

Original Article

Does progesterone-induced endometrial withdrawal bleed before ovulation induction have negative effects on IUI outcomes in patients with polycystic ovary syndrome?

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Abstract: Polycystic ovary syndrome is a common heterogeneous endocrine disorder in reproductive-age women, with prevalence around 4-12%. The present study was performed to investigate whether progesterone-induced endometrial bleed before ovulation induction affects pregnancy in patients with PCOS who underwent intrauterine insemination (IUI) treatment. A total of 241 IUI cycles were retrospectively analyzed. Patients enrolled in this study underwent ovulation induction with IUI treatment from Jan. 2011 to Dec. 2012. The study group consisted of 184 cycles with progesterone-withdrawal bleed before ovulation induction. The control group included 57 cycles with spontaneous menses. The clinical characteristics, ovulation induction parameters and IUI outcomes, such as pregnancy rate and live birth/ongoing pregnancy rate, were compared between the two groups. We found that patients in induced shedding group had thinner peak endometrium in ovulation induction cycles. Additionally, the ratio of peak endometrial-thickness to baseline endometrial-thickness was lower in induced menses patients. However, the pregnancy rate and live birth/ongoing pregnancy rate per cycle were similar with the control group. Excluding the peak E_2 level, peak E_2 /number of follicles > 15mm and peak endometrial-thickness/baseline endometrial-thickness, no differences were found in ovulation induction or IUI results between patients used Letrozole or Clomiphene Citrate. In patients undergoing administration with Letrozole, those taking progesterone had thinner endometrium and lower peak endometrial-thickness/baseline endometrial-thickness. However, the pregnancy rate and live birth/ongoing pregnancy rate were not statistically different from patients with spontaneous menses. In conclusion, our study showed that progesterone exerted a negative effect on endometrial development, which seemed to be associated with reduced pregnancy results in ovulation induction with IUI cycles.

Keywords: Polycystic ovary syndrome, ovulation induction, endometrial withdrawal bleed, pregnancy rate, live birth/ongoing pregnancy rate, letrozole

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive-age women, with prevalence around 4-12% [1]. Patients with PCOS typically present oligo-ovulation or anovulation, clinical and/or biochemical hyperandrogenism, polycystic ovaries on ultrasonography and various endocrine disturbances such as obesity, diabetes mellitus, insulin resistance [2-4]. Moreover, PCOS has been linked to infertility, which is the common reason for women with PCOS to visit gynecologists.

Patients with PCOS who failed to achieve pregnancy can be treated with Assisted Reproductive Technology. Ovulation induction (OI) with or without intrauterine insemination (IUI) were widely used in managing PCOS related infertility. However, controversies still remain on the issue of the optimal OI plans. In traditional OI protocols, clomiphene citrate (CC) was used as first line agent. In addition, progesterone-induced endometrial withdrawal bleed before administration with CC is recommended by The American Society of Reproductive Medicine Practice Guidelines [5]. During the last decade,

Table 1. Demographic

	SWB group	PWB group	P value
No. of cycles	184	57	
Mean age (years)	28.7±3.3	29.0±3.5	NS
BMI (kg/m ²)	22.4±3.3	21.5±2.9	NS
Duration of infertility (years)	3.7±1.9	3.5±2.0	NS
Primary infertility (%)	140/184 (76.1)	34/57 (59.6)	0.016

Note: NS = not significant; SWB = spontaneous withdrawal bleed; PWB = progesterone-withdrawal bleed.

OI with aromatase inhibitors, such as letrozole (LE), were rapidly expanded [6]. Further, recent studies suggested that progesterone-induced withdrawal bleed before OI reduced the ovulation rate, pregnancy rate and live birth rate [7, 8]. Several hypotheses were proposed to explain the mechanism for the possible detrimental effect of progesterone-induced endometrial shedding on pregnancy. However, none of the suggested mechanisms has been identified to be totally correct. The present study was performed to investigate whether progesterone-induced endometrial shedding affects OI or/and IUI outcomes in patients with PCOS.

Material and methods

Patients

This was a retrospective, noninterventional, single-center cohort study of patients undergoing OI with IUI treatment at reproductive medicine center in Tongji hospital between January 2011 and July 2012. A total of 241 cycles of patients with PCOS were enrolled. All the patients were diagnosed with PCOS according to the Rotterdam 2003 consensus criteria [3]. Exclusion criteria were age > 35 years, other causes of infertility besides PCOS (such as endometriosis or tubal factors infertility), abnormal semen analysis of the spouse. Institutional review Board (IRB) approval was not necessary, since all women in the current study underwent the routine OI with IUI treatment in our center and no additional intervention or sampling was performed.

