of small GnRHα doses before the COH combined with hMG significantly increased the pregnancy rates. In addition, the lower serum P level on the hCG day might ameliorate the receptivity of the endometria assessed as endometrial morphology, thickness, blood flow and improve pregnancy rates. **We believe that the modified ultra-long protocol plus hMG ovarian stimulation regimens are preferable treatments for PCOS patients especially with elevated BMIs.**
research should focus on biological molecular endometrial changes and its mechanisms in an ultra-long protocol.
List of abbreviations

IVF: in vitro fertilization

GnRHα: gonadotropin-releasing hormone agonist

ICSI: intracytoplasmic sperm injection

P: progestogen

HCG: human chorionic gonadotrophin

OHSS: ovarian hyperstimulation syndrome

Gn: gonadotrophin

hMG: human menopausal gonadotropin

PCOS: Poly-cystic Ovarian Syndrome

ART: assisted reproductive technologies

LH: Luteinizing Hormone

rFSH: Recombinant Follicle Stimulating Hormone

hCG: human chorionic gonadotropin

COH: Controlled ovarian hyperstimulation

ET: embryo transfer

ANOVA: analysis of variance

Acknowledgments

None
Conflict of interest statement

The authors have declared that no competing interests exist

Authors’ Contributions:

Fei Gong, Juan Li, Ge Lin and Guangxiu Lu were responsible for the conception and design of the study. Fei Gong, Xihong Li, Shunji Zhang, Hainan Ma, Sufen Cai, Juan Li, Ge Lin and Guangxiu Lu were responsible for acquisition of data. Fei Gong, Xihong Li, Shunji Zhang, Hainan Ma, Sufen Cai and Guangxiu Lu performed the data analysis and drafted the manuscript. All authors participated in interpretation of the findings. Fei Gong and Guangxiu Lu revised and commented the draft, and all authors read and approved the final version of the manuscript.
References


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Page 2 of 4 of hCG injection via transvaginal ultrasound in the midsagittal plane. Studies where gonadotrophins were used in the stimulation protocol. Exclusion criteria were as follows: 1. Studies that evaluated endometrial thickness in natural conception or intravuterine insemination. 2. Studies that did not provide quantitative data on endometrial thickness or pregnancy rates. 3. Studies in which frozen embryos were used 2012, **4**.


### Tables

Table 1 Basic clinical features of the PCOS patient in two groups

<table>
<thead>
<tr>
<th></th>
<th>Ultra-long protocol (n=75)</th>
<th>Long protocol (n=72)</th>
<th>P value</th>
</tr>
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<tr>
<td>Age</td>
<td>28.01±2.03</td>
<td>27.89±2.91</td>
<td>0.7652</td>
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<tr>
<td>Infertility duration (years)</td>
<td>4.49±2.43</td>
<td>3.88±2.44</td>
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<td>BMI</td>
<td>22.61±2.85</td>
<td>21.71±2.85</td>
<td>0.0580</td>
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<tr>
<td>Basal FSH (mIU/ml)</td>
<td>5.89±1.21</td>
<td>6.12±1.17</td>
<td>0.2460</td>
</tr>
<tr>
<td>Basal LH (mIU/ml)</td>
<td>8.41±5.08</td>
<td>7.58±6.20</td>
<td>0.3753</td>
</tr>
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Table 2. Comparison of clinical outcomes of PCOS patients with two different protocols

