

Luteal start vaginal micronized progesterone improves pregnancy success in women with recurrent pregnancy loss

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Objective: To assess the effectiveness of luteal start vaginal micronized P in a recurrent pregnancy loss (RPL) cohort.

Design: Observational cohort study using prospectively collected data.

Setting: Not applicable.

Patient(s): Women seen between 2004 and 2012 with a history of two or more unexplained pregnancy losses <10 weeks in size; endometrial biopsy (EB) performed 9–11 days after LH surge; and one or more subsequent pregnancy(ies). Women were excluded if concomitant findings, such as endometritis, maturation delay, or glandular-stromal dyssynchrony, were identified on EB.

Intervention(s): Vaginal micronized P was prescribed at a dose of 100–200 mg every 12 hours starting 3 days after LH surge (luteal start) if glandular epithelial nuclear cyclin E (nCyclinE) expression was elevated (>20%) in endometrial glands or empirically despite normal nCyclinE (≤20%). Women with normal nCyclinE (≤20%) who did not receive P were used as controls.

Main Outcome Measure(s): Pregnancy success was an ongoing pregnancy >10 weeks in size.

Result(s): One hundred sixteen women met the inclusion criteria, of whom 51% (n = 59) had elevated nCyclinE and 49% (n = 57) had normal nCyclinE. Pregnancy success in the 59 women with elevated nCyclinE significantly improved after intervention: 6% (16/255) in prior pregnancies versus 69% (57/83) in subsequent pregnancies. Pregnancy success in subsequent pregnancies was higher in women prescribed vaginal micronized P compared with controls: 68% (86/126) versus 51% (19/37); odds ratio = 2.1 (95% confidence interval, 1.0–4.4).

Conclusion(s): In this study, we found that the use of luteal start vaginal micronized P was associated with improved pregnancy success in a strictly defined cohort of women with RPL. (Fertil Steril® 2017;107:684–90. ©2016 by American Society for Reproductive Medicine.)

Key Words: Recurrent pregnancy loss, recurrent miscarriage, progesterone, endometrium, cyclin E

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Recurrent pregnancy loss (RPL), defined as two or more pregnancy losses at less than 10 weeks in size (1), is a challenging clinical problem with few evidence-based treatment options. Factors associated with RPL are numerous, including genetic, endocrine,

anatomic, immunologic, infectious, and autoimmune. Evaluation and management of RPL patients involves comprehensive testing and close monitoring of subsequent pregnancies.

The role of endometrial factors in early gestation and pregnancy loss is an

area of increasing interest. Studies of uterine secretions by Burton et al. have revealed that the endometrial glands may play a larger role in early pregnancy than previously thought. Burton et al. demonstrated that endometrial glands remain active until at least 10 weeks of pregnancy. Glycoproteins MUC-1 and glycodeilin A secreted by the endometrial glands are phagocytized by the placental syncytiotrophoblast, indicating a nutritive role in early embryonic development (2, 3). Additionally, multiple studies have demonstrated a decreased concentration of MUC-1 in endometrial biopsies (EBs) obtained from women with RPL

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compared with fertile controls, indicating an association between RPL and deficiencies in endometrial glandular activity (4, 5).

Glandular development and endometrial maturation can be assessed based on the appearance of hematoxylin and eosin- (H&E-) stained endometrial tissue using the eight morphologic markers proposed by Noyes et al. in 1950 (6). Unfortunately, this morphologic classification has been shown to have high interobserver and intraobserver variation (7). Coutifaris et al. demonstrated that the ability of histologic evaluation to discriminate between fertile and infertile couples is poor (8). The investigators suggested that continued research focusing on molecular markers of endometrial development should be pursued.

This study uses the endometrial molecular marker, nuclear cyclin E (nCyclinE), a cell cycle regulator that changes in intensity and subcellular localization throughout the menstrual cycle. Dubowy et al. reported that abnormal nCyclinE expression in endometrial glands, defined as greater than 20% after day 20 of the menstrual cycle, correlates with a history of infertility and may be a useful molecular marker of endometrial development (9).