Protocol of OI and IUI

OI was initiated with administration of Letrozole (LE, Hongrui, China) 5mg/d or Clomiphene Citrate (CC, FERTILAN, CODAL SYNTO, Cyprus) 50mg with or without FSH (Lishenbao, Lizhu, China) 75IU/d intramuscularly from the cycle

day 5 until day 9 after a spontaneous or induced withdrawal bleed by progesterone (Medroxyprogesterone 17-acetate, Xianju, China). The FSH dosage was adjusted according to ovarian response which was examined by ultrasound and serum E₂ level. Ovulation was assessed by transvaginal ultrasound and serum P assessment. When at least one

follicle reached a mean diameter of 18mm, recombinant hCG (Serono, Switzerland) was given to trigger the final follicle maturation. IUI was performed approximately 34-36 hours after hCG infection. Administration with 60mg progesterone intramuscularly was applied as luteal phase support from the day of IUI. Biochemical pregnancy was considered when serum hCG > 20IU/L 2 weeks after IUI but then declined. A clinical pregnancy was defined as a serum hCG level > 20IU/L and gestational sac(s) present on ultrasound scan 5-7 weeks after IUI. Ongoing pregnancy was defined as a pregnancy with positive heartbeat seen on ultrasound after 12 weeks of gestation. All pregnant women were followed up to obtain the live birth, miscarriage or ectopic pregnancy outcomes.

Blood samples and hormone measurements

All blood samples were analyzed at the laboratory of the reproductive medicine center, Tongji hospital, Wuhan, China. Serum FSH, LH, E₂, PRL and total T were measured by radioimmunoassay (Biotechnology Institute of the North, Beijing, China). The inter-assay and intra-assay coefficients of variation were 6.3% and 10.8%, 7.1% and 11.4%, 5.8% and 11.0%, 6.5% and 10.9%, 7.2% and 11.5%, respectively.

Statistical analysis

Shapiro-Wilks test was used to evaluate the distribution of the data. The Continuous data were given as mean and SD. Groups were compared with student's *t*-test or Mann-Whitney *U*-test as appropriate. Categorical variables were presented as number and percentage. Differences between proportions were evaluated with chi-square test and the Fisher exact test. Missing data were excluded per test. A *P* value < .05 was considered statistical significant. SPSS version 13.0 (SPSS Inc.) was used for statistical analysis.

Table 2. Clinical characteristics, OI parameters and IUI outcomes of PCOS patients

	SWB group	PWB group	P value
No. of cycles	184	57	
Day 3 endometrial-thickness (mm)	5.6±1.8	5.9±1.6	NS
Day 3 T (ng/mL)	40.5±18.7	44.4±18	NS
Day 3 E ₂ (pg/mL)	53.9±26.5	70.2±24.5	< 0.01
Day 3 PRL (ng/mL)	11.7±5.7	10.3±6.4	NS
Day 3 FSH (mIU/mL)	6.1±1.5	6.1±1.5	NS
Day 3 LH (mIU/mL)	8.2±5.9	13.7±8.1	< 0.01
Peak endometrial-thickness (mm)	10.1±2.2	9.2±2.3	< 0.01
No. of follicles > 15 mm	1.5±0.9	1.6±1.8	NS
Peak diameter of leading follicle (mm)	20.3±2.5	20.6±2.6	NS
Peak E ₂ level (pg/mL)	336.8±292.6	257.0±164.7	NS
Peak E ₂ /No. of follicles > 15mm (pg/mL)	68.8±124.8	69.6±122.0	NS
Peak endometrial-thickness/day 3 endometrial-thickness	2.0±0.7	1.6±0.5	< 0.01
Postwash sperm motility (%)	91.3±6.3	91.5±6.9	NS
Postwash sperm concentration (X10 ⁶ /mL)	29.9±12.3	30.5±13.4	NS
Biochemical pregnancy rate (%)	3/184 (1.6)	2/57 (3.5)	NS
Implantation rate (%)	47/184 (25.5)	12/57 (21.1)	NS
Live birth/ongoing pregnancy rate (%)	39/47 (83.0)	12/12(100.0)	NS
Miscarriage rate (%)	8/47 (17.0)	0/12 (0.0)	NS
Live birth/ongoing pregnancy rate per cycle (%)	39/184 (21.2)	12/57 (21.1)	NS

Note: NS = not significant; SWB = spontaneous withdrawal bleed; PWB = progesterone-withdrawal bleed.

Results

Demographic data were shown in **Table 1**. Proportion of primary infertility was lower in patients in P-induced withdrawal bleed (PWB) group, as compared to those in spontaneous withdrawal bleed (SWB) group. No differences were found in terms of age, BMI, duration of infertility between the two groups.

