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Endometrial Shedding Effect on Conception and Live Birth in Women With Polycystic Ovary Syndrome

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Abstract

Objective—To estimate whether progestin-induced endometrial shedding, prior to ovulation induction with clomiphene citrate, metformin, or a combination of both, affects ovulation, conception, and live birth rates in women with polycystic ovary syndrome (PCOS).

Methods—A secondary analysis of the data from 626 women with PCOS from the National Institutes of Child Health and Human Development Cooperative Reproductive Medicine Network trial was performed. Women had been randomized to up to six cycles of clomiphene citrate alone, metformin alone, or clomiphene citrate plus metformin. Women were assessed for occurrence of ovulation, conception, and live birth in relation to prior bleeding episodes (after either ovulation or exogenous progestin-induced withdrawal bleed).

Results—While ovulation rates were higher in cycles preceded by spontaneous endometrial shedding than after anovulatory cycles (with or without prior progestin withdrawal), both conception and live birth rates were significantly higher after anovulatory cycles without progestin-induced withdrawal bleeding (live birth per cycle: spontaneous menses 2.2%; anovulatory with progestin withdrawal 1.6%; anovulatory without progestin withdrawal 5.3%; $p < 0.001$). The difference was more marked when rate was calculated per ovulation (live birth per ovulation: spontaneous menses 3.0%; anovulatory with progestin withdrawal 5.4%; anovulatory without progestin withdrawal 19.7%; $p < .001$).

Conclusion—Conception and live birth rates are lower in women with PCOS after a spontaneous menses or progestin-induced withdrawal bleeding as compared to anovulatory cycles without progestin withdrawal. The common clinical practice of inducing endometrial shedding with progestin prior to ovarian stimulation may have an adverse effect on rates of conception and live birth in anovulatory women with PCOS.

INTRODUCTION

In oligo-ovulatory and anovulatory women with polycystic ovary syndrome (PCOS) who wish to conceive, first line therapy is usually ovulation induction with clomiphene citrate (CC). (1, 2) The American Society of Reproductive Medicine Practice Guidelines describes

the use of progestin to induce a withdrawal bleed prior to initiation of CC administration in women with PCOS (3). However, there is scant literature on the value or possible detrimental effect of such a practice. Further, if a patient remains anovulatory in response to the initial clomiphene dose, another dose of progestin is recommended by a leading reproductive endocrinology textbook (4) to induce a withdrawal bleed prior to dose escalation. Others have skipped routine withdrawal with progestins after anovulatory cycles and instead proceeded immediately to repeat CC administration in women with PCOS. One group has labeled this a “stair step protocol” to accelerate treatment progression and time to ovulation with clomiphene. However their report did not describe pregnancy or live birth rates associated with dosage escalation (5).

Our original study protocol entitled Pregnancy in Polycystic Ovary Syndrome I (PPCOSI) (6) utilized either clomiphene, metformin, or the combination of the two for ovulation induction in women with PCOS. In this report, we have performed a secondary analysis of this study to estimate whether progestin administration to induce a withdrawal bleed immediately prior to initiation of ovulation induction (with CC alone, metformin alone, or combination CC and metformin) would influence the subsequent likelihood of ovulation, conception, and live birth in the ensuing cycle.

METHODS

This study represents an analysis of the database of the NIH/NICHD Cooperative Reproductive Medicine Network PPCOSI study. This analysis was approved by the Wayne State University Institutional Review Board; the conduct of the original study was approved by Institutional Review Boards at each participating institution (6). The study protocol has been published previously (6–11), and is briefly reviewed here. Six hundred twenty-six women diagnosed with PCOS had oligomenorrhea (history of eight or less spontaneous menses/year) or amenorrhea, and evidence of hyperandrogenemia (defined as an elevated serum testosterone level). All women were documented to have a normal uterine cavity with patency of at least one fallopian tube, with a partner who had a semen analysis within a year with a sperm concentration of at least 20 million sperm/ml.