P induces a secretory transformation of the endometrium; it is essential to achieve and maintain pregnancy. Therefore, vaginal micronized P is commonly used empirically in women with RPL of <10 weeks in size. Several studies have examined the clinical utility of vaginal, oral, and/or IM P in improving the live-birth rate in women with either first trimester spotting or a history of RPL (10–12). A Cochrane review and meta-analysis suggested that P supplementation could improve the live-birth rate in women with three or more pregnancy losses (10). However, these studies had heterogeneous cohorts, variable routes of P administration, and the use of P after women were already symptomatic with vaginal bleeding.

In 2015, Coomarasamy et al. performed a randomized trial of vaginal P in women with recurrent miscarriage, defined as three more pregnancy losses in the first trimester (13). Women were randomized to 400 mg of vaginal micronized P twice daily or matched placebo, starting after a positive pregnancy test but no later than 6 weeks of gestation. The live-birth rate was similar between groups, 66% versus 63%. Although the trial was well conducted, we question the late start and high daily dose of P administered.

It is well known that the midcycle LH surge promotes luteinization of the granulosa cells with consequent increased P production. P supplementation is routinely prescribed in assisted reproduction to improve endometrial development, starting shortly after the LH trigger. Therefore, if nCyclinE, a marker of endometrial development, is abnormally elevated in the luteal phase in a cohort of women with a history of RPL of <10 weeks in size, we hypothesize that vaginal micronized P starting 3 days after the LH surge may be effective in improving their subsequent pregnancy outcomes.

MATERIALS AND METHODS

Patients

Approval was obtained from the University of Chicago Institutional Review Board (IRB) to prospectively collect data and

tissue from women and their partners seen in the University of Chicago Recurrent Pregnancy Loss Program for future research in RPL. All of the subjects gave written informed consent. Approval was obtained from the University of Illinois IRB for this specific study.

Subjects were identified using the University of Chicago Recurrent Pregnancy Loss Database (Microsoft Access 2007), created by one of the authors (M.D.S.). The database was queried for all women seen between July 2004 and April 2012 who had a history of RPL, defined as two or more “unexplained” (miscarriages with chromosome errors excluded) pregnancy losses of less than 10 weeks in size, an RPL evaluation including an EB 9–11 days after LH surge, and at least one subsequent pregnancy, conceived without the use of fertility drugs, closely monitored in the University of Chicago Recurrent Pregnancy Loss Program. Women with histologic findings on EB, including maturation delay, glandular-stromal dyssynchrony, and intraglandular neutrophils and macrophages were excluded (n = 32). Any discrepancies or omissions in the data set were corrected by chart review.

The RPL diagnostic screening protocol was previously described with definitions of positive and negative results (14). In brief, a laboratory evaluation for RPL included TSH, PRL, cytogenetic analysis of both partners, and antiphospholipid antibodies (lupus anticoagulant, anticardiolipin and beta-2-glycoprotein IgG, IgM). An office hysteroscopy was also performed to evaluate the uterine cavity.