Clinical characteristic with respect to baseline endometrial-thickness and serum hormones, as well as the results of OI and IUI treatment were presented in **Table 2**. Day 3 E₂, LH level were higher in patients in PWB group compared with those in SWB group, but no significant differences were found in baseline endometrial-thickness and other serum hormones. The peak endometrial-thickness and the ratio of peak endometrial-thickness to day 3 endometrial-thickness in patients undergoing P-withdraw bleeding preparation were lower than the counterparts in the control group. However, there were no differences in other OI parameters, as indicated by number of follicles with diameter > 15mm, peak diameter of the leading follicle, peak serum E₂ level and the ratio of peak E₂ level to the number of follicles >

15mm. Moreover, the implantation rate, live birth/ongoing pregnancy rate, miscarriage rate and live birth/ongoing pregnancy rate per cycle were similar between the two groups.

According to the regimens used in OI treatment, PCOS patients were divided into LE group and CC group (**Table 3**). The Peak E₂ level, the ratio of peak E₂ to the number of follicles > 15mm, and the ratio of peak endometrial-thickness to day 3 endometrial-thickness of patients in LE group were lower than patients in CC group. As for IUI results, no differences were found between the two groups.

Patients who underwent administration with LE were further divided into two subgroups, according to whether menses were induced with progesterone (**Table 4**). Patients in PWB group had lower peak endometrial-thickness and ratio of peak endometrial-thickness to day 3 endometrial-thickness, as compared to patients who did not use progesterone. Nevertheless, the number of follicles with diameter > 15mm, peak E₂ level, peak diameter of leading follicle and the ratio of peak E₂ level to the number of follicles > 15mm were comparable. The pregnancy outcomes of patients in

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Table 3. OI parameters and IUI outcomes in LE and CC groups

	LE group	CC group	P value
No. of cycles	204	34	
No. of follicles > 15 mm	1.5±1.2	1.7±1.0	NS
Peak E ₂ level (pg/mL)	288.7±247.4	478.8±336.2	0.016
Peak diameter of leading follicle (mm)	20.35±2.6	20.68±2.6	NS
Peak endometrial-thickness (mm)	9.9±2.2	10.0±2.7	NS
Peak E ₂ /No. of follicles > 15mm (pg/mL)	59.2±112.7	120.3±161.9	0.042
Peak endometrial-thickness/day 3 endometrial-thickness	1.8±0.6	2.2±0.7	< 0.01
Biochemical pregnancy rate (%)	5/204 (2.5)	0/34 (0.0)	NS
Implantation rate (%)	51/204 (25.0)	6/34 (17.6)	NS
Live birth/ongoing pregnancy rate (%)	43/51 (84.3)	5/6 (83.3)	NS
Miscarriage rate (%)	7/51 (13.7)	1/6 (16.7)	NS
Live birth/ongoing pregnancy rate per cycle (%)	43/204 (21.1)	5/34 (14.7)	NS

Note: NS = not significant; LE = Letrozole; CC = Clomiphene Citrate.

SWB group, as indicated by implantation rate and live birth/ongoing pregnancy rate per cycle, seem to be better than those in PWB group (27.3% versus 18.5%, 22.0% versus 18.5%, respectively). However, the differences were not statistically significant.

Discussion

The present study suggested that patients in induced endometrial shedding group had thinner peak endometrium during the course of OI with IUI cycles. Additionally, the ratio of peak endometrial-thickness to baseline endometrial thickness was lower in induced menses patients. However, the pregnancy rate and live birth/ongoing pregnancy rate per cycle were similar with the control group. Excluding the peak E₂ level, peak E₂/number of follicles > 15mm and peak endometrial-thickness/baseline endometrial-thickness, no differences were found in OI or IUI results between patients used Letrozole or Clomiphene Citrate. In patients undergoing administration with Letrozole, those taking progesterone had thinner endometrium and lower peak endometrial-thickness/baseline endometrial-thickness. However, the pregnancy rate and live birth/ongoing pregnancy rate were not statistically different.

The OI with CC is considered as the first-line therapy of PCOS related infertility. In Traditional management protocols, P-induced withdrawal bleed were recommended prior to CC administration [5]. However, neither the advantage nor