Subjects received in a double blind double dummy manner both encapsulated clomiphene (purchased from Teva Pharmaceuticals), 50 mg or placebo, and extended release metformin (Glucophage XR), 500 mg or placebo (provided by Bristol Myers Squibb). Subjects were randomized to treatment arms (clomiphene alone, metformin alone, or combination of clomiphene plus metformin) by means of an interactive voice system, with assignment stratified based on the study center and presence or absence of prior exposure to either clomiphene and/or metformin. Administration of the medications was initiated concurrently and continued for up to six cycles, with the metformin dose increased gradually up to a dose of four tablets/day. Clomiphene was administered as one pill/day for five days beginning cycle day 3 in the initial cycle; the dose was increased one tablet/day in subsequent cycles if there was no evidence of ovulation (as assessed by weekly serum progesterone level of 5 ng/ml), up to a maximum dosage of 3 tablets/day. A negative pregnancy test was confirmed each cycle prior to clomiphene administration. As previously described, baseline demographic characteristics were comparable in each treatment group (6).

Couples were instructed to have intercourse every two to three days, to record vaginal bleeding in their diaries, and to have serum progesterone measured every one to two weeks to diagnose ovulation. For women who failed to have a spontaneous menses, progestin administration to induce withdrawal bleeding was performed at the discretion of each site's principal investigator. Women could receive up to six cycles of treatment if they had failed to conceive. Women with a positive pregnancy test underwent ultrasonography to determine the number of fetuses and their viability, but there were no routine ultrasounds of follicular response during the study. Pregnancy outcomes up till the completion of pregnancy were ascertained.

Methodology for measurement of markers of the androgen milieu (total testosterone, free androgen index, sex hormone-binding globulin (SHBG) and glucose homeostasis (serum glucose, insulin, and proinsulin) have been described previously (6). Similarly, the HOMA algorithm to assess insulin sensitivity has been described previously (7, 8). Briefly, the HOMA index is a measure of insulin action and is derived from fasting glucose and insulin levels based on the formula $[(\text{fasting insulin in } \mu\text{IU/mL} \times \text{fasting glucose in nmol/L}) / 22.5]$. These measures were obtained on a monthly basis during the study.

Analysis was conducted on the outcome variables of ovulation, conception, and live birth in cycles in which women had a spontaneous menstruation preceding administration of study drug, were anovulatory and underwent a progestin-induced withdrawal bleed prior to initiating clomiphene study drug, or were anovulatory and received no progestin prior to receiving study drug. (Menstrual like flow was termed a "spontaneous menses", but because of the retrospective nature of this study it is not possible to be certain whether such bleeding episodes represented endometrial shedding following ovulation or spontaneous shedding in association with anovulation). This analysis was conducted for all three arms (clomiphene alone, metformin alone, and clomiphene and metformin combined) together, as well as separately. Menstrual cycle status of the preceding cycle was categorized: spontaneous menses, anovulatory with progestin withdrawal, and anovulatory without progestin withdrawal. Data were available in 2,809 of 2,925 cycles of therapy during the study (96%) and are the basis for analysis in this article.

Statistical Analysis

Data were analyzed using SPSS v. 18.0 for Windows. Statistical analyses were conducted using Fisher's Exact Test and Pearson Chi-Square Test; hormonal and metabolic parameters were analyzed by two way analysis of variance (ANOVA) and one way ANOVA for menstrual cycle status and treatment condition with Student-Newman-Keuls (SNK) post-hoc comparisons. BMI was analyzed by two way and one way ANOVA for menstrual cycle status and either ovulation, conception and live birth status with SNK post-hoc comparisons. All statistical tests were two-tailed with a significance level of $p < 0.05$ considered statistically significant. Data are expressed as mean $\pm$ SD.

RESULTS

Ovulation after treatment occurred significantly more often in cycles after a previous spontaneous menses, than in cycles after progestin withdrawal, or cycles without progestin

withdrawal ($p < 0.001$, Table I). There was no significant difference in the rate of ovulation between the anovulatory cycles with and without progestin withdrawal. Consistent ovulation rate observations were observed in each of the treatment arms [(clomiphene alone: spontaneous menses 280/386 (72.5%), progestin withdrawal 62/178 (34.8%), and no progestin withdrawal 106/336 (31.5%) respectively, ($p < 0.001$); metformin alone spontaneous menses 202/329 (61.4%), anovulatory with progestin withdrawal 28/201 (13.9%), and anovulatory without progestin withdrawal 60/446 (13.5%) ($p < 0.001$); and clomiphene plus metformin spontaneous menses 371/470 (78.9%), anovulatory with progestin withdrawal 76/172 (44.2%), and anovulatory without progestin withdrawal 123/291 (42.3%), ($p < 0.001$)]. Comparing the individual treatment arms, there was a significant difference in ovulation rates in cycles preceded by spontaneous menses ($p < 0.001$), as well as in prior anovulatory cycles (with ($p < 0.001$) or without progestin withdrawal ($p < 0.001$)). Ovulation rates were lower in the metformin arm than the clomiphene alone and clomiphene plus metformin arms in cycles with a previous spontaneous bleed ($p = 0.006$ and $p = 0.003$ respectively) and in anovulatory cycles with ($p = 0.003$ and $p = 0.003$ respectively) and without ($p = 0.003$ and $p = 0.003$ respectively) progestin withdrawal. The ovulation rate of the clomiphene arm was significantly less than the combined clomiphene plus metformin arm only in cycles with anovulation without progestin ($p = 0.018$).