Criteria for Abnormal nCyclinE Expression

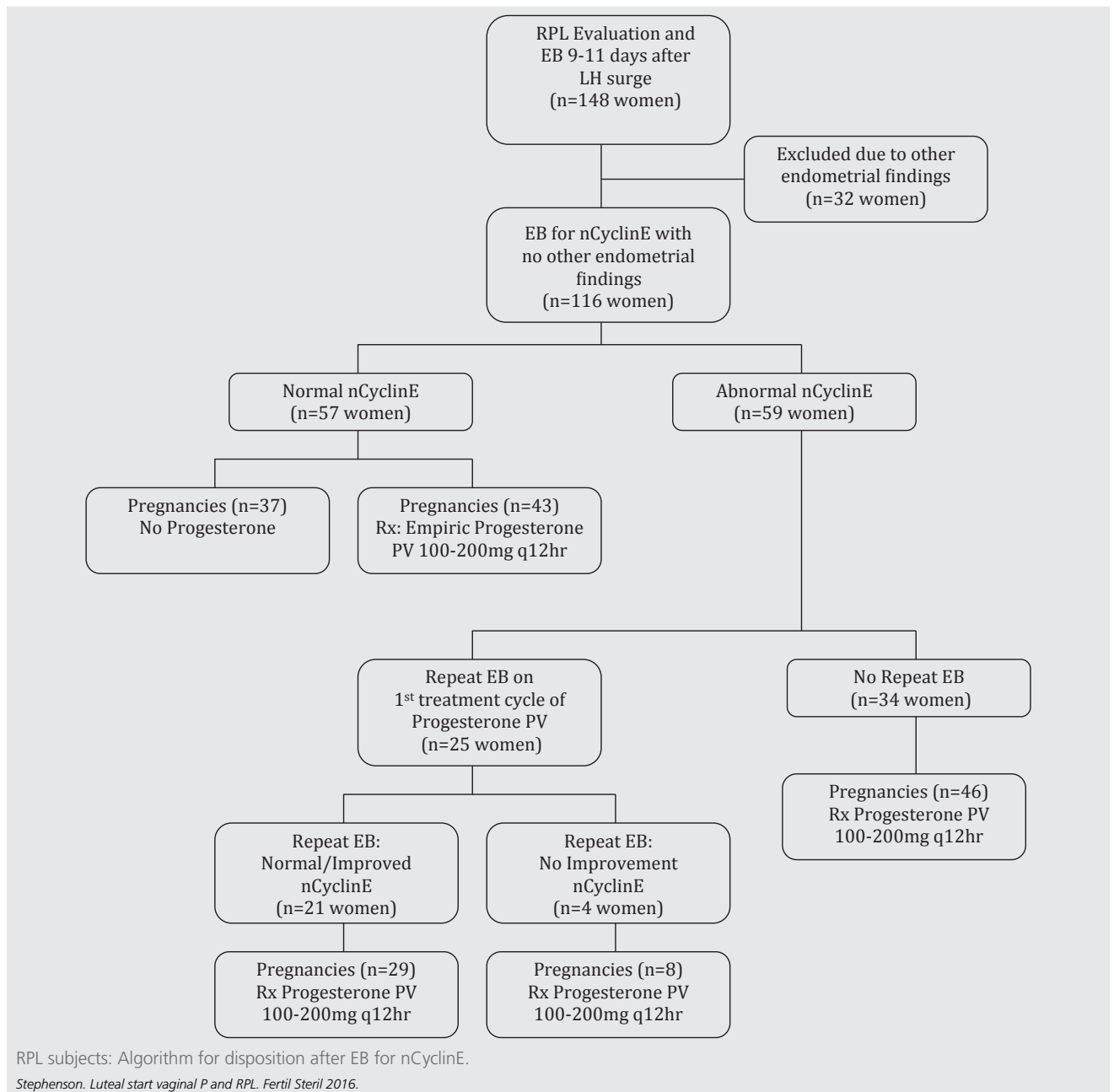
The EB was performed 9–11 days after the LH surge, documented by self-administered urinary LH testing; patients were advised to use condoms or abstain from intercourse in this cycle.

The LH surge was defined as day 13 of the menstrual cycle. The endometrium was evaluated histologically after H&E staining, according to the Noyes et al. criteria (6). In addition, immunohistochemical staining for nCyclinE expression in endometrial glands was performed and interpreted by one of the authors (H.J.K.), as described elsewhere (9). Reliability was assessed in a cohort of 100 patients whose samples underwent repeated immunohistochemical staining (mean of six per patient). Excellent reliability was established with an intraclass correlation of 0.76 (95% confidence interval [CI], 0.70–0.82). An abnormal result was defined as greater than 20% nCyclinE by maximizing the odds ratio (OR) for pregnancy rates above and below different cutoffs. To determine the appropriate cutoff value, testing was performed in a group of 118 women seeking infertility evaluation at Yale University. There was a 42% pregnancy rate among women with nCyclinE ≤20% and an 8% pregnancy rate for women with nCyclinE >20% (OR = 0.12, 95% CI, 0.02–0.99; P = .027).

Management Strategy

Commercially available vaginal micronized P (Endometrin or Prometrium) was prescribed at a dose of 100–200 mg every 12 hours starting 3 days after the LH surge (luteal start) and continued until 10 weeks of gestation in women with elevated nCyclinE (>20%). Some women with normal nCyclinE (≤20%) insisted on using empiric vaginal micronized P,

FIGURE 1



prescribed at a dose of 100–200 mg every 12 hours, as stated above. Women with normal nCyclinE ($\leq 20\%$) who did not use P were used as controls.