possible detrimental effect of such practice of endometrial preparation was described. To date, impact of P-induced withdrawal bleed on pregnancy remains a controversial issue. Hurst et al. [9] reported that the clomiphene stair-step protocol decreased the time to ovulation, as compared to commonly used protocols. They concluded that induced menses before CC administration was not necessary. However, they did not report the conception and live birth rates. Another study showed that progesterone had an adverse effect on the rates of pregnancy and live birth in anovulatory women with PCOS [7]. Casper et al. [8] proposed that the decreased possibility of pregnancy or live birth may be associated with the fact that the patients, who underwent induced menses, initiated their OI cycles with thinner endometrium. The direct and indirect effect of progesterone administration on the hypothalamic-pituitary-ovarian axis and endometrial receptivity may also contribute to impaired pregnancy outcomes [10, 11]. Our study showed that peak endometrial-thickness and the ratio of peak endometrial-thickness to baseline endometrial-thickness were lower in patients with induced menses than patients who had spontaneous menses, which paralleled the hypothesis by Casper and Collages. Multiple studies indicated that pregnancy success were associated with adequate endometrial receptivity, which can be partially assessed by endometrial thickness [12-14]. Therefore, we may conclude that P-induced withdrawal bleed have negative effect on endometrial-thickness and receptivity.

Table 4. OI parameters and IUI outcomes in SWB and PWB subgroups of patients undergoing OI with LE

	SWB group	PWB group	P value
No. of cycles	150	54	
No. of follicles > 15 mm	1.4±0.7	1.6±1.8	NS
Peak E ₂ level (pg/mL)	246.0±203.0	260.0±171.4	NS
Peak diameter of leading follicle (mm)	20.2±2.5	20.6±3.0	NS
Peak endometrial thickness (mm)	10.0±2.0	9.1±2.0	< 0.01
Peak E ₂ /No. of follicles > 15mm (pg/mL)	59.5±108.9	67.9±124.1	NS
Peak endometrial-thickness/day 3 endometrial thickness	1.9±0.6	1.6±0.5	0.022
Implantation rate (%)	41/150 (27.3)	10/54 (18.5)	NS
Live birth/ongoing pregnancy rate per cycle (%)	33/150 (22.0)	10/54 (18.5)	NS

Note: NS = not significant; SWB = spontaneous withdrawal bleed; PWB = progesterone-withdrawal bleed; LE = Letrozole.

In the present study, we used the ratio of E₂ to the number of follicles > 15mm to assess the quality of each follicle and ratio of peak endometrial-thickness to baseline endometrial-thickness to assess the endometrium proliferation, development and response to OI regimens. To our best knowledge, these parameters were seldom or not used in previous studies. Our results suggested that the endometrial-thickness were affected in patients with P-induced menses. However, the quality of follicles and pregnancy parameters were not significantly altered by progesterone. Such results might derive from the fact that although the endometrial-thickness of patients in PWB group were reduced, this endometrial-thickness (9.2±2.2 mm) may be sufficient for embryo-implantation. Our hypothesis was supported by the study by Okohue et al. [12]. They reported that an endometrial-thickness between 7 and 14mm was suitable for conception.

CC was considered as the first line regimen in OI treatment for more than 4 decades, but some recent studies showed that LE may be an acceptable alternative to CC as OI agent in managing PCOS [15, 16]. Moreover, compared with CC, LE was reported to have more positive influences and/or less negative influences on several markers of endometrial receptivity, such as LIF, LIFR, HOXA10, Integrin [17-19]. Indeed, administration with LE was commonly applied in our center. The present study showed that although the ratio of peak endometrial-thickness to baseline endometrial-thickness was lower in patients in LE group than those in CC group, the peak endometrial-thickness was similar. In addition, no differences were found

in pregnancy parameters. Our results were in line with the previous studies [15, 16, 20].

In the context of impact of P-induced withdrawal bleed on pregnancy, the previous study by Diamond et al. [7] was performed in patients underwent CC administration. To our best knowledge, impact of progesterone-induced shedding on conception in patients with LE treatment has not been reported. In the current study, patients who underwent administration with LE were divided into P-induced menses and spontaneous menses group. Patients taking progesterone had lower peak endometrial thickness and ratio of peak endometrial-thickness to baseline endometrial thickness. Furthermore, implantation, live birth/ongoing pregnancy rates seemed to be lower in patients with P-induced menses, as compared to patients with spontaneous menses (27.3% versus 18.5% and 22.0% versus 18.5% respectively), whereas the differences were not statistically significant. Such results may relate to the relatively small size of the cohort in our study, further study with large population was needed. Therefore, we proposed that the progesterone may adversely influence pregnancy by affecting endometrial receptivity.

Limitations of the study are its retrospective nature and the relatively small size of the cohort. Results in the present study need to be interpreted with caution, because several cofound factors may interfere with the results: number of times of the same patients, the delay between treatments, the option of OI regimens and other management besides OI taken by some patients (such as surgery, herbal therapy).

In conclusion, the present study showed that the progesterone exerted a negative effect on endometrial development, which seemed to be associated with reduced pregnancy results in OI with IUI cycles.

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Declaration

There were no conflicts of interest. All authors have nothing to declare.

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