In the 2809 cycles, conception in all treatment arms occurred in 131 women, with live births in 118. In the 131 conception cycles, 29.8% followed spontaneous menses, 8.4% followed anovulatory cycles with progestin withdrawal, and 61.8% followed anovulatory cycles without a progestin induced withdrawal bleed ($p < 0.001$).

Among all the cycles with ovulation, conceptions occurred in 4.5%, and 6.6%, of the cycles which were preceded by spontaneous menses, and anovulatory cycles with progestin withdrawal respectively; in contrast, the conception rate was dramatically increased (27.7%) in the cycles with anovulation without progestin withdrawal ($p < 0.001$; see table 1). These relationships persisted when examined by each of the individual treatment arms (clomiphene alone, metformin alone, and clomiphene plus metformin) separately (each $p < 0.001$). (Data not shown)

Similarly, when evaluating live births (table 1) as a function of the preceding cycle menstrual status (spontaneous menses, anovulatory with progestin withdrawal, and anovulatory without progestin withdrawal), the rates were 3.0%, 5.4%, and 19.7% respectively ($p < 0.001$). Again, these significant relationships were all manifested when examining each of the clomiphene alone, metformin alone, and clomiphene plus metformin arms separately (each $p < 0.001$). (Data not shown)

To reduce the effect of including different numbers of cycles from each subject and to assess the impact of correlated data from the same subject, we repeated the analysis by including only the initial treatment cycle in the six hundred twenty six women and identified similar results. Thus, the use of data from the multiple cycles of the same patients does not alter our conclusion. Specifically, among women who ovulated who had a preceding spontaneous menses, conceptions occurred in 8/70 cycles (11.4%), while previously anovulatory women

who did or did not undergo progestin withdrawal conceived in 11/159 (6.9%) of cycles and 3/10 (30%) of cycles, respectively ($p=0.037$). Examining live births in the initial cycles, a total of seventeen occurred. Among women with previous spontaneous menses, or anovulation with or without progestin withdrawal, the rate of live births among women who ovulated were 5/70 (7.1%), 9/159 (5.7%) and 3/10 (30%) respectively ($p=0.015$).

We examined the effects of several possible confounding variables. As previously noted (6), a significant relationship of BMI to ovulation, conception, and live birth was observed, with women with lower BMI being more likely to have each of these outcomes (Table 2). This relationship persisted within each of the three groups when considering the menstrual status of the preceding cycle. However, there was no significant interaction between the menstrual status of the preceding cycle with respect to BMI and either ovulation, conception, or live birth. Testosterone and SHBG were both significantly different as a function of menstrual groupings of the preceding cycles; those with spontaneous menses had significantly lower total testosterone levels, as well as higher SHBG levels ($p < 0.001$ and $p < 0.008$ respectively). Levels in the women with anovulatory cycles with or without progestin were comparable. Assessing markers of glucose homeostasis, the preceding menstrual status was not associated with any difference in plasma glucose. However plasma insulin and proinsulin were both significantly different ($p = 0.011$ and $p < 0.001$, respectively), (Table 2). Post hoc analysis revealed that cycles in women with a preceding spontaneous menses had lower insulin and proinsulin levels as compared to cycles preceded by anovulation (with or without progestin induced menses), and had greater insulin sensitivity, as assessed utilizing the HOMA formula (Table 2).

DISCUSSION

Our secondary analysis of the RMN PPCOS I study data, looking at the administration of progestin in an anovulatory cycle prior to ovulation induction, has found that a menstrual bleed immediately prior to the cycle, whether induced or not, is associated with a reduced chance of conception and live birth in the subsequent cycle of ovulation induction. We noted this association both in the initial cycle as well as all cycles combined, and identified it as independent of ovulation induction agent. Further, we noted that this effect was unrelated to ovulation in the previous cycle, which was significantly more likely in cycles with spontaneous menses compared to anovulatory cycles. Importantly, these findings challenge the widespread clinical practice tradition of routinely administering progestin to induce a withdrawal bleed in the context of anovulatory women undergoing ovulation induction.