Women with an elevated nCyclinE were given the option of having a repeat EB on their first cycle of vaginal micronized P, or attempting pregnancy, as shown in Figure 1. If the nCyclinE remained elevated on the repeat EB, vaginal micronized P was increased to a maximum of 200 mg every 12 hours, prescribed as stated above. The pathologist reviewing the biopsy was not aware of whether or not the patient was treated with P. Concomitant factors associated with RPL were managed per protocol as previously published (14).

Subsequent pregnancy was defined by a serum β hCG of ≥ 5 mIU/mL, drawn 1–2 days after a missed menses; the β hCG was repeated 1 week later. Transvaginal ultrasound was performed at 6 weeks of gestation. All women were offered close monitoring and supportive care until the end of the first trimester, with transvaginal ultrasound and physician visits every 1–2 weeks, with 24-hour emergency coverage. At the end of the first trimester, ongoing prenatal care was transferred to the local obstetrician or a maternal fetal medicine subspecialist, if indicated. Pregnancy outcomes were obtained from the obstetrician and/or hospital records.

Definitions

A pregnancy loss of less than 10 weeks in size included miscarriage (with embryo size based on crown-rump length), resolved pregnancy of unknown location (PUL), and biochemical pregnancy loss. Based on the Consensus Statement from the European Society for Human Reproduction and Embryology Early Pregnancy Special Interest Group, a biochemical pregnancy loss was defined as decreasing serum or urinary hCG levels without an ultrasound evaluation (15). The estimated gestational age was calculated based on the last menstrual period unless a difference of 3 days was documented by crown-rump length on first trimester ultrasound (16).

Outcome Measures

Pregnancy success was defined as a term, preterm, or ongoing pregnancy of greater than 10 weeks in size. The secondary outcome was the prevalence of abnormal nCyclinE among women with RPL.

Data Analysis

The data were collected prospectively and entered into the University of Chicago Recurrent Pregnancy Loss Database (Microsoft ACCESS 2007) and transferred to Microsoft Excel for analysis. Discrepancies and omissions were corrected by chart review.

Demographics were compared between women with normal and abnormal nCyclinE expression in endometrial glands. Categorical variables were compared by Student's *t*-test. Continuous variables were compared by χ^2 -test or Fisher's exact test, as appropriate. A two-sided $P < .05$ was considered statistically significant.

A multivariate generalized estimating equation (GEE) model was used as this data set includes multiple pregnancy outcomes recorded for a single subject. Backward selections were performed for age, body mass index (BMI), race, and

concomitant factors, including parental translocation, hypothyroidism, intrauterine adhesions, uterine septum, and antiphospholipid syndrome; no factor was found to be significant in the multivariate model ($\alpha = 0.05$).

RESULTS

One hundred sixteen women met the inclusion criteria. The cohort had a total of 499 prior pregnancies, of which 425 (85%) ended in pregnancy loss of less than 10 weeks in size. The demographics of the cohort are presented in Table 1. There were no significant differences in maternal age, ethnicity, BMI, and incidence of concomitant factors associated with RPL between women who received P and controls.

Of the RPL cohort, 51% (59/116) had abnormally elevated nCyclinE on the initial EB and 49% (57/116) were normal. There were no significant differences in maternal age, ethnicity, BMI, and incidence of concomitant factors associated with RPL between women with abnormal and normal nCyclinE on initial EB.

All 59 women with elevated nCyclinE expression in endometrial glands were prescribed vaginal micronized P, 100–200 mg every 12 hours starting 3 days after the LH surge. Twenty-five of the women with initially elevated nCyclinE had a repeat EB on the first treatment cycle of vaginal micronized P, of which 84% ($n = 21$) showed a decrease in nCyclinE and 16% ($n = 4$) did not. Three of the four women with no decrease in nCyclinE were prescribed an increased dose of vaginal micronized P of 200 mg every 12 hours; two women corrected at this dose.

Representative H&E- and nCyclinE-stained endometrium are shown in Figure 2 and Supplemental Figure 1.