<table>
<thead>
<tr>
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<th>Long protocol (n=72)</th>
<th>P value</th>
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<td>LH level on the day before Gn (mIU/ml)</td>
<td>0.63±0.69</td>
<td>1.89±0.87</td>
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<td>Dosage of Gn (IU)</td>
<td>2104.9±908.92</td>
<td>2020.76±899.76</td>
<td>0.5737</td>
</tr>
<tr>
<td>Duration of Gn(day)</td>
<td>11.52±2.15</td>
<td>11.85±2.13</td>
<td>0.3562</td>
</tr>
<tr>
<td>P level on HCG day(ng/ml)</td>
<td>0.63±0.22</td>
<td>0.77±0.23</td>
<td>0.0002*</td>
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<tr>
<td>E₂ level on HCG day (pg/ml)</td>
<td>3707.29±1307.97</td>
<td>3667.29±1621.14</td>
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<tr>
<td>P*1000/E₂ on HCG day</td>
<td>0.19±0.08</td>
<td>0.25±0.13</td>
<td>0.0006*</td>
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<tr>
<td>LH level on HCG day (mIU/ml)</td>
<td>1.40±0.45</td>
<td>1.29±0.51</td>
<td>0.1558</td>
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<tr>
<td>mean number of oocytes retrieved</td>
<td>14.63±5.96</td>
<td>15.58±5.00</td>
<td>0.2928</td>
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<td>Fertilization rate (%)</td>
<td>84.11±17.16</td>
<td>82.26±14.95</td>
<td>0.4865</td>
</tr>
<tr>
<td>Good quality embryo rate (%)</td>
<td>71.13±25.10</td>
<td>66.52±25.15</td>
<td>0.2679</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>59.56% (81/136)</td>
<td>44.09% (56/127)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Cancel rate (%)</td>
<td>9.33% (7/75)</td>
<td>13.89% (10/72)</td>
<td>0.388</td>
</tr>
<tr>
<td>the number of transferred embryos</td>
<td>2.0±0</td>
<td>2.05±0.28</td>
<td>0.1595</td>
</tr>
<tr>
<td>Clinical pregnancy rate per transfer (%)</td>
<td>77.94% (53/68)</td>
<td>61.29% (38/62)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Sever OHSS rate (%)</td>
<td>1.33% (1/75)</td>
<td>2.78% (2/72)</td>
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<tr>
<td>Abortion rate (%)</td>
<td>4.76% (3/53)</td>
<td>4.76% (2/38)</td>
<td>1.000</td>
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<tr>
<td>Ectopic pregnancy rate (%)</td>
<td>0% (0/53)</td>
<td>2.38% (1/38)</td>
<td>0.218</td>
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</table>

Note: *there is significant difference between two groups, P<0.05.
Table 3. Endometrial thickness, morphology and blood flow of two protocols

<table>
<thead>
<tr>
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<th>Long protocol (n=72)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial thickness on HCG day (mm)</td>
<td>12.67±1.67</td>
<td>11.78±2.30</td>
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<tr>
<td>Endometrial morphology on HCG day</td>
<td>A 32.0% (24/75)</td>
<td>23.6% (17/72)</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>B 57.3% (43/75)</td>
<td>43.1% (31/72)</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>C 10.7% (8/75)</td>
<td>33.3% (24/72)</td>
<td>0.001</td>
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<tr>
<td>Endometrial blood flow on HCG day</td>
<td>A 50.7% (38/75)</td>
<td>36.1% (26/72)</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>B 49.3% (36/75)</td>
<td>56.9% (41/72)</td>
<td>0.278</td>
</tr>
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<td></td>
<td>C 0% (0/75)</td>
<td>6.9% (5/72)</td>
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<tr>
<td>Endometrial thickness on ET day (mm)</td>
<td>12.91±2.01</td>
<td>11.97±2.85</td>
<td>0.0226</td>
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<tr>
<td>Endometrial morphology on ET day</td>
<td>A 0% (0/75)</td>
<td>0% (0/72)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>B 0% (0/75)</td>
<td>0% (0/72)</td>
<td>1.000</td>
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<tr>
<td></td>
<td>C 100% (75/75)</td>
<td>100% (72/72)</td>
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<tr>
<td>Endometrial blood flow on ET day</td>
<td>A 32.0% (24/75)</td>
<td>23.6% (17/72)</td>
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<tr>
<td></td>
<td>B 49.3% (34/75)</td>
<td>37.5% (27/72)</td>
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<tr>
<td></td>
<td>C 18.7% (14/75)</td>
<td>38.9% (28/72)</td>
<td>0.007</td>
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</table>
**Figure Legends:**

Figure 1. Classification of endometrial morphologic. **A**: three linear or multilayer endometrial, the outer and central are echogenic and the inner is hypoechoic or dark areas, the uterine cavity midline echo significantly;  **B**: the middle is isolated echo, like uterine myometrium image, the uterine cavity midline echo is not obvious;  **C**: the endometrial is homogeneous echogenic, without uterine cavity midline echo.

Figure 2. Classification of endometrial and subendometrial blood flows.  **A**: can be monitored to endometrial and subendometrial blood;  **B**: only can be monitored to subendocardial blood flow;  **C**: endometrium and endometrial blood flow were not detected.