Administration of fertility promoting medication to anovulatory/oligoovulatory women with PCOS is often successful in helping couples conceive. However, multiple cycles of therapy are often required, both to find the proper dose of ovulation inducing medication and to allow for sufficient opportunity for conception when ovulatory. A recent publication has highlighted the time “lost” in successive cycles of attempted ovulation induction when the generally recognized approach is to administer progestin to induce endometrial shedding after each nonresponsive (anovulatory) cycle (5). Compared to historical controls (3, 4), that report suggested that utilization of a stair step protocol increasing the dose of CC reduced the time to ovulation by 32–53 days, and was associated with a three-fold higher overall rate

of ovulation (64% at the 100 mg/d dosage) as compared to the progestin withdrawal group (where the ovulation rate was 22%). That report did not assess conception or live birth rates.

In our report, the rate of ovulation following anovulatory cycles with or without progestin withdrawal in women with PCOS was significantly reduced when compared with cycles with a preceding menses. Thus these findings are inconsistent with the calculations of effect on the rate of ovulation in the report of Hurst et al (5) where use of progestin withdrawal in the historical control group was associated with a reduced rate of ovulation. In our study, we showed that in cycles not preceded by spontaneous menses, ovulation rates were comparable whether or not treatment was preceded by progestin-induced endometrial shedding. However, we found that cycles preceded by endometrial shedding (either spontaneously or following progestin withdrawal) were associated with several fold lower rates of conception and live birth. Thus, our data does not support “conventional wisdom” that progestin induced endometrial shedding in anovulatory women improves the preparation of the endometrium for pregnancy initiation in the subsequent cycle.

This observation of an apparent detrimental effect of endometrial shedding was observed whether evaluating all cycles that women underwent in the study, or just the initial cycle of each subject. This relationship was also manifest in each of the three treatment arms (clomiphene citrate alone, metformin alone, or clomiphene citrate plus metformin). Additionally, while BMI was associated with the likelihood of each of the endpoints (ovulation, conception, and live birth), (6, 12, 13) there was no significant effect of the menstrual status of the preceding cycle with respect to BMI for any of these outcomes.

These observations raise the question of a mechanism by which lack of endometrial shedding could exert a positive effect on subsequent conception and live births. Possibilities include an indirect consequence of the effect of progestin on the hypothalamic-pituitary-ovarian axis, as well as direct or indirect effect of the differences in the androgenic and metabolic milieus. Progestin administration has direct effects on the endometrium which may be deleterious to subsequent conception. Alternatively, it is generally well accepted that women with PCOS have reduced sensitivity to suppression of LH pulse frequency by progesterone and estradiol, compared to non-PCOS women (14–17). Thus, the increased LH pulse frequency observed in women with PCOS may be due to impairment of sensitivity of the GnRH pulse generator to these hormones. Administration of estradiol and progesterone for seven days to PCOS women reduced serum LH, FSH and testosterone levels, as well as the LH pulse frequency (14). Thus progestin exposure (either endogenous or exogenous) may have altered the HPO axis sufficiently to alter effects of sex hormones and protein factors directly on the endometrium in women receiving clomiphene citrate, metformin or a combination of these two agents, and thereby indirectly affecting the endometrium.

There are multiple other possible mediators of the effect of the status of the preceding menstrual cycle. For example, endometrial androgen receptor expression fluctuates throughout the menstrual cycle, with a gradual reduction from the proliferative phase to the mid-secretory phase, and further diminution in the late secretory phase (18, 19). The potential significance of this variation to conception and live birth is highlighted by the observation that expression of androgen receptors in endometrium is elevated in women

with PCOS (20), an observation consistent with reports that androgen receptor expression is androgen dependent (21).

Interestingly, expression of SHBG, which is usually attributed to the liver, is also found in human uterine endometrium (22). In women with PCOS, SHBG expression in endometrial stroma is reduced as compared to fertile women during the proliferative phase of the menstrual cycle (23). Similarly, women with PCOS have been reported to have reduced expression of insulin signaling molecules (such as insulin-receptor substrates-1, and PPAR gamma) as compared to control women (24, 25). Lack of endometrial shedding in the preceding cycle may influence expression of factors considered to be important to endometrial receptivity, such as integrins, osteopontin, leukemia inhibitory factor, glycodeclin, TNF- α , TGF- β , and VEGF (20, 26–31). Importantly, these observations are consistent with evaluation of androgen receptor expression in pig endometrium during early pregnancy, which indicated a role for the androgen receptor in defining the window of implantation and early pregnancy endometrium, thereby affecting the potential for early pregnancy loss (32).