Pregnancy Outcomes

As shown in Table 2, the 59 women with elevated nCyclinE expression on the initial biopsy had 255 prior pregnancies and 83 subsequent pregnancies using either 100 mg every 12 hours ($n = 56$) or 200 mg every 12 hours ($n = 27$) vaginal

TABLE 1

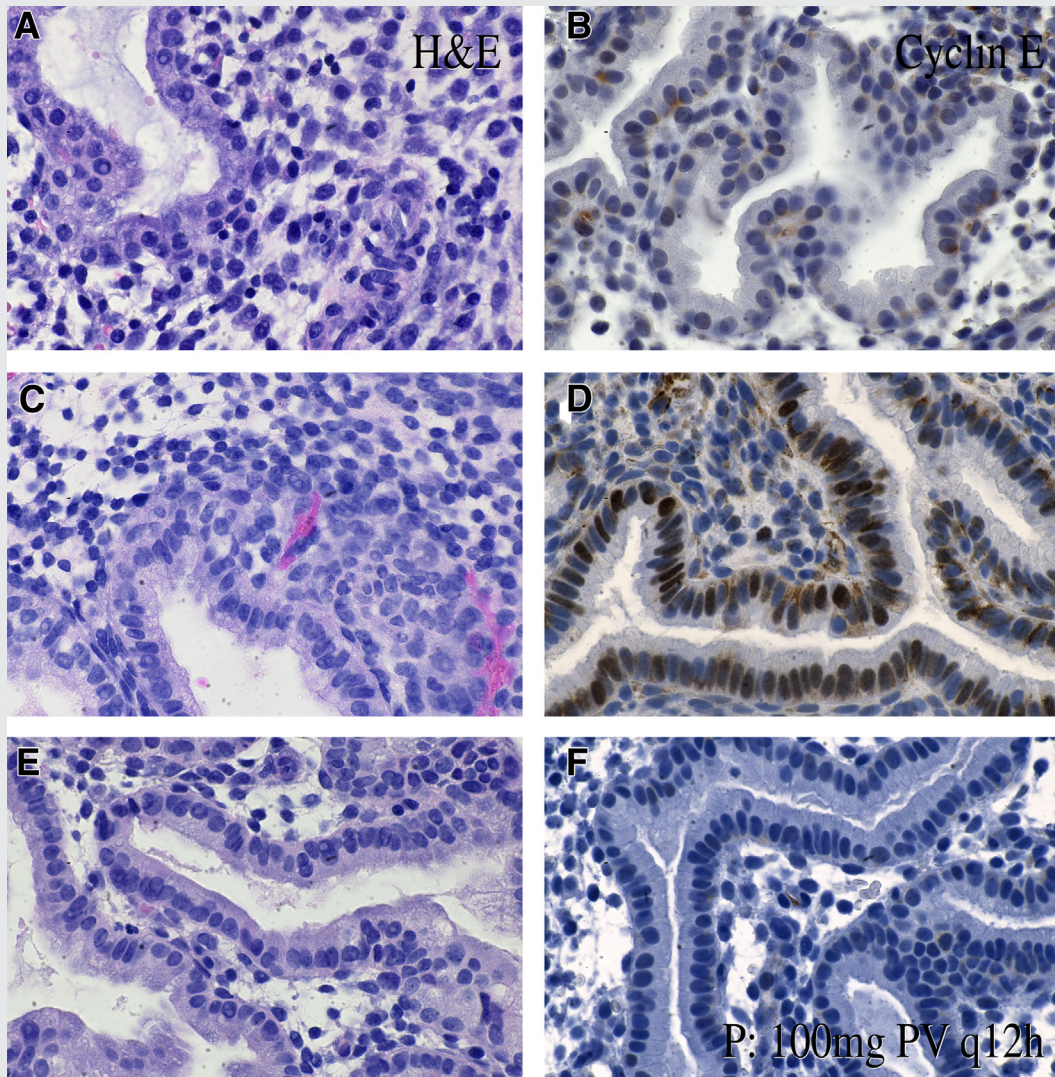
Demographics of recurrent pregnancy loss subjects in their initial subsequent pregnancy ($n = 116$).

Variable	P ($n = 88$)	No P ($n = 28$)
Age (y) at initial consult (SD, range)	33.8 (3.6, 25–43)	35.0 (2.7, 31–42)
Ethnicity, n (%)		
Non-Hispanic	83 (94)	27 (96)
Hispanic	5 (6)	1 (4)
Race, n (%)		
Caucasian	84 (95)	25 (89)
Asian	2 (2)	2 (7)
Black	2 (2)	1 (4)
BMI (kg/m^2) at initial consult (SD, range)	24.6 (4.2, 16.6–38.3)	24.5 (4.1, 18.4–34)
Concomitant factors associated with RPL, n (%)		
Translocation	4 (4.6)	0 (0)
Hypothyroidism	10 (11.4)	2 (7.1)
Intrauterine adhesions	3 (3.4)	0 (0)
Uterine septum	5 (5.7)	1 (3.6)
Antiphospholipid syndrome	4 (4.6)	1 (3.6)

Note: BMI = body mass index; RPL = recurrent pregnancy loss.

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FIGURE 2



H&E- and nCyclinE-stained endometrial biopsies obtained 9–11 days after the LH surge. (A) Biopsy revealing normal histologic dating and (B) normal nCyclinE expression. (C) Biopsy revealing normal histologic dating but (D) abnormally increased glandular epithelial nCyclinE expression. Repeat biopsy of same patient shown in panels C and D treated with 100 mg of vaginal micronized P every 12 hours beginning 3 days after the LH surge, now with (E) normal histology and (F) normal absent glandular epithelial nCyclinE expression.

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micronized P. Pregnancy success improved significantly from 6% (16/255) to 69% (57/83) after treatment with vaginal micronized P and monitoring in the RPL Program ($P < .001$, GEE). As shown in [Supplemental Table 1](#), among the 25 women who had a repeat EB on vaginal micronized P, pregnancy success increased stepwise as nCyclinE expression normalized.

The 57 women with normal nCyclinE expression on the initial biopsy had 244 prior pregnancies and 80 subsequent pregnancies, as shown in [Supplemental Table 1](#). Pregnancy success improved significantly from 11% (27/244) to 60% (48/80) after evaluation and close monitoring through the RPL Program ($P < .001$).

A subset of women with normal nCyclinE expression on the initial biopsy ($n = 28$) requested empiric vaginal micronized P. There were 43 subsequent pregnancies using either 100 mg every 12 hours ($n = 39$) or 200 mg every 12 hours ($n = 4$) vaginal micronized P. There was no statistical difference in the success rate between these groups: 67% (29/43) compared with 51% (19/37; $P = .14$).

There was a total of 163 subsequent pregnancies in 116 women: 126 pregnancies with vaginal micronized P (either empirically or due to elevated nCyclinE) and 37 pregnancies without vaginal micronized P. There was one fetal demise in a woman treated with P (15 weeks in size) and one fetal demise in a woman who did not use P (12 weeks in size), as shown in

TABLE 2

Prior and subsequent pregnancy outcomes of cohort with elevated and normal nCyclinE expression in endometrial glands and no other endometrial findings (n = 116 women).

Variable	Initial EB at 9–11 d after LH surge		
	Abnormal nCyclinE (> 20%) (n = 59 women)		Normal nCyclinE (≤20%) (n = 57 women)
Prior pregnancies	255		244
Success: term and preterm, n (%)	16 (6)		27 (11)
Fetal demise, n (%)	8 (3)		3 (1)
PL (<10 wk) n (%) ^a	219 (86)		206 (84)
PL, mean (SD, range)	3.7 (1.7, 2–11)		3.6 (1.2, 2–6)
Maternal age (y) at PL, mean (SD, range)	32.6 (3.7, 24–42)		32.9 (3.5, 19–41)
Other, n (%) ^b	12 (5)		8 (3)
	Vaginal micronized P	Empiric vaginal micronized P	No vaginal micronized P
Subsequent pregnancies	83	43	37
Success: term, preterm, and ongoing, n (%)	57 (69)	29 (67)	19 (51)
Fetal demise, n (%)	1 (1)	0	1 (3)
PL (<10 wk) n (%) ^a	24 (29)	14 (32)	14 (38)
PL, mean (SD, range)	1.1 (0.5, 1–3)	1.4 (1.0, 1–3)	1.3 (0.6, 1–3)
Maternal age (y) at PL, mean (SD, range)	35.8 (2.9, 30–43)	34.5 (3.3, 31–40)	36.4 (3.7, 31–42)
Other, n (%) ^b	1 (1)	0	3 (8)

Note: EB = endometrial biopsy; LH = luteinizing hormone; PL = pregnancy loss.