Although the RMN PPCOS I study was well designed to examine efficacy of clomiphene citrate, metformin or a combination of these agents for ovarian stimulation and live birth rates in women with PCOS, this post hoc evaluation has many limitations which should lead to a cautious interpretation of the finding. The investigator(s) at each site made the decision if, and when, to administer progestin in each subject, as well as the type and duration of progestin therapy. Additionally, while inclusion criteria mandated a normal uterine cavity, at least one patent fallopian tube, and the male partner having at least 20 million sperm/ml, variability existed in parameters related to fertility prognosis, including age of the female partner.

In summary, this secondary analysis has suggested that endometrial shedding whether spontaneous or progestin-induced prior to ovarian stimulation results in a reduction in the subsequent rate of conception and live birth. Taken together with another recent report which estimated a shorter time to ovulation if progestin therapy was withheld, these reports suggest that there may be an advantage with respect to conception and live birth in not initiating progestin withdrawal following an anovulatory cycle in women with PCOS who are about to undergo ovarian stimulation.

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Table 1
Pregnancy Outcomes in the Pregnancy in Polycystic Ovary Syndrome Trial as a Function of the Menstrual Status of the Preceding Cycle*

Menstrual Status group	Cycles		Ovulation		Conception			Live Birth		
	n		n	Ovulation per Cycle	n	Conception per Cycle	Conception per Ovulation	n	Live Birth per Cycle	Live Birth per Conception
Spontaneous menses	1185		853	72.0	39	3.3	4.5	26	2.2	3.0
Anovulatory with progestin withdrawal	551		166	30.1	11	2.0	6.6	9	1.6	5.4
Anovulatory without progestin withdrawal	1073		289	26.9	81	7.5	27.7	57	5.3	19.7
Total	2809		1308	46.6	131	4.7	9.9%	92	3.3	7.0
P				<.001		<.001	<.001		<.001	NS

Data are % unless otherwise specified.

* Spontaneous menses, anovulation with progestin induced withdrawal bleed, and anovulation without progestin induced withdrawal bleed.

Table 2

Age, Body Mass Index, Sex Hormone–Binding Globulin, Glucose, Insulin, Proinsulin and Insulin Sensitivity (Assessed by Homeostasis Model Assessment of Insulin Resistance) at Baseline in Each Cycle, Preceded by a Spontaneous Menses or Anovulation With or Without Progesterin-Induced Menses

Menstrual Status Group	Age (years)	Body Mass Index (kg/m ²)	Total Testosterone (ng/dl)		SHBG (nmol/L)		Glucose (mg/dl)		Insulin (mU/ml)		Proinsulin (pmol/L)		HOMA	
			n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Spontaneous menses	29.5 ± 4.2*	33.3 ± 9.0*	978	50.9 ± 31.5	979	46.7 ± 35.5*	978	87.1 ± 19.5	979	22.7 ± 30.1*	976	20.0 ± 21.1*	978	5.50 ± 9.4
Anovulatory with progestin withdrawal	27.8 ± 3.9	35.4 ± 8.4	514	53.9 ± 30.6	513	32.6 ± 21.1	513	88.8 ± 16.9	513	26.9 ± 32.5	512	24.7 ± 24.2	513	6.6 ± 9.9
Anovulatory without progestin withdrawal	28.2 ± 4.0	38.2 ± 9.9	839	62.2 ± 37.3*	839	35.6 ± 27.1	836	89.3 ± 22.1	839	26.5 ± 32.7	834	26.8 ± 28.8	836	6.5 ± 10.0
Total	28.1 ± 4.0	35.2 ± 8.7	2331	55.6 ± 33.9	2331	39.6 ± 30.5	2237	88.3 ± 20.4	2331	25.0 ± 31.6	2322	23.5 ± 25.0	2327	6.1 ± 9.7
P	<0.001	<0.008		<0.001		<0.001		NS		0.011		<0.001		0.050

SHBG, sex hormone-binding globulin; HOMA, homeostasis model assessment of insulin resistance; SD, standard deviation; NS, not significant.

* Significantly different from each of the other two groups.