^a Miscarriage, resolved pregnancy of unknown location, and biochemical pregnancy loss.

^b Ectopic pregnancy, termination or pregnancy, and/or lost to follow-up before 10 wk of gestation.

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Table 2. There were 38 pregnancy losses <10 weeks in size in women treated with P: 14 biochemical pregnancy losses, two anembryonic miscarriages, four yolk sac miscarriages, and 18 embryonic miscarriages. There were 14 pregnancy losses <10 weeks in size in women not treated with P: five biochemical pregnancy losses, one anembryonic miscarriage, one yolk sac miscarriage, and seven embryonic miscarriages.

The mean gestational age at the time of first trimester pregnancy loss in women treated with P was 5.3 weeks (SD, 1.4) versus 5.8 weeks (SD, 1.9) in women not treated with P ($P=.31$). The mean maternal age at the time of first trimester pregnancy loss was similar in both groups, 35.4 years (SD, 3.1) versus 36.4 years (SD, 3.7; $P=.33$).

Twenty-three pregnancy losses underwent chromosome testing. The female-to-male ratio was 1.09. Among women treated with P, the euploid miscarriage rate was 47.1% (8/17) versus 33.3% (2/6) in women not treated with P ($P=.66$).

Pregnancy success was higher in the women prescribed P: 68% (86/126) versus 51% (19/37; $P=.05$); OR = 2.1 (95% CI, 1.0–4.4). Backward selections were performed for age, BMI, race, and concomitant factors; no factor was significant ($\alpha = 0.05$). The number needed to treat with luteal start vaginal micronized P to achieve one additional pregnancy success was six pregnancies.

DISCUSSION

This observational cohort study found the use of luteal start vaginal micronized P was associated with improved pregnancy success in a strictly defined cohort of women with RPL. We propose that luteal start vaginal micronized P leads to improved endometrial gland development, which optimizes the local environment for early maintenance of pregnancy. Although the use of the molecular marker,

nCyclinE, is exploratory, and there is no control group of women without RPL, vaginal micronized P resulted in decreased or normalization of nCyclinE expression in 84% of women with initially elevated expression of this molecular marker. In addition, there was a stepwise increase in pregnancy success with decreased or normalization of nCyclinE expression with the use of vaginal micronized P. Therefore, we recommend a repeat EB in the first treatment cycle of luteal start vaginal micronized P, with a dosing adjustment if nCyclinE is persistently elevated. Immunohistochemical staining of molecular markers such as nCyclinE in endometrial glands, as opposed to histology based on H&E staining alone, may be more reliable in determining an “endometrial factor” in women with a history of RPL; further study is needed.

As stated above, we found that women with a history of RPL and elevated nCyclinE expression of endometrial glands subsequently had a high rate of pregnancy success after luteal start vaginal micronized P. Interestingly, women with normal nCyclinE expression who insisted on using luteal start vaginal micronized P also had a higher rate of pregnancy success than women who did not use P. This could have been due to a placebo effect. Alternatively, since nCyclinE is just one of many molecular markers of endometrial development, luteal start vaginal micronized P may normalize other molecular markers, such as beta3 integrin expression in endometrial glands, which have been reported to be associated with a history of otherwise unexplained RPL (17). Further study of other endometrial molecular markers is needed.

Close monitoring and supportive care has been shown to markedly improve subsequent pregnancy outcome in women with RPL (18, 19). This may explain why women with normal nCyclinE who did not receive P also had a significant improvement in subsequent pregnancy success.

The benefit of using vaginal micronized P in this study is in contrast to the results of the recent randomized trial of Coomarasamy et al. that included a more heterogeneous cohort without evaluation of the endometrium (13). This can be explained by the differences in timing and dosing of P used. In the Coomarasamy et al. trial, P (Utrogestan) 400 mg or matched placebo twice daily was initiated after a positive pregnancy test but no later than 6 weeks of gestation. In contrast, we prescribed luteal phase vaginal micronized P 100–200 mg every 12 hours starting 3 days after the detection of the urinary LH surge. As stated by the Practice Committee of the American Society for Reproductive Medicine, luteal support with P yields significantly higher pregnancy rates compared with placebo or no treatment in assisted reproduction (20). Based on two randomized trials, the benefit of P supplementation is limited to the luteal phase until the day of a positive hCG test, not beyond (21, 22). These randomized trials, albeit in assisted reproduction cohorts, support our finding that luteal start vaginal micronized P improves subsequent pregnancy success.

We recognize our study is limited by its study design and limited sample size, but the results are encouraging and require further investigation. Concomitant factors associated with RPL, except for other endometrial findings, were included in both groups; however, these factors were similarly distributed so the potential for bias is limited. The strengths of this study are that the cohort is well characterized and consistently evaluated and managed by a single provider.

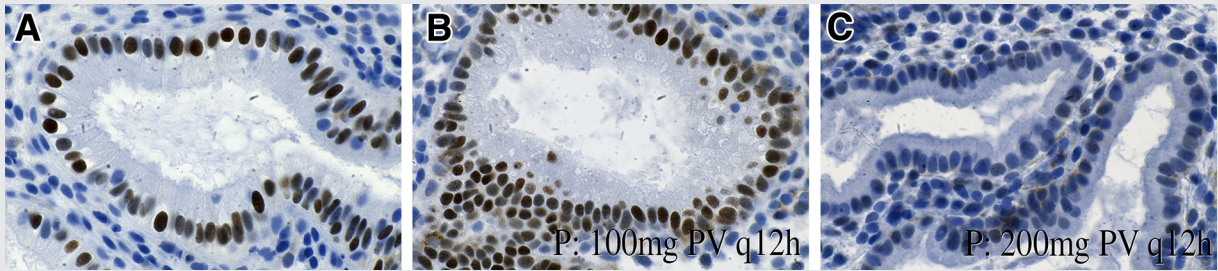
Based on our positive results, a randomized trial with a placebo-matched control group is urgently needed to determine whether luteal start vaginal micronized P improves the subsequent live-birth rate in women with RPL. Since micronized P appears to be safe and is relatively inexpensive, we recommend for the interim the empiric use of luteal start vaginal micronized P in women with RPL. Additionally, further molecular analysis of luteal phase endometrium is warranted to identify women who may benefit the most from the use of luteal start P.

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SUPPLEMENTAL FIGURE 1



Cyclin E immunohistochemistry of a patient who normalized glandular epithelial nCyclinE expression after increasing the dose of vaginal micronized P. (A) Initial abnormal glandular epithelial nCyclinE expression. (B) Persistently abnormal glandular epithelial nCyclinE expression using 100 mg every 12 hours vaginal micronized P beginning 3 days after the LH surge. (C) Normalized glandular epithelial nCyclinE expression after dose was increased to 200 mg of vaginal micronized P every 12 hours beginning 3 days after the LH surge.

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SUPPLEMENTAL TABLE 1

Subsequent pregnancy outcomes of women with elevated nCyclinE expression in endometrial glands on initial biopsy, stratified according to whether nCyclinE expression improved on repeat endometrial biopsy with use of vaginal micronized P (n = 25 women).

Variable	Repeat EB 9–11 d after LH surge (n = 25 women)		
	No change in nCyclinE (n = 4)	Decreased nCyclinE (n = 10)	Normalized nCyclinE (n = 11)
Subsequent pregnancies,	8	14	15
Success: term, preterm, and ongoing, n (%)	5 (63)	10 (71)	12 (80)
PL (<10 wk), n (%) ^a	3 (38)	4 (29)	3 (20)

Note: No fetal demise was reported for any pregnancy. EB = endometrial biopsy; LH = luteinizing hormone; PL = pregnancy loss.

^a Miscarriage, resolved pregnancy of unknown location, and biochemical pregnancy loss.

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